THE EFFECTS OF FEED RESTRICTION AND LIGHT CONTROL IN TURKEY REPRODUCTIVE PHYSIOLOGY

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ABSTRACT

THE EFFECTS OF FEED RESTRICTION AND LIGHT CONTROL IN TURKEY REPRODUCTIVE PHYSIOLOGY

by Edsel Jorge Bixler

Individual seminal volume, sperm counts, spermatocrits, motility scores, testicular weights, ductus deferens weight and sperm reserves were obtained for turkey toms under three different nutritional regimes. Data were also collected on fertility, hatchability, feed consumption, body weight, follicle stimulating hormone content and follicle stimulating hormone releasing factor concentration in the hypothalami.

The time of oviposition and its relation to the light regime was also studied.

Semen volume and sperm counts were increased when feed was withheld twice a week for a period of 24 hours prior to semen collection as compared to ad libitum fed toms and those that had feed withheld 36 hours twice a week prior to semen collection.

No significant differences were detected among groups with respect to motility scores, testicular weights and ductus deferens weights. An increase in spermatogenesis over that of the ad libitum fed toms was observed in the testicular homogenates of the toms where the feed was withheld.

The follicle stimulating hormone concentration of the pituitary and the follicle stimulating hormone releasing factor found in the

hypothalamic extracts were increased in those treatment groups in which the feed was withheld when compared to the ad libitum fed toms.

Feed intake was significantly depressed when the toms were fasted twice a week for periods of 36 hours prior to semen collection when compared to those toms fed ad libitum or deprived of feed for 24 hours twice a week prior to semen collection. No statistical difference ($P > 0.05$) was detected between the last two treatment groups.

Heavier body weights were recorded for the ad libitum fed toms when compared to the two treatment groups in which feed was withheld prior to the collection of semen.

From the data collected, it appears that restricting the feed intake for 24 hours prior to the collection of semen stimulates spermatogenesis and produces higher semen volumes.

Oviposition time was changed by means of light manipulation. Using a windowless house and a light regime in which the lights came on at 8:00 p.m. and went off at 12 noon, it was possible to obtain approximately ninety percent of all eggs before noon.

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By

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It is better not to know so much than to know so many things that are not so.

Josh Billings

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INTRODUCTION

The poultry industry has become a highly competitive business and its ever increasing technical and scientific problems have become a continuous challenge for the scientist and husbandman. Breeding highly efficient birds for some desired traits has been done at the expense of some other desirable traits. Loss of these traits has resulted in new challenges to other fields of the industry such as physiology, nutrition, etc.

For example, development of the Broad Breasted turkey, emphasizing a well developed breast and short legs to obtain meat type conformation, resulted in anatomical disadvantages in the toms. That this type of turkey is widely distributed among commercial farms has made artificial insemination imperative in almost every breeding flock. But, intense use of artificial insemination in commercial operations has created many problems. For example, the majority of the known nutritional requirements of breeding turkeys are based on females and little or no information is available for feeding males. There are also problems in collection of clean semen, in semen quality, evaluation techniques and in insemination methods.

Field and experimental observations have shown that when both sexes receive the same breeder ration, the females lose weight during the laying season while the males gain weight. Thus, the nutritional requirements of males may differ from those of females during the

breeding season. Therefore, the main purpose of this research was to evaluate, under controlled laboratory conditions, a practice of some turkey producers. In this system, poultrymen remove the feed from toms the night before semen is to be collected. Some claim to obtain larger volumes of semen with less contaminants at the following ejaculation. However, other producers report disastrous results with the same practice. The toms tend to go out of semen production.

It is well accepted that obesity is negatively correlated with sexual drive and efficiency in farm animals. The fact that turkey toms become overweight during the breeding season prompted the following study of the effect of feed deprivation before semen collection upon the subsequent production of semen.

LITERATURE REVIEW

A. Effect of Feed Restriction on Semen Output and its Characteristics

Since the development of a technique for artificial insemination in the fowl by Quinn and Burrows (1936) and its application to turkeys by Burrows and Marsden (1938), there has been increasing interest in avian reproduction. The use of artificial insemination in the turkey industry has been accepted as a common practice in the United States because the Broad Breasted type turkey is often physically unable to mate efficiently because of its conformation (Cooper, 1965). Artificial insemination has been shown by various workers to improve fecundity in these turkeys and be a useful tool in turkey breeding (McCartney, 1951; MacIlraith, 1952).

Cooper (1965) stated that the amount of semen which can be collected from a tom is dependent upon the influence of nutrition, management and the normal physiological processes regulating spermatogenesis as well as the training of the male and efficiency of man in obtaining the semen. None of these factors has been extensively studied. The influence of nutrition on semen production or its characteristics in birds has not been thoroughly investigated (Chubb, 1960). This can be appreciated by a review of the National Research Council publication 1345 (1966).

