PERIPHERAL BLOOD ANALYSIS AS AN INDEX TO REPRODUCTIVE CONDITION IN THE CANADA GOOSE

Thesis for the Degree of M. S.
MICHIGAN STATE UNIVERSITY
WILBUR C. JOHNSON
1967

ABSTRACT

PERIPHERAL BLOOD ANALYSIS AS AN INDEX
TO REPRODUCTIVE CONDITION
IN THE CANADA GOOSE

by Wilbur C. Johnson

Two exogenous estrogen experiments were conducted, December 1966 and January 1967, using different daily dosage levels of 17-"B"-estradiol. Blood serum samples were taken from the brachial vein at three day intervals from each treatment group prior to, during, and after the treatment period. At dosage levels between 125 μg and 1 mg, mean treatment values for serum calcium (mg%), measured fluorometrically, total protein (g%), albumin (g%), globulin (g%), determined by electrophoretic separation, and the albumin/globulin ratio (A/G) showed significant ($P<.05$) changes from pre-treatment and post-treatment means. There appeared to be a correlation between increasing dosage levels and the relative changes in serum calcium, total protein and the A/G ratio. As a result of estrogen treatment, an average increase in serum calcium to 16.0 mg% or above, an average increase in serum protein to above 5.6 g% and a decrease in the A/G ratio to 0.8 or less were noted. Blood samples were ob-

tained from adult, two-year, and one-year old geese at weekly intervals during pre-season (unstimulated), pre-egg laying (stimulated) and egg laying (oviposition) time periods. Pre-season values for serum calcium, total protein, albumin, and A/G ratio were obtained from samples taken from December 31, 1966 to February 17, 1967. Pre-egg laying values for the same parameters were obtained from February 24, to March 17, 1967, and egg laying values were obtained during the period of March 24, to April 7, 1967. The adult geese were divided into normal, suppressed - egg laying and suppressed - non-egg laying after observations on behavior and oviposition were recorded. During the pre-season time period there was no significant difference ($P>.05$) between the five groups. The three adult groups, one year and two year old female geese could not be distinguished on the basis of peripheral blood constituents. During the pre-egg laying period serum calcium was the only parameter to increase significantly over pre-season values. This increase occurred only in the adult groups and there appeared to be a difference among mean calcium levels in normal adults and those adults that were considered suppressed. During the egg laying period the normal adults appeared to have a higher mean serum calcium level than the suppressed adults. Significant changes in total protein, globulin and the A/G ratio occurred during the egg laying period in adult geese. One and two-year old geese did not show significant changes in any of the parameters measured. The A/G ratio in those adult females that successfully nested did not decrease below 1.00 until approximately one week prior to the oviposition of the first egg. Suppressed non-laying adult females exhibited an increase in serum calcium but the A/G ratio did not decrease below egg laying values obtained from the normal adults. The time, order and magnitude of changes in peripheral blood constituents are of prime importance in their use as an index to reproductive condition. Calcium was the first constituent to demonstrate decernable changes. Increases in total protein followed and just prior to oviposition the A/G ratio decreased to below 1.00.

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By

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A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Fisheries and Wildlife

1967

G46892
12-8-67

ACKNOWLEDGMENTS

The author wishes to express appreciation to the following:

Dr. Jack S. Wood, Western Michigan University, for his supervision, encouragement, and guidance throughout the study.

Dr. L. W. Gysel for his advice and direction during the study.

Dr. Peter I. Tack, Dr. George Lauff, and Dr. Robert Ringer for their time and patience in reviewing the manuscript. Special thanks to Dr. Robert G. Wetzel for his editorial comments.

Mr. R. D. VanDeusen, Kellogg Bird Sanctuary, for the access to the Kellogg Flock and use of the Sanctuary's facilities and equipment.

Mr. Morton Rickless and Mr. Nick Smyrnios for their help with the analysis of the serum samples.

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INTRODUCTION

Wild populations of the Canada goose, *Branta canadensis*, in the Mississippi flyway normally leave wintering grounds in mid-March, and reach nesting areas by the third week in April. Within two weeks after arrival, breeding pairs have established territories, selected a nesting site, constructed a nest, and produced a complete clutch of eggs (Hanson and Smith, 1950).

At a time prior to and during the northward spring migration of wild populations, potential breeding female geese of the Kellogg flock progress from a state of sexual rest (non-breeding condition) to one of sexual activity (breeding condition). A number of research projects on domestic fowl and more recently the mallard duck, *Anas platyrhynchos*, indicate that there are distinct changes in the levels of certain blood chemical constituents in birds associated with the transition from a non-breeding to breeding condition. Smoes (1966), Wood and Hofman (1967) suggested that changes in peripheral blood constituents are related to the reproductive state in the mallard and that analysis of these changes may yield an index to reproductive condition.

This study, conducted from December 2, 1966, to April, 1967, was designed to provide data on the normal quantitative changes of selected blood chemical constituents (serum calcium, total protein, albumin, globulin and the albumin/globulin ratio) in the Canada goose and to measure the effects of estrogenic hormones on these constituents. If changes in the selected peripheral blood chemical constituents are decernable, then it may be possible to develope an index to reproductive state that can be used to determine reproductive potential in wild populations.

Since the Canada goose is migratory and its geographic range is in temperate North America, the gonadal and behavioral changes associated with reproduction are probably controlled and regulated by endogenous hormones which are triggered in response to external stimuli (Witschi, 1959). Much evidence now indicates that temperature and photoperiod may act to synchronize an inherent rhythm of activity of the pituitary (Bissonette, 1937; Burger, 1949; Benoit, 1938; Wolfson, 1952, 1959; Welty, 1962).

According to Marshall (1955) and others, the anterior pituitary, controlled by the hypothalamus, is the site of gonadotropin secretion that affects ovarian growth and egg laying. The gonadotropins in fowl are follicle stimulating hormone (FSH) and luteinizing hormone (LH).

Follicle stimulating hormone stimulates ovarian follicular growth which results in increased amounts of gonadal hormones, particularly estrogen, which has been demonstrated to have a pronounced effect on avian metabolism (Nalbandov, 1953; Lorenz, 1954; and Sturkie, 1965). Estrogen treatment in birds has been shown to cause enlargement of oviduct and pronounced changes in blood composition. A recent study on the mallard duck demonstrated both morphological and blood chemical changes induced by estrogen (Hofman, 1966). A number of other researchers have demonstrated similar estrogenic effects, but only those pertinent to this study will be discussed.

A marked increase in serum calcium has been noted in the mallard duck when daily doses of 17-"B"-estradiol were administered (Hofman, 1966; Rickless, 1967; Smyrnios, 1967). A number of other investigators have observed similar increases in serum calcium in domestic fowl of both sexes when estrogen treatments were administered (Landauer, _et al._, 1941; Fleischman and Fried, 1945; Common, _et al._, 1948; Polin and Sturkie, 1958; and Urist, _et al._, 1960). The increases were believed to be caused by the estrogen in the presence of parathormone. Benoit, et al. (1941) reported that parathyroidectomized ducks did not demonstrate an elevation in calcium in response to estrogen treatment. Polin and Sturkie (1958) and Urist, et al. (1960) reported similar results in the chicken.

Estrogen has also been reported to have a profound effect on serum protein. Hofman (1966), Rickless (1967) and Smyrnios (1967) demonstrated a marked increase in total serum protein following estrogen treatment in the mallard duck. Urist (1959) has reported a similar increase in chickens. The increase was related to the globulin fraction; the albumin fraction remained stable or slightly lower than pre-treatment values (Hofman, 1966). Hofman (1966) also demonstrated a significant reduction in the albumin/globulin ratio (A/G) with daily exogenous estrogen treatment. Smoes (1967) has reported a similar reduction during normal endogenous estrogen stimulation. According to Rickless (1967), exogenous and endogenous stimulation increases in globulin can be attributed to the beta fraction; however, under stress conditions, the alpha and gamma fractions increase. An increase in gamma globulin occurs just prior to egg laying (Rickless, 1967). Because increase in total protein and decrease in A/G ratio occur both under estrogenic stimulation and stress conditions, the two parameters alone can not be used to indicate reproductive state. However, an examination of the subdivisions of the globulin fraction of the serum protein can be used to determine whether the total protein and A/G ratio are responding to "stressors" or estrogen (Rickless, 1967).

Therefore a qualitative analysis of the globulin fraction along with quantitative changes in serum calcium, total protein, albumin, globulin, and the A/G ratio were used in this study to determine the reproductive state and probability of egg laying in selected female Canada geese.

This project was supported by the W. K. Kellogg Biological Station and a grant from the U. S. Fish and Wildlife Service, Contract Number 14-16-0008-773, to Western Michigan University.

METHODS AND MATERIALS

Semi-wild female Canada geese were used in this study. The individuals were selected according to age (one-year, two-year and three-years or older) from the research flock at the W. K. Kellogg Bird Sanctuary, Hickory Corners, Michigan. One-year female geese are immature, two-year geese are on the verge of sexual maturity, and three-year geese are normally sexually mature (Wood, 1964). Black plastic and aluminum leg bands with routed numerals were used as code numbers for individuals during the blood sampling periods. Primary feathers on one wing were clipped to prevent escape from experimental pens.

Blood sampling technique

To prevent the blood samples from freezing during the first portion of the study, December 31, 1966 to March 10, 1967, all samples were taken in a laboratory. The geese were transported by truck from the experimental pens to the laboratory in a 3 ft. x 3 ft. x 5 ft. wire holding crate. From March 17, 1967 to April 28, 1967, blood samples were taken in the field.

Because of the size of the geese two men were required to obtain the samples. After the bird was removed from the holding crate, its head was placed under its wing to aid in quieting. It was then turned ventral side up and the outside wing extended exposing the underside of the wing. By lifting the axillary feathers and removing the down from the humeral area, the brachial vein was exposed (Fig. 1). Pressure was applied to the brachial vein causing it to enlarge. The sampling area was then cleaned with 70% ethanol and a 2 ml blood sample obtained.

External and internal hemorrhaging were minimized by applying pressure with a dry cotton swab directly on the sampling point for several seconds. The blood sample was then placed in a labelled 5cc plastic vial and allowed to stand for about 20 minutes. Samples that did not readily clot were cooled in a water bath at 10 C for an additional 5-7 minutes.

Clotted samples were centrifuged at 3000 rpm for 10 minutes. The supernatant serum was removed with clean disposable pipettes and transferred to labelled plastic vials. All serum samples were stored frozen at -13 C until time of analysis.

<u>Serum analysis</u>

Blood serum samples were not held for analysis more than six weeks. Decker (1966) demonstrated that no material change

Figure 1. Blood sample being taken from the brachial vein, which has been enlarged by application of pressure above the sample point.

occurred in frozen samples up to that period. The serum was analyzed for calcium, total protein, albumin, and globulin in the following manner:

Calcium analysis

Calcium levels expressed in mg per 100 ml (mg%) were obtained by the use of a Turner Fluorometer with a primary filter 110-816(2A) and a secondary filter 110-818(2A-12). The calcein reagent was prepared by removing a 7 ml portion from 1000 ml 0.8N KOH, then replacing the 7 ml with calcein, yielding a 1 mg/ml concentration. (Calcein, 3,6-dihydroxy-2, 4-Bis [N,N-carboxy-methyl-aminomethyl] fluran, is a product of the Fisher Scientific Company.)

Three mixtures of known calcium concentration (9, 16, and 21 mg%) were used to establish standard curves. The fluorometer was adjusted to zero with the 9 mg% mixture and checked against the curve for the remaining two mixtures. A commercial serum (Versatol, 10.7 mg% calcium) was used as a control during the analysis.

Five ml of the calcein reagent was placed in a plastic vial along with a 20 lambda serum sample, shaken ten times and placed in 27 C water bath, oscillated for ten minutes and the fluorescence recorded.

Protein analysis

Quantitative values for total protein were obtained with the use of an American Optical T.S. Meter. Serum protein fractions, albumin and globulin were separated using cellulose acetate strips in a Gelmen cell (1.5 ma per strip for 40 min.) with a barbitone barbitol buffer solution (pH 8.6). The strips were then placed in Ponceau S stain for 6 minutes, cleared with 12% acetic acid in 95% ethanol and placed on glass to dry. A Densicord densitometer and integrator were used in the albumin and globulin determinations [grams per 100 ml (g%)].

Endogenous Estrogen Stimulation Experiments

This experiment consisted of ten pairs having previous breeding records, and two 18-24 month old females and two 8-12 month old females that were sampled at weekly intervals to obtain data pertaining to normal fluctuations of peripheral blood constituents during the progression from nonbreeding to breeding conditions.

The geese were fed a daily diet of whole corn, wheat, oats, buckwheat and alfalfa pellets. Several feeding locations within the pen were utilized to prevent intraspecific competition for food. Ample grazing areas were available, except during periods of snow cover. Lettuce trimmings were also fed during the winter months. Feeding was postponed on sample dates until after samples were taken. Blood samples were obtained between 11:00 and 12:00 a.m. on Fridays, from December 31, 1966 to April 28, 1967.

During the non-breeding season from December 25, 1966 to March 10, 1967, the entire flock was maintained in a 300 ft. x 150 ft. pen in close proximity to the laboratory. Approximately one-half of the pen consisted of water averaging in depth from two to three feet. During periods of severe cold, a water area about ten feet in diameter was kept open by a yearling pair of mute swans (Cygnus olar). An ice saw was used to enlarge the water area once a week (Fig. 2).

Ten adult pairs were transferred to breeding pens when they were believed to be progressing toward a reproductively stimulated condition. The experimental breeding pens were approximately 200 ft. long and 150 ft. wide. About one-fourth of each pen consisted of a shallow narrow pond with one small earthen nesting island in the south end (Fig. 3). In spite of the fact that the ground was still snow-covered and the ice-filled ponds did not thaw until March 18, 1967, nine of the breeding pairs were placed in their respective breeding pens on March 10, 1967.

Figure 2. Water area maintained by mute swans and part of goose flock used in endogenous estrogen experiment. February 11, 1967.

Figure 3. Experimental breeding pen III, approximately 200 ft. long and 150 ft. wide, with one earthen nesting island in the south end.

In order to obtain data on suppressed individuals the listed densities and non-random combinations of pairs were used because of facts known from past reproductive seasons and behavior exhibited by the pairs during confinement in the wintering pen. Three pairs were placed in each of the three breeding pens in the following non-random predetermined order: Pairs 5, 12 and 67 were placed in pen I; Pairs 7, 10, 62 were placed in pen II; Pairs 4, 11 and 13 in pen III and Pair 3 were left in the winter pen which was their resident territory the previous year. By using these combinations one or more of the individual pairs in each pen were expected to be suppressed due to overcrowding and lack of more than one suitable nest site.

In breeding pen I, Pair 12 were considered the dominant because of aggressive behavior[1] exhibited during the wintering period and the territory size occupied the previous nesting season. Pairs 5 and 67 were believed to be subordinate because of lack of aggressive behavior during the wintering period.

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[1] The degree of aggressiveness of each pair was based primarily on the behavior of the gander.

In pen II, Pair 10 were the resident birds (its nesting territory in 1966) and exhibited moderate aggressiveness during the wintering period. Pair 7 appeared to be one of the most aggressive during the winter period and past nesting records indicated that the gander was probably a dominant bird in the Kellogg flock. Pair 62 appeared to be submissive during the wintering period.

Pair 13 in pen III appeared to be one of the most dominant and aggressive in the experimental flock. They were also the resident nesting pair in pen III in 1966. Pairs 4 and 11 appeared to be subordinate during the wintering period and neither pair were considered dominant in the experimental flock.

On April 12, 1967, those pairs that had not established a territory, nesting site and begun their egg laying cycle were considered reproductively suppressed and were released as pairs into suitable nesting areas where they were no longer "overcrowded". Pairs 11 and 4 were released on their resident territories from 1966. Pairs 62 and 67 were placed in breeding pens where no other pairs of geese were nesting. Blood sampling was continued until egg laying occurred or until the likelihood of egg laying had passed.

The experiment was designed so that fluctuations in peripheral blood chemicals could be obtained for one-year, two-year, adult and suppressed adult female geese as they progressed from non-breeding to breeding conditions.

The two-year old (18-24 month) and one-year old (8-12 month) female geese were retained in the wintering pen for the duration of the experiment. Four earthen nesting islands were present in the wintering pen in event that the two-year geese progressed to a reproductive condition. Two full winged ganders paired off with the two-year old females.

First exogenous estrogen experiment

On November 22, and 25, 1966, ten two-year or older female geese were live trapped and separated into five groups of two each and placed in pens. The five pens were 40 ft. long and 8 ft. wide; water varying in depth from 6 in. to 2 in. covered approximately three-quarters of the area. An 8 ft. x 8 ft. covered shelter was located on the south end of each pen.

The experimental birds were fed the same diet as those in endogenous estrogen studies. Feeding was postponed until after blood samples were obtained on sample dates. The experimental geese were maintained at normal temperatures and photoperiod for that time of year.

The experiment was conducted during December 2 - 23, 1966. On December 2, two days prior to the first hormone treatment, pre-treatment samples were obtained from all ten geese. Beginning on December 5, additional pre-treatment samples were taken just prior to the administration of 17-"B"-estradiol in peanut oil. Injections were made subcutaneously in the axillary space. This treatment was continued for 14 consecutive days with the following dosage levels for each of the five groups.

Band numbers	Experimental group	Dosage/day
21, 22	1	1cc peanut oil
8, 18	2	250 μg estradiol
4, 77	3	1.0 mg "
79, 95	4	2.5 mg "
29, 94	5	5.0 mg "

Additional blood samples were taken on days 4, 7, 10, and 13 of the treatment period and on day 2 and 4 after the treatment period. All samples were taken between 11:00 and 11:30 a.m., and estrogen injections were made immediately following sampling.

<u>Second exogenous estrogen experiment</u>

Using the methods described above, ten adult female geese were divided into five groups of two each and administered a daily dose of 17-"B"-estradiol according to the following schedule:

Band numbers	Experimental group	Dosage/day
08, 49	1	0.5cc peanut oil
2, 47	2	125 µg estradiol
5, 9	3	250 µg "
8, 7	4	500 µg "
4, 23	5	1 mg "

The experiment began on January 14, 1967, and concluded on February 4, 1967.

Blood samples obtained during the two exogenous experiments were analyzed by the same method as those described in the endogenous estrogen studies.

Statistical analysis

A two-way analysis of variance using f ratios was employed to show significant differences at the .05 level between the age groups in relation to time periods (preseason, pre-egg laying, egg laying, suppressed, and post-season means) in the endogenous estrogen studies. A similar analysis was used for values obtained in both of the exogenous estrogen experiments. The comparison was made between pre-treatment, treatment and post-treatment means for each dosage level. A model 3600 Control Data computer at the Computer center, Michigan State University, was used to obtain the analysis.

RESULTS AND DISCUSSION

Exogenous Estrogen Experiments

Two exogenous estrogen experiments were conducted using 17-"B"-estradiol in a peanut oil carrier. The first experiment was conducted December 2 - 23, 1966 and the second, January 14 - February 4, 1967. The two experiments were conducted in the same manner, differing only in the time of year and dosage levels administered (Tables 1 and 2). Pre-treatment samples were taken three days prior to and on the first day of estrogen treatment just prior to its administration. Treatment samples consisted of four samples taken on days 4, 7, 10 and 13 during estrogen administration. Post-treatment samples were taken 2 and 4 days after cessation of treatment.

First Exogenous Estrogen Experiment

Mean pre-treatment, treatment and post-treatment values for serum calcium, total protein, albumin, globulin and the A/G ratio are given in Table 1. The mean treatment values for serum calcium showed a significant difference ($P < .05$) from mean pre-treatment and post-treatment levels in all but the 250 μg group. Mean pre-treatment values for all groups combined was 10.9 mg%; treatment values were 30.6 mg% and the post-treatment level was 18.8 mg% (Table 2, Appendix A).

Table 1. Mean pre-treatment (Pt), treatment (T), and post-treatment (Po) values for calcium (mg%), total protein (g%), albumin (g%), globulin (g%), and the A/G ratio from the first exogenous estrogen experiment. December 2, 1966 to December 23, 1966.

Group	Bird number	Estradiol dosage	Number of samples	Ca	σ	TP	σ	Alb	σ	Glb	σ	A/G	σ
1	20 & 21	1 ml pea-	4 Pt	11.2	0.26	4.7	0.43	2.9	0.07	1.7	0.41	1.8	0.46
		nut oil	8 T	11.0	0.83	4.7	0.28	2.8	0.33	1.9	0.14	1.5	0.27
			4 Po	11.6	0.53	5.0	0.32	2.9	0.11	2.2	0.27	1.4	0.15
2	8 & 18	250 μg	4 Pt	11.4	0.52	5.0	0.52	3.0	0.17	1.9	0.33	1.6	0.25
			8 T	12.5	1.12	4.9	0.44	2.7	0.29	2.0	0.42	1.4	0.34
			4 Po	12.8	1.00	5.7	0.38	3.1	0.53	2.5	0.54	1.4	0.60
3	4 & 77	1.0 mg	4 Pt	9.9	0.94	4.4	0.41	2.4	0.54	1.8	0.25	1.4	0.38
			8 T	30.9	14.44	6.2	1.06	2.8	0.38	3.3	1.07	0.8	0.20
			4 Po	17.1	5.64	5.6	0.56	3.3	0.61	2.7	0.76	1.3	0.13
4	79 & 95	2.5 mg	4 Pt	10.8	0.40	4.8	0.19	2.8	0.15	1.9	0.11	1.5	0.04
			8 T	50.1	9.88	10.8	1.41	4.0	0.80	6.8	1.12	0.6	0.14
			4 Po	23.0	5.75	6.1	0.67	3.0	0.12	3.1	0.53	1.0	0.13
5	29 & 94	5.0 mg	4 Pt	11.2	0.92	5.3	0.50	3.4	0.58	1.8	0.26	1.9	0.54
			8 T	48.4	13.45	10.4	1.63	3.4	0.35	7.2	1.66	0.5	0.18
			4 Po	29.1	6.04	6.4	0.70	3.0	0.11	3.4	0.64	0.9	0.13
Significance level			(Groups)	0.143		0.219		0.551		0.245		0.129	
Probability of F statistic			(Time)	0.035		0.083		0.665		0.064		0.009	

Thus a significant increase occurred during estradiol treatment and a decrease after treatment was apparent.