Although some commercial turkey breeders follow the practice of removing the feed from the toms prior to semen collection, there

apparently is no research report on this practice. Flock owners claims are contradictory. Some claim that this practice helped to obtain greater volumes of cleaner semen while others indicated disastrous effects with the toms going out of semen production.

1. Species other than aves --

In species other than aves, several research reports deal with the effect of nutrition upon reproduction. The early paper by Evans and Scott (1922) demonstrated that rats raised on certain diets capable of sustaining growth possessed low fertility in the first generation and were wholly sterile in the second generation. They also showed that the deficiency was nutritional because feeding fresh lettuce, wheat germ or dried alfalfa meal corrected the ailment.

Nearly forty years ago, nutritional "pseudohypophysectomy" was demonstrated by Moore and Samuels (1931) in the rat. This effect could be overcome by administering either testicular hormone or anterior pituitary extracts. These results were corroborated by other researchers (Mulinos and Pomerantz, 1941; Pazos and Huggins, 1945).

Rats maintained from four weeks of age to maturity on a high fat diet were found to have underdeveloped accessory reproductive organs (Mann, 1960). Manganese deficiency during the last days of gestation and during lactation in laboratory rats leads to testicular degeneration of the male offspring (Boyer et al., 1942).

Comparison of different fasting times in male mice before mating by McClure (1966) showed that fasting up to thirty hours did not affect libido, mating distribution or fertility. However, fasting for 36 to 48 hours decreased libido for a period of 48 hours, but did not affect fertility.

Nutrition of boars relative to reproduction has been reviewed by Duncan and Lodge (1960). The published data indicate that feed restriction from weaning to puberty prevents normal testicular development and results in retarded tubular development and testicular volume. The authors also stated that restriction of feed intake to 50 and 70 percent of normal intakes from weaning on, decreased seminal volume without affecting either sperm concentration or fertility. Meduzov and Kudrjavcen (1965) found that boars receiving 0.5 mg of zinc sulphate and manganese salts per kilogram of body weight had markedly better semen quality after 30 to 45 days of administration when compared to boars receiving no trace mineral supplement. The piglets sired by those boars receiving the mineral supplement were heavier at birth than those sired by boars receiving no trace mineral supplement. McCance (1960) reported that pigs suffering severe undernutrition develop large adrenals and have spleens lighter than normal swine when expressed as percentage of body weight.

Salamon (1964) showed that in grazing Merino rams a daily supplementation of two pounds of a concentrate containing 165 grams of protein and 600 T.D.N. for six weeks prior to the beginning of intensive collection of semen improved sperm output. The control rams received a supplementation of one pound per day of grain containing 45 grams of crude protein and 300 grams of T.D.N. during the same period of time.

In farm animals, overfattening has been reported to have an adverse effect on fertility (Millen, 1962). It has also been shown that the level of nutrition in young Holstein male calves affects the age at onset of puberty (Bratton et al., 1959). These authors showed that young bulls began ejaculations of viable sperm at 37, 43 and 51 weeks

of age, when fed on a high, medium and low plane of nutrition, respectively. Flipse and Almquist (1961) have shown that overfeeding and, therefore, overweight in dairy bulls produced a decrease in libido and sexual response. However, no effect was demonstrated in total sperm output or extra-gonadal sperm reserves. De Alba (1964) stated that semen output is quite insensible to the plane of nutrition and more so, if the nutritional treatment is of short duration.

In humans, obesity may lead to a deficient production of spermatozoa. On the other hand, malnutrition may cause sterility and appears to be associated with a reduction in testosterone production (Millen, 1962). P-aminobenzoic acid has occasionally been noted to produce a marked increase in libido and potency in human subjects (Ansbacher, 1944). In some cases of human malnutrition, there is a striking correlation between nutritional deficiency and the presence of testicular softening and atrophy associated with diminished libido and potency. These conditions have been reported to be restored to normal by intensive vitamin B-complex therapy (Biskind, 1946). Studies carried on with 529 human subjects suffering from chronic malnutrition showed cases of focal fibrosis, basal membrane thickening, decrease of germinal epithelium and, therefore, total lack of spermatogenesis. The sertoli cells frequently showed vacuolization, and the interstitial cells were constantly of small size and reduced in numbers. In general, the testes showed typical characteristics of senile retrogression and clinical tests for testosterone production showed a deficient function (Zubiran and Gomez-Mont, 1953).

2. Research utilizing poultry --

Regardless of the intensive research that has been carried out in breeding hens with specific nutrients, there are some reports in the literature that cast doubt on the efficacy of the accepted requirements and allowances. As an example of this, Summers et al. (1967) reported superior egg production with no difference in fertility and hatchability when meat-type pullets were feed restricted by using wheat bran. These pullets were lighter in weight at the onset of egg production than the full-fed controls and showed better feed conversion for egg production.