There was not a statistical difference among groups on the various dosage levels. However, there was an apparent correlation between increased estradiol dosages and calcium elevation. As dosage levels increased from 250 µg to 5 mg, an increase in mean calcium from 12.2 to 48.4 mg% occurred (Table 1). It appears that 2.5 mg of estradiol may have been the maximum effective dosage for the geese, as the 5 mg treatment group did not demonstrate any apparent increases over those given 2.5 mg (Table 1).

Mean treatment levels of total protein as compared to pre-treatment and post-treatment levels approached statistical significance at the 0.05 level (Table 1, 0.08). Even though they were not statistically different, there was an increase that appeared to be correlated with the dosage level. The total protein levels attained by the two groups receiving 2.5 mg and 5 mg doses did not appear to be different (Table 1, group 4 and 5). The increases in total protein can be primarily attributed to a marked increase in the globulin fraction. While the globulin increase due to treatment was not statistically significant at the 0.05 level, it approached that level (Table 1, 0.06). The albumin fraction neither increased nor decreased markedly from pre-treatment values. The relatively stable albumin level and the increase in the globulin fraction resulted in a significant ($P < .05$) decrease in the A/G ratio when mean treatment values were compared with pre-treatment and post-treatment values (Table 1). An analysis for differences among dosage levels again was not significant, but mean values for the A/G ratio given in Table 1 show an apparent decrease from control and pre-treatment means at all dosage levels except 250 μg. The lack of statistical differences among the groups on various dosage levels, even though pre-treatment values were equable (homogeneous), can probably be attributed to the wide range of dosages (250 μg to 5 mg) resulting in a great variability in group response to these levels of estradiol. The small number of degrees of freedom resulting from the use of mean values rather than individual sample values reduced statistical sensitivity. Even without statistical significance, it was apparent that increases in serum calcium and total protein occurred in female Canada geese at increased estradiol levels. The increases in total protein resulted from an increase in the globulin fraction which in turn resulted in the noted decrease in the A/G ratio.

With the acknowledged results and problems encountered in the first exogenous estrogen experiment, a second exogenous estrogen experiment was conducted.

Second Exogenous Estrogen Experiment

The range of dosage levels of estradiol was reduced to 125 μg - 1 mg in an attempt to reduce response level and the variability of response among groups. Lower responses increase the accuracy of protein analysis; severe lipemia occurs with high doses.

Mean treatment period values for all five parameters were significantly different ($P<.05$) from means of pre-treatment and post-treatment periods (Table 2, time). The combined means for pre-treatment calcium levels was 11.4 mg%; treatment level, 15.1 mg%; and post-treatment, 14.2 mg%. As in the first experiment, there was a statistically significant difference in serum calcium during estradiol treatment and an apparent decrease after treatment (Appendix B, Table 2).

A significant difference ($P<.05$) among groups for mean serum calcium was also obtained in this experiment. Mean treatment values for each of the five groups were 11.6, 12;5, 13.6, 16.4 and 21.4 mg% respectively (Table 2). There appeared to be a correlation between increased estradiol and

Table 2. Mean pre-treatment (Pt), treatment (T), and post-treatment (Po) values for calcium (mg%), total protein (g%), albumin (g%), globulin (g%), and the A/G ratio from the second endogenous estrogen experiment. January 14, 1967 to February 4, 1967.

Group	Bird number	Estradiol dosage	Number of samples		Ca	σ	TP	σ	Alb	σ	Glb	σ	A/G	σ
1	08 & 49	½ ml pea-	4	Pt	11.0	0.15	4.2	0.05	2.9	0.18	1.4	0.18	2.0	0.48
		nut oil	8	T	11.6	1.01	4.5	0.30	2.7	0.22	1.8	0.33	1.6	0.32
			4	Po	11.8	0.96	4.4	0.44	2.6	0.30	1.8	0.35	1.5	0.36
2	2 & 47	125 µg	4	Pt	11.5	0.58	4.8	0.46	2.8	0.29	2.0	0.33	1.4	0.35
			8	T	12.5	0.69	4.8	0.35	2.8	0.44	2.1	0.23	1.3	0.31
			4	Po	12.3	0.41	4.8	0.30	2.4	0.37	2.4	0.26	1.0	0.22
3	5 & 9	250 µg	4	Pt	11.1	0.48	4.8	0.18	3.0	0.36	1.8	0.27	1.9	0.48
			8	T	13.6	1.56	5.0	0.44	2.4	0.35	2.6	0.47	1.0	0.29
			4	Po	13.3	0.69	5.1	0.61	2.7	0.24	2.4	0.73	1.4	0.34
4	7 & 81	500 µg	4	Pt	11.6	0.23	5.1	0.27	3.0	0.26	1.8	0.36	1.9	0.60
			8	T	16.4	2.22	5.6	0.63	2.6	0.37	3.1	0.73	0.8	0.26
			4	Po	14.8	1.60	5.2	0.30	2.4	0.24	2.8	0.15	0.9	0.09
5	4 & 23	1.0 mg	4	Pt	11.9	0.78	5.1	0.19	3.2	0.26	1.8	0.26	1.8	0.46
			8	T	21.4	4.13	5.6	0.50	2.6	0.34	3.0	0.58	0.9	0.26
			4	Po	18.5	3.85	5.2	0.20	2.9	0.55	2.5	0.66	1.3	0.64
Significance level			(Groups)		0.028		0.0005		0.495		0.023		0.134	
Probability of F statistic			(Time)		0.032		0.015		0.015		0.005		0.003	

mean calcium elevation. The degree of response and the variability encountered among the treatment groups was not as great as those in the first experiment (Table 1 and 2, means and s). This decrease in variability as previously mentioned in experiment one, probably resulted in the statistical significance found among the groups even though the differences among group means were not as obvious.

Increases in mean total protein values among groups and in response time (pre-treatment versus treatment and post-treatment) were significantly different (Table 2, $P<.05$). A correlation between dosage level and increases in mean total protein was not as obvious in this experiment because treatment levels did not show as great a deviation. The group mean values (1-5) were 4.4, 4.8, 5.0, 5.3 and 5.4 g% (Table 2, Appendix B). There was a significant ($P<.05$) difference among the albumin fractions and an even more apparent significant ($P<.01$) difference in the globulin fractions. These resulted in a significant ($P<.05$) difference in the resulting A/G ratios.

There was not a significant difference ($P>.05$) among mean A/G ratios in relation to dosage level (group). This lack of significance can probably be attributed to the low levels of response to the low dosages resulting in less pronounced decreases in the ratio.

As a result of both exogenous estrogen experiments the following changes in the measured parameters were attributed to estradiol stimulation:

1. An average increase in serum calcium to approximately 16 mg% or above.
2. An average increase in serum protein to above 5.6 g%.
3. A general decrease in the A/G ratio to 0.8 or less, due to increased globulin fraction and a decrease in the albumin fraction.

Wood and Hofman (1967) obtained similar results from female mallard ducks given estradiol as in this study. Elevations in serum calcium to 15.0 mg% or above, serum protein above 5.6 g% and a decrease in the A/G ratio to 0.66 or less were attributed to estradiol stimulation. Baldini and Zarrow (1952) using six male and female bobwhite quail administered 100 μg of estradiol for 21 days and obtained a mean serum calcium level of 34.5 mg% (range 25-51). Two geese in the second experiment of this study were given a 125 μg dosage of estradiol, resulting in a mean treatment value of 12.5 mg% calcium. Wood and Hofman (1967) administered 125 μg of estradiol to female mallard ducks and obtained a mean treatment level of 28.2 mg%. Obviously the three species are not equal in the level of response attained at this common dosage level. Perhaps if the dosage levels had been administered on a µg/Kg body weight the discrepancies would not have been as great. Differences in methods of analysis may also be responsible for the discrepancies.

Malbandov (1953) demonstrated that estrogen is capable of increasing plasma proteins in chickens by 60%. In the Canada goose, increases of 100% were obtained on two groups receiving 2.5 mg and 5.0 mg estradiol, respectively. All other dosage levels did not increase serum protein levels by more than 30%.

Wood and Hofman (1967), and Landauer *et al*. (1941) stated that mallards treated with estrogen demonstrate a correlation between increased dosages and calcium elevation. However, both investigators also reported that in subsequent experiments, at a later time in the year (January), the elevation in calcium did not directly correspond to dosage levels as it had previously. In this study both of the experiments appeared to demonstrate a correlation directly between increases in calcium and total protein to increased dosage level. There is also evidence that the A/G ratio decreased linearly in relation to increased dosage level in the first experiment. The results of this study do not agree entirely with those of Wood's and Landauer's.

It appears the parameters measured differ in sensitivity to exogenous estrogen. Calcium seems to respond first and demonstrates the highest percentage of increase. At the high dosage levels in the first experiment there was a three to five fold increase in serum calcium. However, it is not necessarily the degree of change that is the important factor in using peripheral blood constituents as an index to estrogen stimulation. Changes in serum calcium, total protein and the A/G ratio by themselves are meaningless. It is the sequence and combination of changes that occur in the parameters that offer a potentially useful index.

Endogenous Estrogen Experiment

Mean values for serum calcium, total protein, albumin, globulin and the A/G ratio in five age treatment groups were calculated from 172 blood samples taken from 14 individuals over a period of 12 sample dates from December 31, 1966 to April 7, 1967. Group one (1) consisted of five three-year or older female Canada geese. Group two (2) consisted of two three-year or older females that were suppressed during the normal egg laying period but successfully nested after release from crowded conditions. Group three (3) consisted of three three-year or older females that were suppressed during the normal egg laying period and did not successfully nest after release from crowded conditions. Group four (4) consisted of two two-year old females and group five (5) consisted of two one-year old females. The 12 sample dates were divided into three time periods: pre-season (December 31, 1966 - February 17, 1967), pre-egg laying (February 24 - March 17, 1967), and egg laying (March 24 - April 7, 1967) (Tables 1-4, Appendix C). Means and standard deviations are given for each parameter in Table 3 and 4.

Endogenous Estrogen Experiment: Pre-season period

Mean values for serum calcium showed no significant difference ($P > .05$) among any of the five groups. The pre-season means were 11.2, 11.5, 11.2, 11.0 and 11.5 mg% for groups 1-5, respectively (Table 3). These values are only slightly higher than the non-laying serum calcium value of 10.8 mg% for the female mallard duck (Wood and Hofman, 1967). Landauer *et al*. (1941) found serum calcium levels of 9.8 - 10.4 mg% in the male mallard.

As in calcium, there was no significant difference ($P > .05$) among groups for total protein, albumin, globulin and the A/G ratio during the pre-season period. Mean values for each parameter are given in Table 1. An example of pre-season

Table 3. Mean pre-season (Ps), pre-egg laying (Pe), and egg laying (E), values for calcium (mg%), total protein (g%), albumin (g%), globulin (g%), and the A/G ratio in adult, one-year and two-year old female Canada geese.