Lowering the protein content of the feed to ten percent or increasing the fiber content to 15 percent in grower feeds produced a delay in sexual maturity with lower body weights in broiler-type pullets, but no differences were detected in egg production, fertility, hatchability or mortality (Waldroup et al., (1966). Feed restriction in broiler pullets has not given consistent results. For instance, feed restriction during the growing period has been reported to decrease subsequent egg production (Davis and Watts, 1955), increase future egg production (Fuller, 1960) and have no effect on subsequent egg production (Milby and Sherwood, 1953). These findings might be explained by the fact that each researcher used different diets and different methods of feed restriction.

Anderson et al. (1963) reported that feeding Large White turkey hens at 84 percent of ad libitum consumption of a balanced ration from 12 to 24 weeks of age improved hatchability and feed utilization for egg production during the breeding season. However, similar restriction from 24 to 40 weeks of age had no effect on hatchability. Daniels and Pino (1962) obtained better fertility and hatchability in Broad

Breasted Bronze turkeys raised from 8 to 24 weeks of age on clover prairies and supplemented with a mash of white corn and mineral mixture than from those raised on similar conditions but receiving a balanced diet as a supplement. However, sexual maturity was delayed and the birds were significantly lighter in weight at the end of the growing period. Restriction of feed intake from 12 to 28 weeks or from 18 to 28 weeks of age in medium sized white turkeys significantly restricted body weight as well as egg production (Touchburn, 1966). Parker and McSpadden (1943) reported a reduction in turkey semen volume and fertilizing capacity when the toms were subjected to a thirty percent restriction in feed intake. On the contrary, Cooper and Barnett (1962) improved the fertility of a naturally mated flock by controlling the weight of the toms by utilizing high fiber diets containing Coastal Bermuda grass.

Boone et al. (1967) showed that a six-day starvation period was enough to reduce seminal volume and fertilizing capacity of White Plymouth Rocks. When the starvation was prolonged to 17 days, twenty percent of the males produced no semen. However, severe undernourishment of Rhode Island Red cockerels capable of preventing growth beyond 150 to 190 grams at six months of age did not prevent satisfactory reproduction after rehabilitation by ad libitum feeding (McCance, 1960).

Touchburn (1966) reported that restricting feed consumption from 12 to 24 weeks of age in turkey toms brought about an increase in semen production during the breeding season. Furthermore, the author stated that a separate ration for breeder toms based on the assumptions that toms do not lay eggs and, therefore, their requirements for calcium and protein would logically be lower, had given good results. This tom

ration was formulated to furnish 0.9 percent calcium and 15.8 percent protein.

In chickens, Arscott et al. (1965) showed that diets high in linoleic acid and low in vitamin E affect fertility negatively without affecting seminal volume, hatchability, feed consumption, body weight or testicular weight. Further, increasing the number of inseminations per female did not improve fertility. However, vitamin E supplementation of the diet at a level of 162 mg/Kg of feed prevented the drop in fertility. Patrick (1965) fed cocks $Se^{75}O_3$ and measured the amount of selenium deposited in the semen and the sperm. Since selenium was present in the semen and sperm, he made the following conclusions: (1) Selenium could be metabolized; (2) selenium could be pooled and retained in the male's reproductive tract and (3) selenium may play a role in the physiology of germ cells.

Jones et al. (1966) reared cockerels during their growing period under two levels of protein (4.0 and 16.0 percent) and studied the subsequent effects on reproduction. Those males that received the lower protein level had also lower body weights and sexual maturity was delayed for eight weeks. Nevertheless, when the restricted males were placed on a 17.0 percent protein ration at 21 weeks of age, they attained a higher sperm concentration peak which was also maintained for a longer period than the 16.0 percent protein reared controls. The effect of protein level in the breeding ration in relation to fertility in the male chicken has been studied by Arscott and Parker (1963). These authors found that lowering the protein level from 16.9 to 10.7 or 6.9 percent over a 33-week period showed no adverse effect on seminal volume or hatchability. In artificial insemination fertility

trials, the 6.9 percent protein level had significantly higher fertility ($P < 0.55$).

Several authors have reported that a vitamin A deficiency results in underdeveloped testes, reduction in comb size, increased numbers of abnormal sperms and a decrease in sperm number and motility (Craft et al., 1926; Burrows and Titus, 1938; Howe et al., 1957). Later work of Rubio and Garcia (1959) showed that a vitamin A deficient diet fed to 10-month-old Single Comb White Leghorn cockerels produced no change in seminal volume. However, severe detrimental effects such as decreased sperm motility, increased numbers of abnormal sperm and decreased sperm concentration resulted in poor fertility. These adverse changes were not irreversible and the administration of 30,000 I.U. vitamin A per Kg. of body weight resulted in complete recovery in two weeks.

Perek and Snapir (1963) have shown that vitamin C had a beneficial effect upon seminal volume and spermatozoa output. Later, Perek (1966) reported results implying that vitamin C might be involved with thyroid metabolism. Since this work is the only one of its kind and was done under Israeli conditions (hot weather implied) further work in this field is required.