Bird nos.	Group name (Group no.)	No. of samples	Time period	Ca	σ	TP	σ	Alb	σ	Glb	σ	A/G	σ
3,7,	Normal adult	25	Ps	11.1	0.57	4.6	0.42	2.9	0.36	1.6	0.31	1.9	0.49
10,12	(1)	20	Pe	16.6	5.45	4.5	0.59	2.9	0.29	1.7	0.56	1.8	0.52
& 13		15	E	33.7	9.83	6.6	1.20	2.8	0.61	3.8	1.20	0.88	0.42
11 &	Suppressed adult	10	Ps	11.5	0.75	5.0	0.45	3.0	0.59	2.0	0.58	1.7	0.68
62	laying after	8	Pe	13.6	2.16	4.9	0.25	3.4	0.24	1.5	0.24	2.2	0.65
	release	6	E	26.0	7.68	6.1	1.03	2.8	0.39	3.3	1.12	1.00	0.47
	(2)												
5,8	Suppressed adult	15	Ps	11.2	0.60	4.7	0.39	2.9	0.50	1.8	0.35	1.8	0.73
& 67	non-laying	8	Pe	12.7	2.30	4.3	0.28	2.9	0.34	1.3	0.23	2.2	0.64
	(3)	6	E	28.3	11.05	5.8	1.17	3.1	0.51	2.7	0.87	1.4	0.57

Table 3. Continued

Bird nos.	Group name (Group no.)	No. of samples	Time period	Ca	σ	TP	σ	Alb	σ	Glb	σ	A/G	σ
81 &	Two-year old	10	Ps	11.0	0.48	4.5	0.45	2.6	0.35	1.8	0.36	1.5	0.26
88	18-24 months	8	Pe	11.7	0.95	4.0	0.22	2.4	0.20	1.5	0.10	1.5	0.24
	(4)	6	E	12.1	1.83	4.4	0.91	3.0	0.69	1.4	0.24	2.2	0.27
35 &	One-year old	10	Ps	11.5	0.28	5.0	0.23	2.9	0.31	2.1	0.25	1.5	0.37
89	8-12 months	8	Pe	11.4	1.78	4.2	0.20	3.0	0.46	1.3	0.35	2.3	0.45
	(5)	6	E	12.3	1.63	4.7	0.33	3.2	0.34	1.5	0.20	2.2	0.43
Significance level			(Groups)	0.263		0.137		0.207		0.453		0.755	
Probability of F statistic			(Time)	0.022		0.020		0.621		0.077		0.281	

mean values are as follows for the normal three-year or older geese: 4.6 g% total protein, composed of 2.9 g% albumin, 1.6 g% globulin and an A/G ratio of 1.9. Wood and Hofman (1967) have established similar values for the non-laying female mallard: 4.8 g% total protein, consisting of 3.2 g% albumin, 1.6 g% globulin and an A/G ratio of 2.0.

The values appear quite similar between the five groups in this experiment during the pre-season period. However, subsequent differences during the following two time periods can probably be related to the level and time of stimulation attained by each of the groups.

<u>Endogenous Estrogen Experiment</u>: <u>Pre-egg laying period</u>

As mentioned in the experimental methods for the endogenous estrogen experiments, it was necessary to transfer the ten three-year or older pairs to breeding pens at a time when they were believed to be progressing from an unstimulated to a reproductively stimulated condition. This transfer was made on March 10, 1967, after the following observations were made indicating that the pairs were entering a reproductively stimulated condition.

1. The return of established non-experimental pairs to the vicinity of their previous year's nesting territory occurred on March 9,

1967, when 12 pairs were observed defending their last year's territory[2] (Fig. 4). The temperature was above 12 C and sunny.

2. Pair isolation of the flock in general was also used as an indirect method of determining the onset of stimulation. Pair isolation was first noticed on February 22, and became more obvious on March 2, 3, and 9.

3. Agonistic behavior on the part of the dominant pairs in the experimental flock became more frequent during the first week in March, even to the point where threats and fighting occurred when the geese were being driven into the holding pen for sampling on March 10.

4. Fence walking by the experimental pairs in the wintering pens occurred on March 4 and 9, indicating that the pairs probably would have returned to their previous

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[2]Past records at Kellogg on nesting site selection by Canada goose pairs indicated that if their nesting attempts were successful they returned to the same territory and often the same nest site year after year.

Figure 4. Non-experimental pair of geese defending the vicinity of their previous year's nest, March 9, 1967.

year's nesting territory had they not been retained by the fence (Fig. 5).

The use of behavioral characteristics as indices to reproductive stimulation has been questioned. Wood (1964) has suggested that the presence of behavioral patterns related to reproductive stimulation do not necessarily mean that Canada geese will successfully reproduce; conversely, the absence of these patterns does not mean that they are not in a stimulated reproductive condition. Behavioral motor patterns that occur and are often associated with reproductively stimulated adult geese are also performed by known immature geese (Wood, 1964; Sherwood, 1966). Thus, the use of behavioral characteristics as an index to reproductive stimulation is probably not suitable.

In this study the occurrence of behavioral attitudes in the mature pairs occurred from one to three weeks prior to any decernable peripheral blood response. Had the blood samples been analyzed on the day of release in an attempt to determine the precise time for release from the wintering pen, it would have been noted that March 17,was the first sample date on which all of the experimental mature female geese demonstrated an increase in serum calcium over previous samples, this was the first indication of estrogen stimula-

Figure 5. Some of the experimental pairs walking on the east border fence of wintering pen on March 9, 1967.

tion (Tables 1-3, Appendix C). Individual females, 7 and 13, demonstrated marked increases in serum calcium much earlier (March 3). The first individual to demonstrate a decernable change in serum calcium was number 10 on February 24, (Table 1, Appendix C). Thus, subsequent analysis of the data collected substantiates the selection of March 10, as a release date coinciding with the transition from an unstimulated to a reproductively stimulated condition.

The variability observed in the start of decernable changes in serum calcium for individual females can probably be attributed to individual variation in response to photoperiod. However, there was some indication that a relationship may exist between social status and the time and the degree of stimulation at the beginning of the reproductive season. On March 10, those females in group one (dominant pairs) had a mean calcium level of 16.6 mg% while those females in group two and three (subordinate pairs) had a mean calcium level of 13.2 mg%. The small number of samples make the interpretation of the data difficult.

Mean pre-egg laying serum calcium values for one-and two-year old geese were 11.4 and 11.7 mg%, respectively (Table 3). These values do not appear to differ from preseason values, indicating the birds were not reproductively stimulated.

An analysis of variance for pre-egg laying means of each parameter for all groups (Table 3, 1-5) did not show any significant differences among the groups. The variability about the means during this period, particularly group one, probably accounts for the lack of significance (Table 3). Lack of statistical significance can also be attributed to the heterogeneity of the groups (one-year, two-year and three-year olds or older geese) that are not comparable physiologically during this period. Another important factor in the significance of an F statistic is the degrees of freedom. These were small because an analysis of variance requires independence among the samples. Continuous sampling of a single group results in dependency. This was corrected by using means which are independent.

Even though the mean values were not statistically different, there were noticeable differences in the mean serum calcium between the adult geese (groups 1-3) and the one and two-year old geese (groups 4 and 5) (Table 5, Appendix C). Mean serum calcium values for the mature geese were 20.5 mg%, 17.0 mg% and 17.4 mg% in groups 1, 2, and 3, respectively. The mean values for one-year old and two-year old geese were 11.6 mg% and 11.7 mg%, and are not different from the pre-season means established for these two age groups (Table 5, Appendix C). It is apparent that the older geese were in a stimulated condition while the one-year and two-year females were not. There were no obvious differences between the other parameters measured during this period.

Endogenous Estrogen Experiment: Egg laying period

Observations were made on 23 non-experimental nesting pairs during the nesting season in 1967. Of the 23 nests started by these pairs, 95% laid the first egg between March 29, and April 10. Thus, this time period was designated as the peak egg laying period. Whether or not the experimental pairs began their egg laying cycle between these dates determined whether or not they were considered reproductively suppressed during the egg laying period.

At this time in the experiment the adult female geese were divided into three groups: normal (unsuppressed), suppressed (not laying during peak "egg laying" period), and suppressed (non-egg laying after release).

Pair behavior, consisting of overt fighting, head shaking, threats, and calling were used as a direct method of determining the social status of the pairs in each pen. According to the investigator's observations, dominant pairs of geese exhibit, for the lack of a better term, affirmation bouts which consist of synchronous gabbling, head shaking and bowing, and threat postures directed toward all other geese. Subordinate pairs were not observed behaving in this manner under the crowded conditions.

Prior to laying there was a decernable enlargement of the abdominal region in the female goose (Fig. 6). Daily observations on this condition as well as the results of "daily egg hunts" were recorded.

The non-random predetermined combinations of pairs that were unequal in social status as described in the methods were used to affect suppression of one or more pairs due to overcrowding and lack of more than one suitable nesting site.

In pen I, where Pairs 5, 12 and 67 were placed, only Pair 12 established a nest, produced a clutch of eggs and successfully hatched them (first egg laid on April 5). The nesting territory established by the male of Pair 12 included the entire water area and the nesting island.

The mean serum calcium, total protein and A/G ratio for goose number 12 during the egg laying period was 31.4 mg%, 5.7 g% and 0.85, respectively (Table 1, Appendix C). Since Pairs 5 and 67 did not begin egg laying during the established egg laying period they were considered suppressed. For comparison, the mean calcium, total protein and A/G ratio

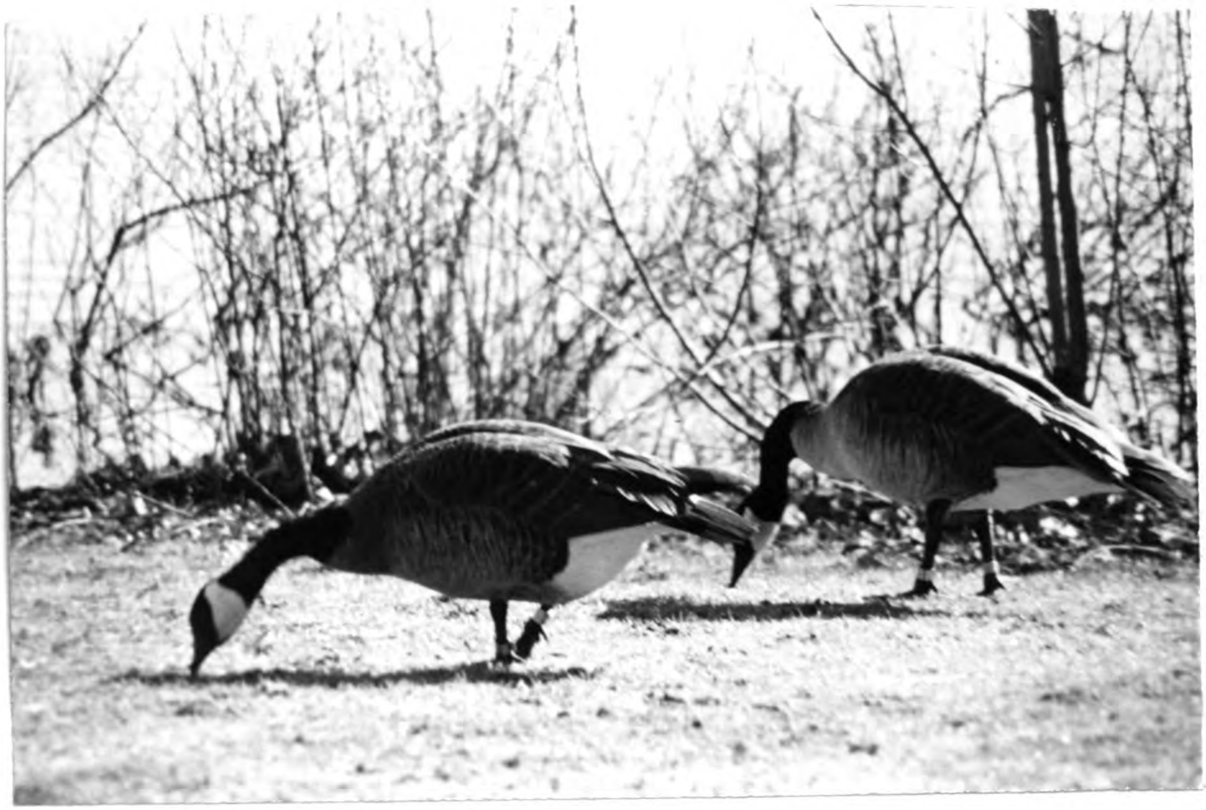

Figure 6. Female in foreground with enlarged abdominal region that occurs a short time prior to egg laying.