B. Size of Testes and Semen Output

1. Species other than birds --

Utilizing the exhaustion technique of collecting a large number of ejaculates per week for four consecutive weeks, Edwards (1940) found a correlation of 0.95 in rabbits between testicular weight and the number of spermatozoa in eight consecutive ejaculates. Ortavant (1952)

obtained a correlation of 0.80 between the size of ram's testes and the number of spermatozoa therein after electro-ejaculating these males at weekly intervals. Willet and Ohms (1957) reported that the correlation between scrotal circumferences in situ of bulls and the weight or volume of the excised testes is 0.94. The correlation between scrotal circumferences and testes spermatozoal production is 0.92 for young bulls and 0.53 with aged bulls when the animals were exhaused with four ejaculations. However, if the bulls were not exhausted, but only maintained in routine service, the correlations were 0.17 and 0.46 for dairy and beef bulls, respectively, with no significant difference between figures, giving a combined correlation of 0.32 ($P < 0.01$).

2. Research utilizing poultry --

Parker and McSpadden (1943) showed that when cocks were subjected to feed restriction so that the birds lost weight, testicular size and seminal production decreased, however, no correlations were calculated between testicular weight and seminal output. The author explained the decrease in testicular weight as a response of diminished output of gonadotropins by the pituitary due to malnutrition.

No report on the correlation between testicular size and spermatozoal output or production in either chickens or turkeys could be found.

C. Evaluation of the Sperm Reserves

1. Species other than birds --

The distribution of sperm in the testes, ductus deferens, and epididymides has been studied in mammals (Amann and Almquist, 1962; Kennelley and Foote, 1964; Kirton et al., 1967). It has also been

shown that the frequence of ejaculation in bulls changes this distribution of spermatozoa (Almquist and Amann, 1961). That there is spermatozoa resorption from the cauda epididymis has been demonstrated by Orgebin-Crist (1964).

2. Research utilizing poultry --

No report could be found of any study of sperm reserves or distribution in turkeys or roosters. Nevertheless, some studies have been done on the spermatogenesis of the duck (Clermont, 1958), the rooster (Kumaran and Turner, 1949) and the turkey (Macartney, 1942). The latter author also showed that left and right testes have the same mitotic activity per gram of tissue. However, it has been reported that in turkeys, the right testicle is larger than the left testicle (Law and Kosin, 1958), this being opposite to the female where the left ovary becomes functional and the right ovary usually does not develop (Nalbandov, 1964).

In the domestic fowl, there is an increasing number of degenerating spermatozoa in the ductus deferens when the frequency of ejaculation is not maximal (Lake, 1966). This observation may indicate that sperm reabsorption could be taking place at this location in fowl which differ from most mammals, in that their epididymidal development is small relative to the ductus deferens.

D. Other Physiological Parameters Affected by Periods of Feed Deprivation

It has been reported that in chickens a 24-hour starvation period decreases blood glucose by approximately ten milligrams percent (Golden and Long, 1942). Prolonged fasting periods in adult chickens produced a decline in blood glucose beginning 24 to 36 hours after the fasting

period started and an increase in blood glucose of a maximum of 22 milligram percent about six days later (Hazelwood and Lorenz, 1959). Fasting also caused adrenal enlargement in chickens (Sure, 1938). The same was true in a deficiency of thiamine (Beznak, 1923), or in a calcium-vitamin D deprivation (Urist, 1959). The effect of diet has a definite effect on the chicken thyroid as it has been shown that a deficiency of iodine causes thyroid enlargement (Patton, Wilgus, and Harshfield, 1939). Feeding of 10.0 to 14.5 percent fat to chickens produced smaller thyroids than did diets containing only 2.5 percent fat (March and Biely, 1957). However, restricted caloric intake had no effect on thyroid weight even though thyroid function was diminished as was metabolic rate (Premachandra, 1962; Mellen, Hill and Dukes, 1954).

Level of cholesterol in blood plasma is directly affected by the thyroid in chickens and its destruction by I^{131} causes a rise in blood cholesterol. This condition can be reversed by thyroxine administration (Clegg et al., 1959). Similar effects of increased cholesterol and lipemia have been observed in ducks (Benoit and Bogdonovitch, 1937) in turkeys (Reineke et al., 1946) under hypothyroidism.

The observations of the changes in the chemical constituents of the adrenal gland following administration of ACTH led to the development of new tests for the study of adrenal stimulation either by endogenous or exogenous ACTH (Sayers et al., 1944). In the rat, the decrease in adrenal cholesterol is sensitive enough to serve as an assay technique for corticotropin preparations and to measure the rapidity of adrenal response to stressors. However, these changes do not appear to occur in all species (Elton et al., 1959). Contradictory to the results of Elton et al. (1959), with respect to chickens, Howard

and Constable (1958) and Siegle and Beane (1961) produced adrenal cholesterol depletion in chickens following ACTH injection. However, Wolford and Ringer (1962) concluded that adrenal cholesterol content in mature Single Comb White Leghorn hens seemed to be an unreliable test as a stress indicator. Furthermore, no statistical differences were detected in adrenal weight between treatments.

The effect of nutrition on the blood corpuscles of the chickens is dependent on the specific nutritional deficiency under observation. For instance, it has been shown that copper or iron deficiencies are capable of producing a decrease in hemoglobin and anemia (Hart, Elvehjem and Kemmerer, 1930). A riboflavin deficiency can produce a significant decrease in lymphocytes and an increase in heterophils (Good et al., 1953). A folic acid deficient diet caused the development of anemia and decreased numbers of leucocytes of all types in young poults (Gomez and Hogan, 1948).

Newcomber (1958) reported that physical restraint in chickens or ACTH or adrenal cortical hormones produced an increase in the numbers of heterophils. Basically, the same results were reported by Wolford and Ringer (1962), except that the latter authors required an extreme stressor such as forty hours of fasting plus an exposure to cold (0^{o} F.) for 15 hours to obtain results similar to those of administering ACTH.

Bioassays of turkey pituitary (Witschi, Stanley and Riley, 1937), as well as that of other birds (Leonard, 1937; Burrows and Byerly, 1936) have shown that FSH, LH and Prolactin are present in avian pituitaries.

Removal of the anterior pituitary in different avian species has led to a sharp decrease in testicular size. This response plus the fact

that gonadotropin hormone preparations contain FSH have proven to be effective in producing reinitiation of spermatogenesis, increased diameter in semineferous tubules and an increase in testicular size, indicate endocrine regulation of the testes (Benoit and Aron, 1934; Nalbandov, Meyer and McShan, 1946; Coombs and Marshall, 1956; Lofts and Marshall, 1956; Chu, 1940).

There is abundant experimental evidence in aves that FSH is the gametogenic hormone (Marshall, 1961) and that chronic malnutrition in the chick is capable of inducing nutritional pseudohypophysectomy and, therefore, impaired reproduction (Breneman, 1940 and 1941).

E. FRF

1. Species other than birds --

As far as mammalian species are concerned, there is no longer any question as to whether there are neurosecretions of hypothalamic origin, which are usually called "releasing factors". The existence of these substances and their control upon the secretion of the hormones of the anterior lobe of the pituitary, is now well established by many experiments in vivo and in vitro (Guillemin, 1967). This author stated that crude acidic extracts of ventral hypothalamus contain substances which stimulate the secretion of pituitary gonadotropins. These stimulating substances have been named according to their function and gonadotropin involved. Therefore, that substance which stimulates the secretion of the pituitary luteinizing hormone (LH) is called LRF for LH-releasing factor. That which stimulates follicle stimulating hormone (FSH) secretion, is named FRF, for FSH-releasing factor. Another

substance in these extracts inhibits rather than stimulates the secretion of prolactin and it is referred as PIF, for prolactin-inhibiting factor.

2. Research utilizing poultry --

In birds, it has been proposed that environmental factors such as light are capable of causing changes in the activity of specialized hypothalamic cells, the so-called neurosecretory cells (Benoit and Assenmacher, 1955 and 1959). These authors observed neurosecretory material of apparent hypothalamic origin in the hypothalamico-hypophyseal nerve tract of ducks (Assenmacher, 1958; Benoit and Assenmacher, 1959). The same authors have given evidence that sectioning of the portal vessels or sectioning of the hypothalamico-hypophyseal tract in the median eminence without damage to the portal system caused testicular atrophy and lack of gonadal response to light stimulation. Further evidence for the possible role of neurosecretory regulation of gonadotropins in birds was presented by Oksche et al. (1959). These authors showed that an increase in daily illumination capable of inducing testicular weight in White-crowned sparrows would also produce a decrease in neurosecretory material in the hypothalamic nuclei and median eminence. These results have been corroborated by Matsui (1966) who classified nine groups of neurosecretory cells in the tree sparrow.

However, specific neurosecretory principles or releasing factors in birds have only been shown for prolactin and LH. Kragt and Meites (1964) reported that in pigeons there is a prolactin releasing factor and not a prolactin inhibiting factor as in mammals. Opel and Lepore (1967) have shown the presence of an ovulating releasing factor in the stalk median eminence of the chicken by intrapituitary infustion and

premature ovulation. Clark and Fraps (1967) obtained similar results with median eminence extracts infused through a stereotactically placed cannula into the anterior pituitary.

F. Effect of Light on Oviposition

It is general knowledge among poultrymen that females under natural conditions lay more eggs during the spring when day length is increasing. Furthermore, it has been shown that birds in different parts of the world but at the same latitude show similar seasonal peaks of production (Wheatham, 1933). This effect of light causes gonadotropin release and has been shown to be mediated through the hypothalamus (Benoit and Assenmacher, 1959). The stimulatory effect of light upon the gonads can be appreciated by evaluating the positive effects of light on egg production that have been demonstrated by several authors (Van Albada, 1962; Byerly and Moore, 1941; King, 1959; Palmer, 1966).