for these two females during this period were 26.4 mg%, 5.5 g% and 1.34 (Table 3, Appendix C). The two suppressed females exhibited calcium and total protein values slightly lower than those for the egg laying female; these values indicate that they were probably reproductively stimulated. The increase in total protein was primarily attributed to beta globulin, indicating that the geese were under estrogenic stimulation, however, the maintenance of a high albumin fraction resulted in an A/G ratio above that of the egg laying females. Some minor fluctuations in gamma globulin were noted, but were not consistent. Pair 67 were released from pen I, but did not successfully nest after release.

The combination of pairs in pen II resulted in the establishment of two nesting territories by Pairs 7 and 10. The single nesting island was occupied by Pair 10 and the base of a willow tree (<u>Salix sp</u>.) was the nest site of Pair 7 (Fig. 7). It appeared that these two pairs were equal in social status and the lack of dominance resulted in the division of the available nesting habitat into two equal portions. Both females began their egg sequence on April 7. The remaining pair (62) did not establish a nesting territory during the egg laying period and were considered suppressed.

Figure 7. Pair 7 at nest site at the base of willow tree, Salix sp. (pen II), May 14, 1967.

However, on April 3, an egg was found next to the feeding trough used by this pair.(three separate feeding troughs were placed in each breeding pen because the aggressive behavior and defended nesting territories of the pairs excluded the subordinate pairs from large portions of the pens). Thus, female 62 was possibly in an egg laying condition at this time. No other "dropped" eggs were found and Pair 62 was released from the breeding pen on April 12.

The mean serum calcium, total protein and A/G ratio for females 7 and 10 during the normal egg laying period were 32.2 mg%, 6.8 g% and 0.86, respectively. Means for female of Pair 62 for the same period were 29.0 mg% calcium, 6.6 g% total protein and 0.77 for the A/G ratio. On March 31, four days prior to the date when the egg was found by the feed trough, the individual sample for number 62 indicated that serum calcium was 36.0 mg%, total protein 7.4 g% and the A/G ratio was 0.85 (Table 2, Appendix C). An analysis of the globulin distribution demonstrated that on March 31, there were elevations in gamma, beta, and alpha globulins. The albumin fraction was reduced. These changes correspond with the noted elevation in total protein and reduced A/G ratio. These observations combined with the high calcium level indicate a general egg laying condition. Female 62 laid the first egg in a latent successful nesting attempt on April 18, seven days after release. The enlarged abdominal region of this female also indicated that she was in an egg laying state. However, the other suppressed females that did not lay also exhibited this condition, leading to the conclusion that it is not necessarily indicative of egg laying.

Of the three pairs in pen III, only Pair 13 established a territory and began egg laying during the egg laying period (first egg oviposited on March 31). The territory defended by the gander of Pair 13 included the nesting island and most of the water area. Pairs 08 and 11 appeared to be suppressed during the egg laying period (Fig. 8).

The mean serum calcium, total protein and A/G ratio for the goose of Pair 13 during the egg laying period were equal to the values listed for the other three egg laying females in pens I and II (Table 1, Appendix C, egg laying values). Neither of the females of Pairs 08 or 11 were known to lay eggs during the egg laying period and were considered suppressed. Both pairs were released from pen III on April 12. Upon examination of the values obtained on April 7 and 12, for goose 11, it was apparent that she was stimulated and within the range of values of the normal egg laying females

Figure 8. An example of aggressive behavior exhibited by the gander of Pair 13 (pen III), March 17, 1967.

(Table 2, Appendix C). Prior to release, increases in total protein were primarily a result of increases in alpha globulins. Beta globulin increased just prior to, and following release and appeared to be greater than either the alpha or gamma fraction. Female of Pair 11 began a normal egg laying cycle three days after release. Similarly, goose 08, on April 7 and 12, had serum calcium and total protein values that were within the range of the normal egg laying female. The A/G ratio was not as low and continued to increase in subsequent samples after release (Table 3, Appendix C). An analysis of the globulin distribution indicated that goose 08 was in a stimulated, but non-egg laying condition. Beta globulin increased during the egg laying period, however, alpha globulin appeared to exceed beta and gamma globulins during this period. The maintenance of high alpha globulins is probably indicative of prolonged "stress". After release the protein fractions returned to a non-reproductive condition. Calcium and total protein values decreased after release. These two observations seem to indicate that female 08 was in a non-egg laying condition accompanied by prolonged "stress". She apparently regressed to a non-reproductive condition after release, as indicated by the return of total protein and A/G ratio to pre-season values on April 28.

(Appendix C, Table 3).

Adult Pair 3 was not transferred to the breeding pens on March 10. By retaining this pair in a situation where there was no competition for a nesting territory, it was thought that they would serve as a "control" for normal egg laying values in the event that none of the other nine adult pairs began their egg laying cycle within the previously defined peak egg laying period. Four of the nine pairs under "crowded" conditions did begin to lay within the peak egg laying period, as did Pair 3. And comparing the egg laying values obtained from Pair 3 with those of the other four females, it seems apparent that there was no difference between the five females (Table 1, Appendix C). On the basis of the similarity in sample values and the time at the onset of the egg laying period, all five geese and their sample values were considered representative of the flock.

A comparison of mean "egg laying" values for one and two-year old geese did not show any marked changes from pre-season values (Table 3). The one-year old females were considered immature and no changes were expected. However, a number of investigators have reported that in the populations they studied a percentage of the two-year female geese are mature and successfully nest (Wood, 1964 - less than 5%; Balham, 1950 - less than 7%; Sherwood, 1966 - 20%; Hanson, 1952 - 80%). Only two two-year females were used in this study and neither of them produced any eggs. There was an apparent latent stimulation in the two-year old geese as serum calcium levels increased on April 21 and 28 (Table 4, Appendix C). Even though there were increases in serum calcium, values for total protein and the A/G ratio did not change from previous samples, indicating that the geese were not in an egg laying condition. No samples were taken after April 28, to determine if the latent stimulation continued.

Mean values for serum calcium showed no significant differences (P>.05) among any of the five groups. The mean egg laying values for serum calcium were 33.7, 26.0, 28.3, 12.1 and 12.3 mg% for groups 1-5, respectively (Table 3). Even though they were not statistically different, there were obvious differences between the normal adult and the one- and two-year old females, and mean values for suppressed adult females were lower than the means of the egg laying adult.

Mean serum calcium values did show a significant difference (P<.05) between pre-season, pre-egg laying and egg laying time periods indicating that endogenous estrogen stimulation at the onset of the reproductive season resulted in a change in the peripheral calcium levels. This significance should not be interpreted as implying that all of the groups are different (Table 5, Appendix C). It is quite apparent upon examination of the means for the one-and two-year olds that there is little difference in mean values among time periods; therefore, significant differences can be attributed only to the adults.

Mean total protein values among groups were not significant ($P > .05$); however, they were significantly different ($P < .05$) among the time periods. As with calcium, this difference is applicable only to the adult geese. The three adult groups 1, 2 and 3 had mean total protein values of 6.6, 6.1 and 5.8 g%, respectively, whereas the one-and two-year old female mean values were 4.7 and 4.4, respectively (Table 3).

Means for albumin, globulin and the A/G ratio were not significantly different ($P > .05$) among groups or time periods, but again there were apparent differences in the globulin fraction and the A/G ratio among adults when compared to one- and two-year olds and pre-season values (Table 3).

Mean egg laying values for the mallard duck were reported as 20.2 mg% calcium, 5.6 g% total protein consisting of 2.1 g% albumin and 3.5 g% globulin, with an A/G ratio of 0.60 (Smoes, 1966). In this study the mean egg laying values for the five normal adult female geese were 33.7 mg% calcium, 6.6 g% total protein consisting of 2.8 g% albumin and 3.8 g% globulin and an A/G ratio of 0.88 (Table 3). It appears that the goose has slightly higher calcium and total protein levels during the egg laying period than other species. Winget and Smith (1958) determined that the serum calcium level of the laying Leghorn chicken was 21.5 to 28.1 mg%. Sendroy et al. (1961) found that the laying pigeon exhibited a serum calcium level of 22.6 mg%. Baldini and Zarrow (1952) using 13 female bobwhite quail determined that the mean egg laying value for serum calcium was 29.3 mg% with a range of 23 to 40.2 mg%.

Sturkie (1965) states that the total protein level for the laying mallard is 3.5 g%. This value is somewhat lower than the 5.6 mg% reported by Smoes (1966) and is considerably lower than the 6.6 mg% established for egg laying Canada geese in this study. Sturkie and Newman (1951) reported a total protein range of 4.6 to 4.9 g%, albumin range of 2.2 to 2.4 g%, globulin range of 2.5 to 2.7 g%, and a range for the A/G ratio of 0.84 to 0.93 for egg laying values in 18-month old White Leghorn chickens. In their study it was stated that they used the biuret method for total protein and albumin analysis. This method reportedly produces more variable results than electrophoretic separation (Wood and Hofman, 1967). Brandt *et al.* (1951) found that the total protein level for the laying chicken was 5.4 ± 0.71 g%. The discrepancies between reported total protein levels for egg laying periods are possibly explainable on differences between species and methods of analysis.

SUMMARY

Two exogenous estrogen experiments were conducted using different daily dosage levels of estradiol. Serum samples were collected from each treatment group prior to, during, and after the treatment period.

In the first exogenous estrogen experiment, significant changes ($P < .05$) attributable to estrogen treatment were found for serum calcium and the A/G ratio. An analysis for differences among treatment groups on increasing dosage levels was not significant ($P > .05$). The lack of statistical differences among the groups was attributed to the wide range of dosages (250 μg to 5 mg) that resulted in a great variability in group response; further, the small number of degrees of freedom resulting from the use of mean values rather than individual sample values reducing statistical sensitivity.