The time of oviposition in the turkey hen is generally during the hours of the day in which they receive light (Woodward et al., 1963). However, most of the eggs are laid in the afternoon as observed by several investigators (Stockton and Asmundson, 1950; Kosin and Abplanalp, 1951; Woodward et al., 1963; Wolford et al., 1964). However, if the light pattern is modified, there is an effect on the time of oviposition (Albright and Thomson, 1933; Marsden and Fraps, 1960; Davis, 1948; Asmundson and Moses, 1950). In laying chickens, light has a rather strong influence on oviposition as demonstrated by Warren and Scott (1936) in a series of experiments. These authors found that birds subjected to normal lighting laid during the daytime but if these same birds were subjected by artificial means to continuous lighting,

oviposition was distributed uniformly throughout the 24 hours of the day. On the contrary, if the pens were darkened during the day and illuminated during the night, totally shifted their laying time to the illuminated period within four days. When 24 hours of darkness intervened, followed by another shift back to normal daytime, change in oviposition time was almost complete within three days after the change had been made. These results demonstrate that under normal cycles of day and night, hens lay during the day. However, if they are illuminated throughout the 24-hour day, the hens will distribute their laying during the 24-hour period. Under reversed conditions of light and darkness, the hens will tend to lay during the illuminated period of artificial daylight. These results have been corroborated by several researchers (Fraps, 1955; Bastian and Zarrow, 1955; Lanson and Sturkie, 1958; Biellier and Ostmann, 1960).

The time of oviposition and time of mating undoubtedly affect fertility as indicated by the results obtained by a series of investigations reported in the literature. In 1943, Gracewski and Scott showed that better fertility is obtained if the matings take place in the afternoon instead of the morning hours. Moore and Byerly (1942) reported that they obtained better fertility when the hens were inseminated after laying and that fertility was lowest when inseminations were made with a hard shell egg in the uterus. Wyne et al. (1959) also showed that the presence of a hard shell egg in the hen's uterus during insemination produces a significant drop in fertility. Parker (1945) also obtained better fertility with afternoon inseminations. In turkeys, Parker and Barton (1946) found only a slight decrease in fertility with morning inseminations as compared to afternoon inseminations.

The above results showing that the presence of a hard shell egg in the uterus affects fertility negatively, plus the fact that egg laying time has been controlled in other aves (Arrington et al., 1962; Warren and Scott, 1936) encouraged testing the same idea with turkey hens. In addition to the increase in fertility expected if hard shell eggs in utero were to be avoided, it would be to the advantage of the turkey breeders to control the time of oviposition in such a way that he could inseminate at a more convenient time rather than at night as is now generally practiced.

OBJECTIVES

1. To study the effect of feed deprivation prior to semen collection from male turkeys upon the semen volume and its characteristics.
2. To study the correlations of testes size and semen output and production.
3. To evaluate the amount of sperm in the testes, epididymi and ductus deferens.
4. To study the feasibility of altering the oviposition time through proper light control so that artificial insemination can be performed at a convenient time.

MATERIALS AND METHODS

General

In two successive years, 1966 and 1967, a total of 45 male and 130 female Anderson strain Broad Breasted Large White turkeys were housed under 16 hours of artificial light and eight hours of darkness. The lights came on at 8:00 p.m. and went off at noon from December 15 until the end of the experimental period. This light pattern was designed to study the possibility of changing the oviposition time in such a way that a hard shell egg would not be present in the uterus at the time of insemination. The turkeys were June hatched from a commercial hatchery and were range-reared during the growing period.

All toms were handled and trained for collection of semen for three weeks prior to the beginning of the experiments.

During the experimental periods, the semen was collected twice per week on Mondays and Fridays between 7:00 and 9:00 a.m., using the technique described by Burrows and Quinn (1938), with the modification that the semen sample was directly collected in a disposable plastic tuberculin syringe. The use of such a syringe permitted the measuring of the volume of semen produced by a tom to the nearest 1/100 ml. This procedure was followed twice a week and the volumes were recorded for each individual. The individual semen samples were divided into two groups and used alternatively for artificial insemination of hens or for laboratory evaluation. In other words, the semen obtained from

one-half of the toms in each experimental group was utilized for artificial insemination on Mondays and the other half for laboratory evaluation. On Fridays, the procedure was reversed, the semen samples obtained from those toms whose semen samples were used the previous Monday for laboratory procedures was inseminated and the semen obtained from the other half was taken to the laboratory for evaluation. When the semen sample was utilized for artificial insemination, the hens were inseminated immediately following collection. However, the semen that was used for laboratory evaluation was kept in the syringe or in a small vial and placed in a tray previously filled with crushed ice. The sample was kept in crushed ice for one hour, on the average, before evaluation.

The following laboratory tests were made: Spermatozoa motility was determined by examining a hanging drop of semen on a standard size coverslip at room temperature with a light microscope under low-power (10X). The sample was rated from 0 to 5 (Table 1) according to the procedure of Allen and Champion (1955). The motility scoring rates with the highest figure (5) were for samples that showed 80 to 100 percent of the spermatozoa in vigorous movement and the formation of eddies, while the lowest figure (0) was used for no motility at all.

Determination of sperm concentration in the collected semen was then made by utilizing two different methods. First, spermatocrit values expressing the percentage of spermatozoa[1] in semen volume were

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[1]Analogous to the usage of "hematocrit". Spermato referring to the spermatozoa and "crit" originally spelled "krite" meaning to judge.

Table 1. Criterium for motility evaluation[a]

Scale	Characteristics
0	No motility
1	1 - 20% motility, weak oscillatory and undulating movement
2	20 - 40% movement but no waves or eddies formed
3	40 - 60% of the sperm showing progressive motility and vigorous motion slowly moving waves or eddies present
4	60 - 80% sperm motility with waves and eddies of rapid formation and movement
5	80 - 100% of the spermatozoa showing progressive movement and extremely rapid formation of waves and eddies

[a]Slightly modified from Allen and Champion (1958).

determined following the procedure of Hickman (1958) with two variants. The first modification was that the capillary tube was sealed with Critoseal® instead of by means of heat. The second variant was that in the spinning of the capillary, it was done twice for a period of 15 minutes, totalling thirty minutes at 11,500 rpm instead of 10,000 rpm for ten minutes as used for dairy bulls. The second method of sperm concentration determination consisted of using precisely the same procedure as used in making red blood cell counts. For this purpose an AO Spencer Bright-Line Hemacytometer was used to determine the sperm concentration per cubic millimeter. For each individual determination, a sample of well-mixed semen was drawn up to the 0.2 mark of the capillary in a red blood cell pipette and then to the 101 mark with a

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mixture of 1:1 physiological saline and eosin solution (Appendix Table I) used as a diluting fluid. The reason for using such a preparation was to dye the spermatozoa and facilitate their counting. This dilution of 1 to 500 instead of 1 to 200 used by most investigators (Wakely, 1949; Burrows and Kosin, 1953; Parker, 1946) was based on the findings of Kosin and Wheeler (1956) who demonstrated that a dilution of 1 to 500 produced more accurate counts and estimates of spermatozoal density. Then, the pipettes were agitated by means of a Clay-Adams Yankee pipette shaker for 45 seconds to make an even mixture. A drop of this fluid was applied to each chamber of the hemacytometer and two microscopic spermatozoal counts were made after allowing the preparation to settle for at least five minutes. The settling period permitted the sperm to fall to the bottom of the counting chamber and, therefore, made more precise counts possible. Two counts such as the one described above were made for each semen sample giving a total of four values. The mean value of these four counts was recorded as the spermatozoa density of that tom's semen.

The spermatozoa were counted in five groups of 16 small squares, usually at the center and four corners of the central square millimeter. To avoid counting a spermatozoon twice, those on a line were counted only when on the top or left lines, but not when on the bottom or right lines.

The calculations for estimating the number of spermatozoa were done as follows: The number of spermatozoa representing the mean value obtained for a sample was multiplied 500 times since the semen had been diluted 1 to 500 as stated before. This multiplication brought the figure to its original density. Since each chamber consisted of 25

large squares and only 5 were counted, a factor five had to be used in multiplying the above figure to obtain the total number of spermatozoa in the whole field. However, the chambers in which the counts were made measured one millimeter X one millimeter X 0.1 millimeter in depth the figure obtained represents the concentration of sperm in 1/10 of a cubic millimeter. Thus, by multiplying this last figure by a factor of ten, the spermatozoal concentration could be expressed in sperm per cubic millimeter which is the conventional form. In other words, if the mean value of the sample was eighty, the following calculations had to be made:

80 X 500 X 5 X 10 = 4,000,000 spermatozoa per cubic millimeter.

All hens were artificially inseminated one week prior to the beginning of the experiment and artificial insemination was continued throughout the experiments as described previously. All eggs were marked with a wax pencil indicating the date, the hen number and time of the day when the egg was collected. The eggs were collected three times a day, just at the onset of light, 12 hours later and just before the lights went off. This system was designed to properly identify the eggs for pedigree purposes and to be able to identify those eggs laid during darkness. For this purpose, each hen had a card where a record was kept of all eggs laid and the time of oviposition as to the light or dark period.

All eggs, except those with either soft or broken shells, were incubated weekly in a Jamesway-252 forced-draft incubator which was operated at a temperature of 99.5° to 100° F. and 70 percent relative humidity. On the 25th day of incubation all eggs were candled for the

determination of fertility. Those eggs containing live embryos were transferred and basketed by pedigree for future observations on hatchability. All poults were individually weighed and recorded after hatching. The recording of these data was designed to obtain specific information on fertility, hatchability and poult weight as affected by the feed restriction of the sire.