In the second exogenous estrogen experiment the range of dosage levels were reduced to 125 μg to 1 mg. Mean treatment values for all five parameters measured were significantly different ($P < .05$) from mean pre-treatment and post-treatment means. Significant differences ($P < .05$) among treatment groups for serum calcium, total protein, and globulin were also obtained in this experiment. The increases

in total protein were attributed to an increased globulin fraction. This relationship resulted in a reduction in the A/G ratio.

In both exogenous estrogen experiments there appeared to be a correlation between increasing dosage levels and change in serum calcium, total protein and the A/G ratio. An average increase of serum calcium to 16.0 mg% or above, an average increase in serum protein to above 5.6 g%, and a general decrease in the A/G ratio to 0.8 or less due to the increased globulin and reduced albumin were attributed to estradiol stimulation.

It also appears that there were differences in sensitivity to exogenous estrogen among the parameters measured. Calcium seemed to respond first, followed by increases in serum protein and a decrease in the A/G ratio. The relationship between the sequence and degrees of change in the selected parameters resulting from estrogen stimulation can be seen more clearly in the endogenous estrogen experiment.

Weekly blood samples were taken from ten adult, two two-year old and two one-year old female Canada geese from December 31, 1966 to April 28, 1967. Pre-season values for serum calcium, total protein, albumin, globulin and the A/G ratio were obtained from samples taken from December 31, 1966 to February 17, 1967. Pre-egg laying values for the same parameters were obtained from February 24, to March 17, and egg laying values were obtained during the period of March 24 - April 7.

During the pre-egg laying and egg laying periods the adult geese were divided into normal, suppressed - egg laying and suppressed - non-egg laying groups after observations on behavior and egg laying were obtained.

During the pre-season period there were no significant differences ($P > .05$) among the five groups and values for all parameters measured were similar. The adult, one-year and two-year old geese could not be distinguished from one another on the basis of the peripheral blood constituents measured.

Pre-egg laying values for serum calcium were different among the adult and one-and two-year old geese. The one- and two-year old geese did not demonstrate any signs of reproductive stimulation whereas the normal adult geese demonstrated a mean serum calcium level of 20.5 mg% - almost a two-fold increase over pre-season values. There were no obvious differences among the other four parameters measured during this period, indicating that serum calcium was the first parameter that showed a decernable change over presea

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season (unstimulated) values. There was some indication that there may be a relationship between social status and the time and degree of stimulation at the beginning of the reproductive season. This statement is based on the apparent difference in mean serum calcium values between the dominant adults and the subordinate adult females during the pre-egg laying period.

The mean egg laying value for serum calcium in the normal adult female goose was 33.7 mg%. Suppressed adults were somewhat lower with 28.0 mg% and the one-and two-year old females did not show a significant increase in serum calcium over pre-season values. During the egg laying period significant changes in total protein, globulin and the A/G ratio occurred. The A/G ratio in the adult females did not decrease below 1.00 until approximately one week prior to the laying of the first egg.

As in the exogenous estrogen experiments, it was apparent that the time, order and magnitude of changes in peripheral blood constituents are of prime importance in their use as an index to reproductive condition. The normal adult females first demonstrated an increase in serum calçium. This was followed by an increase in total protein resulting from the increased globulin fraction and reduced albumin. This in turn resulted in a reduction of the A/G ratio. Suppressed non-laying adult females demonstrated some reproductive stimulation when calcium values increased, but the lack of increased total protein and the maintenance of an A/G ratio above egg laying levels indicated that they were not in an egg laying condition. In contrast to the increased beta globulin and reduced albumin of estrogen stimulation, the non-laying females tended to demonstrate increases in alpha globulin. Some increases in gamma globulin also occurred but they were not consistent. Immature geese did not demonstrate changes in the measured peripheral blood constituents.

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APPENDIX A

Tables of raw data and statistical values from First Exogenous Estrogen Experiment.

Table 1. Changes in serum calcium (mg%), total protein (g%), albumin (g%), globulin (g%), and albumin/globulin ratio, in the first exogenous estrogen experiment beginning on December 2, 1966 and terminating on December 23, 1966.

			Sample Period (Days*)							
Bird Number	Dosage		-2	1	4	7	10	13	+2	+4
21	Control	Ca	11.3	10.8	11.8	11.8	12.1	10.0	12.5	12.0
		TP	4.3	4.2	4.5	4.3	4.7	4.6	4.9	4.6
		Alb	2.8	3.0	2.6	2.4	2.5	2.8	2.9	2.7
		Glb	1.5	1.2	1.9	1.9	2.2	1.9	2.2	1.9
		A/G	1.87	2.51	1.37	1.26	1.14	1.48	1.36	1.47
20	Control	Ca	11.5	11.2	9.7	10.5	11.2	10.8	11.1	11.5
		TP	5.0	5.2	4.8	4.6	5.1	5.2	5.0	5.5
		Alb	2.9	2.9	3.0	2.6	2.9	3.5	3.0	2.9
		Glb	1.8	2.3	1.8	1.8	2.0	1.7	2.0	2.6
		A/G	1.61	1.26	1.67	1.45	1.41	2.08	1.50	1.12
8	250 ug	Ca	12.1	11.7	13.0	13.0	13.1	14.3	14.5	12.5
		TP	5.4	5.7	4.5	4.8	5.2	5.5	6.3	5.6
		Alb	3.3	3.1	2.5	2.9	2.3	3.2	3.3	2.8
		Glb	2.0	2.4	2.0	1.9	1.9	2.3	3.0	2.8
		A/G	1.65	1.29	1.23	1.61	1.21	1.39	1.11	1.00
18	250 ug	Ca	11.2	10.7	10.8	10.8	12.4	12.9	12.3	11.9
		TP	4.7	4.4	4.5	4.4	5.6	4.8	5.3	5.5
		Alb	2.9	2.9	2.9	2.9	2.6	2.4	2.5	3.9
		Glb	1.8	1.5	1.6	1.3	2.8	2.2	2.5	1.6
		A/G	1.61	2.00	1.87	1.95	0.93	1.09	1.00	2.41
4	1.0 mg	Ca	9.5	8.6	19.1	42.0	50.0	54.5	26.8	14.2
		TP	4.4	3.7	5.6	8.2	7.0	7.1	6.0	4.7
		Alb	2.2	1.7	2.9	3.1	2.5	2.7	3.2	2.5
		Glb	2.2	1.6	2.7	5.1	4.5	4.3	2.8	1.9
		A/G	1.00	1.06	1.07	0.62	0.56	0.63	1.14	1.32

Table 1. Continued

			Sample Period (Days*)							
Bird Number	Dosage		-2	1	4	7	10	13	+2	+4
77	1.0 mg	Ca	10.6	11.0	16.4	22.6	24.0	18.4	14.4	12.9
		TP	4.7	4.7	5.3	5.6	5.1	5.3	6.1	5.4
		Alb	3.1	2.8	2.8	3.5	2.8	2.1	4.2	3.2
		Glb	1.6	1.9	2.5	2.1	2.3	3.0	3.9	2.2
		A/G	1.94	1.47	1.12	0.65	0.81	0.70	1.16	1.45
79	2.5 mg	Ca	10.2	11.0	27.4	49.5	59.0	49.6	27.8	16.7
		TP	4.5	4.9	7.7	12.7	11.9	10.3	6.6	5.2
		Alb	2.6	2.8	3.4	5.2	5.2	3.2	3.1	2.8
		Glb	1.7	1.9	4.3	7.5	6.7	7.1	3.3	2.4
		A/G	1.53	1.48	0.79	0.69	0.78	0.45	0.94	1.17
95	2.5 mg	Ca	10.8	11.3	44.0	56.0	57.8	57.5	29.6	17.9
		TP	4.8	5.0	10.4	11.5	10.6	11.6	6.9	5.8
		Alb	2.7	3.0	4.2	4.3	3.2	3.3	3.1	3.0
		Glb	1.9	2.0	6.2	7.2	7.4	8.3	3.8	2.8
		A/G	1.42	1.50	0.68	0.59	0.43	0.40	0.82	1.06
29	5 mg	Ca	10.6	10.1	26.6	51.5	50.2	51.2	36.0	23.4
		TP	5.3	4.8	8.2	10.9	11.3	10.7	7.5	6.1
		Alb	3.9	2.7	3.9	3.5	3.3	3.0	3.0	3.0
		Glb	1.4	1.9	4.3	8.4	8.0	7.7	4.5	3.1
		A/G	2.78	1.42	0.91	0.41	0.41	0.39	0.66	0.98
94	5 mg	Ca	12.2	12.1	26.4	60.0	64.5	57.0	34.2	22.8
		TP	6.1	5.0	7.5	10.4	12.6	11.8	6.5	5.6
		Alb	4.0	2.9	3.0	3.4	4.0	3.2	3.1	2.8
		Glb	2.1	1.9	4.5	7.2	8.6	8.6	3.4	2.8
		A/G	1.91	1.52	0.67	0.68	0.46	0.37	0.91	1.00

* -2, 1 Pre-treatment values, 4-13 Treatment values, +2, +4 Post-treatment values

Table 2. Group and period means, statistical data for analysis of variance for the first exogenous estrogen experiment.

Dependent variable	Treatment group	Time period	Mean	Source of variance	Sum of squares	Degrees of freedom	Mean square	F statistic
Calcium	1		11.3	Group	874.17	4	218.54	2.34
	2		12.2					
	3		19.3					
	4		30.0					
	5		29.6					
		1	10.9	Time	979.69	2	489.84	5.24
		2	30.6					
		3	18.8	Rem. error	748.27	8	93.53	
Total protein	1		4.8	Group	17.88	4	4.47	1.81
	2		5.2					
	3		5.4					
	4		7.2					
	5		7.4					
		1	4.8	Time	16.97	2	8.48	3.44
		2	7.4					
		3	5.8	Rem. error	19.70	8	2.46	
Albumin	1		2.8	Group	0.52	4	0.13	0.81
	2		3.0					
	3		2.8					
	4		3.3					
	5		3.2					
		1	2.9	Time	0.14	2	0.07	0.43
		2	3.1					
		3	3.0	Rem. error	1.28	8	0.16	

Table 2. Continued

Dependent variable	Treatment group	Time period	Mean	Source of variance	Sum of squares	Degrees of freedom	Mean square	F statistic
Globulin	1		1.9	Group	12.66	4	3.16	1.67
	2		2.1					
	3		2.6					
	4		3.9					
	5		4.2					
		1	1.8	Time	14.78	2	7.39	3.94
		2	4.2					
		3	2.8	Rem. error	15.01	8	1.88	
A/G	1		1.5	Group	0.67	4	0.17	2.46
	2		1.5					
	3		1.1					
	4		1.0					
	5		1.1					
		1	1.6	Time	1.21	2	0.60	8.87
		2	1.0					
		3	1.2	Rem. error	0.54	8	0.07	

Group: 1 - unsuppressed; 2 - suppressed egg laying; 3 - suppressed non-egg laying; 4 - two-year; 5 - one-year.
Time : 1 - pre-season; 2 - pre-egg laying; 3 - egg laying.

APPENDIX B

Tables of raw data and statistical values from Second Exogenous Estrogen Experiment.

Table 1. Changes in serum calcium (mg%), total protein (g%), albumin (g%), globulin (g%), and albumin/globulin ratio, in the second exogenous estrogen experiment beginning on January 14, 1967 and terminating on February 4, 1967.

Bird Number	Dosage		Sample Period (Days*) -2	1	4	7	10	13	+2	+4
08	Control	Ca	10.7	11.0	10.2	11.0	10.6	11.3	10.4	11.6
		TP	4.3	4.3	4.4	4.3	4.0	4.4	3.8	4.6
		Alb	2.7	3.1	3.0	2.7	2.2	2.9	2.4	3.0
		Glb	1.6	1.2	1.4	1.6	1.8	1.5	1.4	1.6
		A/G	1.54	2.62	2.12	1.79	1.21	2.00	1.77	1.91
49	Control	Ca	11.0	11.1	11.5	13.2	13.0	12.1	12.3	13.0
		TP	4.2	4.2	4.3	5.1	4.7	4.6	4.2	5.0
		Alb	2.7	3.0	2.7	2.7	2.8	2.8	2.2	2.7
		Glb	1.5	1.2	1.6	2.4	2.2	1.8	2.0	2.3
		A/G	1.58	2.41	1.69	1.21	1.41	1.65	1.09	1.15
2	.125 mg	Ca	12.2	11.5	12.6	13.6	13.0	12.9	12.0	13.0
		TP	5.3	5.1	5.2	5.4	4.7	5.1	4.6	5.2
		Alb	2.9	2.8	3.2	3.5	2.4	3.0	2.0	3.0
		Glb	2.4	2.3	2.0	1.9	2.3	2.1	2.6	2.2
		A/G	1.20	1.21	1.60	1.79	1.11	1.42	0.78	1.34
47	.125 mg	Ca	11.7	10.6	11.9	11.8	11.4	12.1	12.2	12.0
		TP	4.7	4.1	4.5	4.8	4.4	4.5	4.5	5.1
		Alb	3.1	2.3	2.8	2.4	2.1	2.6	2.3	2.3
		Glb	1.6	1.8	1.7	2.4	2.3	1.9	2.2	2.8
		A/G	2.03	1.23	1.64	0.95	0.88	1.32	1.04	0.83
9	.250 mg	Ca	10.9	10.4	11.9	12.4	12.0	12.2	13.1	12.4
		TP	4.8	4.6	4.7	4.8	4.6	4.3	4.4	4.7
		Alb	3.3	2.8	2.8	2.3	2.0	2.4	2.5	3.1
		Glb	1.5	1.8	1.9	2.5	2.6	1.9	1.9	1.6
		A/G	2.27	1.53	1.47	0.94	0.76	1.28	1.31	1.92

Table 1. Continued

Bird Number	Dosage		Sample Period (Days*) -2	1	4	7	10	13	+2	+4
5	.250 mg	Ca	11.3	11.7	13.8	15.6	15.7	15.1	14.3	13.5
		TP	4.8	5.1	5.7	5.5	5.2	5.0	5.2	6.0
		Alb	2.6	3.5	3.1	2.2	2.1	2.3	2.7	2.5
		Glb	2.2	1.6	2.4	3.3	3.1	2.7	2.5	3.5
		A/G	1.25	2.40	1.29	0.65	0.69	0.94	1.09	1.10
81	.5 mg	Ca	11.7	11.2	15.0	15.4	15.6	18.3	15.9	13.0
		TP	4.9	4.8	5.9	5.1	4.5	5.2	4.9	4.8
		Alb	2.6	3.3	3.2	2.6	2.5	2.1	2.1	2.2
		Glb	2.3	1.5	2.7	2.5	2.0	3.1	2.8	2.6
		A/G	1.15	2.24	1.19	1.07	1.20	0.66	0.76	0.83
7	.5 mg	Ca	11.8	11.7	15.5	14.9	15.0	21.6	16.9	13.6
		TP	5.2	5.5	5.6	6.5	6.3	6.0	5.5	5.4
		Alb	3.1	3.0	2.7	2.3	3.2	2.4	2.5	2.7
		Glb	2.1	1.5	2.9	4.2	4.1	3.6	3.0	2.7
		A/G	1.43	2.64	0.93	0.56	0.61	0.61	0.86	1.00
4	1.0 mg	Ca	11.5	12.4	18.2	21.6	24.2	29.2	21.4	14.7
		TP	4.8	4.9	5.8	6.4	6.0	6.0	5.6	5.1
		Alb	2.9	3.5	3.1	2.8	2.5	2.1	2.4	2.9
		Glb	1.9	1.4	2.7	3.6	3.5	3.9	3.2	2.2
		A/G	1.53	2.61	1.15	0.76	0.70	0.54	0.77	1.31
23	1.0 mg	Ca	13.0	11.0	15.1	18.1	21.0	24.0	23.2	14.7
		TP	5.3	5.1	4.7	5.4	5.3	5.4	5.6	5.4
		Alb	3.4	3.0	2.6	3.0	2.4	2.2	2.5	3.8
		Glb	1.9	2.1	2.1	2.4	2.9	3.2	3.1	1.6
		A/G	1.81	1.44	1.24	1.26	0.83	0.71	0.70	2.30

* -2, 1 Pre-treatment values, 4-13 Treatment values, +2, +4 Post-treatment values

Table 2. Group and period means, statistical data for analysis of variance for the second exogenous estrogen experiment.

Dependent variable	Treatment group	Time period	Mean	Source of variance	Sum of squares	Degrees of freedom	Mean square	F statistic
Calcium	1		11.5	Group	65.52	4	16.38	4.86
	2		12.1					
	3		12.7					
	4		14.3					
	5		17.3					
		1	11.4	Time	36.71	2	18.36	5.45
		2	15.1					
		3	14.2	Rem. error	26.96	8	3.36	
Total Protein	1		4.4	Group	1.89	4	0.47	20.66
	2		4.8					
	3		5.0					
	4		5.3					
	5		5.4					
		1	4.8	Time	0.24	2	0.12	5.20
		2	5.1					
		3	5.0	Rem. error	0.18	8	0.02	
Albumin	1		2.7	Group	0.12	4	0.03	0.92
	2		2.6					
	3		2.7					
	4		2.6					
	5		2.9					
		1	3.0	Time	0.47	2	0.24	7.39
		2	2.6					
		3	2.6	Rem. error	0.26	8	0.03	

Table 2. Continued

Dependent variable	Treatment group	Time period	Mean	Source of variance	Sum of squares	Degrees of freedom	Mean square	F statistic
Globulin	1		1.7	Group	1.52	4	0.38	5.18
	2		2.2					
	3		2.2					
	4		2.6					
	5		2.5					
		1	1.8	Time	1.60	2	0.80	10.91
		2	2.5					
		3	2.4	Rem. error	0.59	8	0.07	
A/G	1		1.7	Group	0.50	4	0.13	2.42
	2		1.2					
	3		1.4					
	4		1.2					
	5		1.4					
		1	1.8	Time	1.36	2	0.68	13.05
		2	1.1					
		3	1.2	Rem. error	0.42	8	0.05	

Group: 1 - unsuppressed; 2 - suppressed egg laying; 3 - suppressed non-egg laying; 4 - two-year; 5 - one-year.
Time : 1 - pre-season; 2 - pre-egg laying; 3 - egg laying.

APPENDIX C

Tables of raw data and statistical values from the Endogenous Estrogen Experiment.

Table 1. Changes in blood serum calcium (mg%), total protein (g%), albumin (g%), globulin (g%), and albumin/globulin ratio, in unsuppressed adult female Canada geese.

Bird Number		12/30	1/6	1/13	2/9	a) 2/17	2/24	3/3	3/10	b) 3/17	3/24	3/31	c) 4/7
	C												
3	Ca	11.0	11.8	11.9	11.8	11.3	10.6	11.4	13.3	14.1	17.7	31.6	31.4
	TP	4.9	4.6	5.1	4.0	4.0	3.8	4.5	4.2	4.1	4.7	7.7	6.8
	Alb	3.6	3.0	3.5	2.7	2.3	3.1	3.3	2.9	2.5	3.0	3.4	3.1
	Glb	1.1	1.6	1.6	1.3	1.7	0.7	1.2	1.3	1.5	1.7	4.3	3.7
	A/G	3.27	1.87	2.11	1.91	1.34	2.62	2.81	2.31	1.56	1.72	0.80	0.87
7	Ca	11.6	11.1	11.1	11.0	11.1	11.3	20.3	24.0	23.8	25.6	33.6	32.6
	TP	4.8	4.7	4.7	4.1	4.2	3.8	4.5	4.9	5.1	5.7	6.4	6.7
	Alb	3.4	3.4	2.7	2.6	2.8	2.7	2.9	2.9	2.9	3.4	2.3	2.3
	Glb	1.4	1.3	2.0	1.5	1.4	1.1	1.6	2.0	2.2	2.3	4.1	4.4
	A/G	2.41	2.46	1.38	1.85	2.02	1.91	1.72	1.53	1.31	1.59	0.58	0.53
10	Ca	12.0	10.9	10.6	11.4	11.4	18.8	25.4	14.9	21.0	20.5	40.8	40.0
	TP	5.3	5.3	4.7	4.6	4.8	4.4	4.9	4.8	6.0	6.1	9.3	6.6
	Alb	2.9	2.9	2.9	3.3	2.6	2.3	2.6	3.3	3.2	3.4	3.9	2.1
	Glb	2.2	1.9	2.2	1.5	2.2	2.1	2.3	1.5	2.8	2.7	5.4	4.5
	A/G	1.32	1.52	1.51	2.20	1.23	1.10	1.10	2.21	1.09	1.31	0.73	0.48

Table 1. Continued

Bird Number		12/30	1/6	1/13	2/9	a) 2/17	2/24	3/3	3/10	b) 3/17	3/24	3/31	c) 4/7
12	Ca	11.0	10.4	10.7	10.4	9.9	10.0	13.0	12.2	13.4	19.6	38.0	36.8
	TP	4.9	4.2	4.7	3.9	3.8	3.6	3.9	4.0	4.3	5.1	4.0	6.9
	Alb	3.2	2.5	3.2	2.2	2.7	2.5	2.6	2.8	2.7	3.0	1.9	2.3
	Glb	1.5	1.7	1.5	1.7	1.1	1.1	1.3	1.2	1.6	2.1	3.1	4.6
	A/G	2.14	1.48	2.21	1.32	2.54	2.29	2.11	2.31	1.69	1.44	0.59	0.52
13	Ca	11.3	9.8	11.0	11.6	11.7	13.3	14.1	18.7	29.0	42.4	37.6	56.8
	TP	5.1	4.5	4.8	4.5	5.0	4.4	4.8	4.9	5.5	6.2	8.3	7.2
	Alb	3.1	2.8	3.2	3.0	3.1	3.1	3.0	3.2	3.3	3.0	2.2	2.9
	Glb	1.7	1.5	1.5	1.9	1.9	1.3	2.8	1.7	2.2	3.2	6.1	4.3
	A/G	1.82	1.86	2.21	1.20	1.58	2.30	1.08	1.88	1.50	0.95	0.37	0.66

a) Pre-season period b) Pre-egg laying period c) Egg laying period

Table 2. *Changes in blood serum calcium (*

Table 2. Changes in blood serum calcium (mg%), total protein (g%), albumin (g%), globulin (g%), and albumin/globulin ratio, in suppressed adult female Canada geese that successfully nested after release from crowded breed-pens.

Bird Number		12/30	1/6	1/13	2/9	2/17 a)	2/24	3/3	3/10	3/17 b)	3/24	3/31	4/7 c)	4/12	4/14 d)
		Sample Dates													
11	Ca	10.2	11.0	11.3	11.0	10.5	10.9	11.1	11.8	12.8	13.4	24.4	31.0	35.6	24.6
	TP	4.3	5.2	5.2	4.8	4.7	4.8	5.1	5.0	4.9	4.4	5.8	6.7	6.9	6.2
	Alb	2.8	3.0	2.8	2.9	2.8	3.5	3.6	3.6	3.5	2.9	3.3	2.5	3.8	2.2
	Glb	1.5	2.1	1.9	1.9	1.3	1.3	1.5	1.4	1.4	1.5	2.5	4.2	3.9	4.0
	A/G	1.87	1.43	1.16	1.53	1.43	2.71	2.39	2.71	2.49	1.91	1.29	0.58	0.76	0.57
62	Ca	12.0	12.5	11.7	12.2	12.3	17.0	14.4	14.5	16.2	19.8	36.0	31.4	34.4	33.0
	TP	5.6	5.6	5.7	4.7	4.7	4.3	4.8	5.1	5.1	5.4	7.4	7.0	8.1	7.8
	Alb	3.9	4.3	2.2	2.6	2.9	2.9	3.4	3.2	3.1	2.7	3.4	2.3	3.5	3.3
	Glb	1.7	1.3	3.5	2.1	1.8	1.4	1.4	1.9	2.0	2.7	4.0	4.7	4.6	4.5
	A/G	2.24	3.41	0.82	1.27	1.71	2.11	2.51	1.68	0.66	1.00	0.85	0.48	0.77	0.74

a) Pre-season period b) Pre-egg laying period c) Suppressed period d) After release egg laying

Table 3. Changes in blood serum calcium (mg%), total protein (g%), albumin (g%), globulin (g%), and albumin/globulin ratio, in suppressed adult female Canada geese that did not successfully nest.

Bird No.		Sample Dates 12/30	1/6	1/13	2/9	a) 2/17	2/24	3/3	3/10	b) 3/17	3/24	3/31	c) 4/7	4/12	d) 4/14	4/21	e) 4/28
08	Ca	11.1	11.3	11.1	11.2	10.4	10.1	11.3	11.8	19.2	16.3	23.6	55.6	28.2	31.0	18.3	19.6
	TP	4.8	4.8	4.9	4.5	4.0	4.0	4.5	4.3	4.0	4.2	5.8	8.4	7.3	7.6	5.6	4.9
	Alb	3.6	2.7	2.8	2.8	2.8	2.7	2.9	3.2	2.9	2.8	3.0	4.2	3.2	4.3	3.6	3.0
	Glb	1.2	2.1	2.1	1.7	1.7	1.3	1.6	1.1	1.1	1.4	2.8	4.2	4.1	3.3	2.0	1.9
	A/G	3.00	1.25	1.31	1.62	1.48	1.88	1.41	2.68	2.51	2.42	1.09	0.99	0.80	1.31	1.79	1.60
5	Ca	10.1	11.1	10.8	11.2	10.4	14.6	11.9	11.9	13.1	26.4	33.2	26.2	--	27.6	12.8	9.7
	TP	4.6	5.1	4.9	4.3	4.0	3.8	4.4	4.5	4.3	4.7	6.6	5.4	--	5.8	4.7	3.6
	Alb	2.4	4.0	3.3	2.4	2.4	2.9	3.2	2.4	3.0	3.3	3.5	2.2	--	3.2	3.5	2.5
	Glb	2.2	1.1	1.6	1.9	1.6	0.9	1.2	1.1	1.3	1.4	3.1	3.2	--	2.6	1.2	1.1
	A/G	1.08	3.64	2.27	1.26	1.52	3.14	3.01	1.20	2.41	2.32	1.15	0.71	--	1.21	2.79	2.19
67	Ca	12.2	12.0	12.0	11.8	11.1	10.8	11.3	12.6	13.8	16.2	30.8	26.6	29.0	28.0	20.2	21.0
	TP	5.2	5.1	5.2	4.5	4.4	4.1	4.3	4.5	4.9	4.9	5.7	6.1	5.8	6.7	7.2	4.6
	Alb	3.6	3.2	2.8	2.4	2.5	2.7	2.5	3.2	3.7	3.0	3.0	3.1	2.8	3.3	3.6	2.8
	Glb	1.6	1.9	2.4	2.1	1.9	1.4	1.8	1.3	1.2	1.9	2.9	3.0	3.0	3.4	3.6	1.8
	A/G	2.30	1.76	1.32	2.63	1.32	2.00	1.42	2.34	3.01	1.62	1.13	1.11	1.10	1.00	1.00	1.62

a) Pre-season period b) Pre-egg laying period c) Suppressed period d) After release
e) Post-season

Table 4. Values for blood serum calcium (mg%), total protein (g%), albumin (g%), globulin (g%), and albumin/globulin ratio, in 8-12 month old (35 & 89) and 20-24 month old (81 & 88) female Canada geese.

Sample Dates

Bird Number		12/30	1/6	1/13	2/9	a) 2/17	2/24	3/3	3/10	b) 3/17	3/24	3/31	c) 4/7	4/14	4/21	d) 4/28
35	Ca	11.3	11.9	11.5	11.3	11.0	11.5	10.5	10.6	13.3	11.9	10.9	10.0	--	--	--
	TP	4.8	5.4	4.9	4.6	4.3	4.3	4.3	4.0	3.9	4.1	4.2	4.6	--	--	--
	Alb	2.8	3.0	3.1	2.6	2.6	3.0	2.9	2.7	2.8	2.8	2.8	3.1	--	--	--
	Glb	1.4	2.4	2.8	2.0	1.7	1.3	1.4	1.3	1.1	1.3	1.4	1.5	--	--	--
	A/G	2.00	1.25	1.70	1.27	1.61	2.21	2.10	2.21	2.11	1.82	2.10	2.11	--	--	--
89	Ca	11.9	11.3	12.2	12.2	10.7	10.3	12.1	12.1	11.2	15.8	12.9	12.1	--	--	--
	TP	5.3	5.7	5.6	4.7	4.6	4.2	4.7	4.2	4.2	6.7	4.1	4.6	--	--	--
	Alb	3.1	3.4	3.5	2.5	2.5	2.9	3.4	2.9	3.1	4.7	2.7	3.3	--	--	--
	Glb	2.2	2.1	2.1	2.2	2.1	1.3	1.3	1.3	1.1	2.0	1.4	1.3	--	--	--
	A/G	1.41	1.62	1.69	1.19	1.21	2.14	2.61	2.32	2.81	2.52	1.91	2.50	--	--	--

Table 4. Continued

Table 4. Continued

Bird Number		Sample Dates				a)				b)			c)			d)
		12/30	1/6	1/13	2/9	2/17	2/24	3/3	3/10	3/17	3/24	3/31	4/7	4/14	4/21	4/28
81	Ca	10.6	10.7	11.0	11.4	11.2	10.9	11.3	11.5	11.3	10.5	12.2	11.8	13.8	16.2	32.4
	TP	4.5	4.6	4.5	4.4	4.3	3.8	4.2	4.4	4.0	4.1	4.2	4.9	4.6	4.6	5.2
	Alb	2.4	3.1	2.3	3.1	2.5	2.2	2.5	3.5	2.1	2.5	2.9	3.3	3.0	2.8	3.5
	Glb	2.1	1.5	2.2	1.3	1.8	1.6	1.7	0.9	1.9	1.6	1.3	1.6	1.6	1.8	1.7
	A/G	1.14	2.03	1.16	2.31	1.33	1.42	1.21	1.81	1.13	1.60	2.32	2.00	1.81	1.56	2.11
88	Ca	11.6	11.0	10.9	11.1	11.0	10.5	16.2	11.9	10.1	9.8	13.6	14.5	13.1	14.4	14.3
	TP	4.9	4.7	4.5	4.4	4.0	3.9	4.0	3.8	3.8	4.0	4.2	4.7	4.1	4.8	4.5
	Alb	2.9	2.7	2.6	2.5	2.1	2.0	2.3	2.8	2.2	2.9	2.7	3.5	3.0	3.2	3.1
	Glb	1.7	1.8	1.9	1.9	1.8	1.9	1.5	1.0	1.6	1.1	1.5	1.2	1.1	1.6	1.4
	A/G	1.70	1.50	1.43	1.40	1.20	1.12	1.53	2.56	1.36	2.52	1.71	2.80	2.49	1.91	1.80

a) Pre-season period b) Pre-egg laying period c) Egg laying period d) Post-egg laying period

Table 5. Group and period means, statistical data for analysis of variance for the endogenous estrogen experiment.

Dependent variable	Treatment group	Time period	Mean	Source of variance	Sum of squares	Degrees of freedom	Mean square	F statistic
Calcium	1		20.5	Group	179.15	4	44.79	1.61
	2		17.0					
	3		17.4					
	4		11.8					
	5		11.6					
		1	11.3	Time	357.74	2	178.87	6.42
		2	13.2					
		3	22.5	Rem. error	222.88	8	27.86	
Total Protein	1		5.2	Group	2.31	4	0.58	2.39
	2		5.4					
	3		4.9					
	4		4.6					
	5		4.3					
		1	4.8	Time	3.24	2	1.62	6.70
		2	4.4					
		3	5.5	Rem. error	1.94	8	0.24	
Albumin	1		2.9	Group	0.30	4	0.07	1.88
	2		3.1					
	3		2.9					
	4		3.0					
	5		2.7					
		1	2.9	Time	0.04	2	0.02	0.51
		2	2.9					
		3	3.0	Rem. error	0.32	8	0.04	

Table 5. Continued

Dependent variable	Treatment group	Time period	Mean	Source of variance	Sum of squares	Degrees of freedom	Mean square	F statistic
Globulin	1		2.4	Group	1.61	4	0.40	1.02
	2		2.3					
	3		1.9					
	4		1.6					
	5		1.6					
		1	1.9	Time	2.84	2	1.42	3.59
		2	1.4					
		3	2.5	Rem. error	3.16	8	0.39	
A/G	1		1.5	Group	0.40	4	0.09	0.47
	2		1.6					
	3		1.8					
	4		2.0					
	5		1.7					
		1	1.7	Time	0.63	2	0.32	1.49
		2	2.0					
		3	1.5	Rem. error	1.69	8	0.21	

Group: 1 - unsuppressed; 2- suppressed egg laying; 3- suppressed non-egg laying; 4 - two-year; 5 - one-year.
Time : 1 - pre-season; 2 - pre-egg laying; 3 - egg laying.