All birds were fed a pelleted turkey breeder ration in the following manner: The toms were divided and sorted into three groups as described later in more detail for each particular year. The groups were named A, B and C. In both years, group A received feed ad libitum. In group B, the feed was removed twice a week for periods of 24 hours preceding the collection of the semen. In group C, the feed was removed for periods of 36 hours twice a week previous to semen collection (Table 2).

The ration used was calculated to fulfill all known nutrient requirements when fed ad libitum to a turkey breeding flock (Table 3). Fresh water was available at all times, except 12 hours before semen collection, with the intention of avoiding overdrinking by the fasted birds. Feed consumption was recorded weekly for the toms, while their body weights were recorded on a monthly basis as well as at the beginning and termination of the experiments.

The hens received the same pelleted diet plus oyster shell ad libitum and fresh water was available to them at all times.

All hens were assigned to individual cages that measured 18 inches wide X 24 inches long X 24/26 inches in height, while the toms were housed and kept in conventional floor pens that measured 17.0' X 15' 4" and had cement floors with shavings.

The data were analyzed by means of the analysis of variance technique and Duncan's multiple range test for comparisons among treatment means (Steel and Torrie, 1960), except as otherwise specified in the particular experiment.

A. First Year

The 1966 trial was designed to investigate the feasibility of conducting a second study with a larger number of toms. The study was initiated on January 14, 1966 and ended on May 13 of the same year; thus, it had a duration of 17 weeks.

A total of 15 males and 70 females were distributed at random in the following manner: The males were divided into three different groups of five toms each to be subjected to the treatments listed above and shown in Table 2. For each tom in group A, four hens were assigned at random for artificial insemination. Groups B and C had five hens per tom and were also assigned at random for the same purpose.

Table 2. Experimental design of feeding patterns for both years

Group	Feeding Program
A	Full feeding at all times
B	Feed removed twice a week for 24 hours prior to semen collection
C	Feed removed twice a week for 36 hours prior to semen collection

At the end of the experimental period an extra sample of semen was collected individually from each tom in sterile tuberculin disposable

syringes and sent to the Department of Microbiology and Public Health for Escherichia coli counts.

After an additional fasting period for treatment groups B and C, blood was drawn from the brachial vein of each bird in all groups for blood smear differentials as well as for blood cholesterol and glucose determinations which were made according to the methods of Zlatkis, et al. (1953) and Folin and Wu (1920), respectively (Appendix Tables II and III). The purpose of making these measurements was to determine if the fasting periods could be regarded as stressors and if decreased feed intake would affect the thyroid.

The toms were sacrificed by bleeding and the adrenals, thyroids, testes, crop and rest of the gastrointestinal tract were removed, washed, trimmed and weighed individually on a Roller-Smith or triple-beam balance. The total cholesterol content of the adrenals was also determined according to the method of Zlatkis et al. (1953) as an indirect measurement of stress. The thyroids were fixed in ten percent formalin and stained by hematoxylin and eosin as described in the Manual of Histologic and Special Techniques (1960) for later histological evaluation of the physiological state of the gland. The testes were also prepared for histological observation by means of Allen's fixative (Gray, 1958) and then stained with the periodic acid-Schiff-hematoxylin technique (Biological Stain Commission, 1960).

B. Second Year

A total of 30 toms and 60 hens were used for this experiment. The hens were assigned to individual cages similar to those described before. The toms were divided into five weight blocks of six birds

each. Two toms per weight block were sorted at random into the three treatment groups and housed in conventional pens similar to those described for the first year. The pens were assigned at random into treatment groups A, B and C (Table 3). Each pen had ten toms which were kept under the conditions of the experiment for a period of 20 weeks, from January 16 to June 5, 1967.

In addition to the procedures described previously for both experiments, at the end of the experimental period all the toms were sacrificed by decapitation and the pituitaries were carefully removed and frozen at -20° C. A portion of each hypothalamus was removed and placed in a vial, which contained adequate ice cold 0.1N HCl so that each portion of hypothalamus had a ratio of 0.2 milliliters of solution per hypothalamic portion, and stored at -20° C. A similar volume of cerebral cortex was also removed from those toms who belonged to treatment group A. The cerebral cortex was also placed in ice cold 0.1N HCl and stored at -20° C. The hypothalamic extracts and cerebral extract were later used for Follicle Stimulating Hormone Releasing Factor (FRF) bioassay as described later in this thesis.

The testes were located in the body cavity, then the epididymus-ductus deferens and ductus deferens-cloaca junctions were located and each tightly tied with a thread to avoid sperm movements along the genital tract. Then the entire reproductive tract was dissected free from the surrounding tissues and used for sperm concentration determinations.

The left testicle and ductus deferens from each tom were used to calculate spermatozoal concentrations in these organs, while the right testicle was fixed and prepared as described previously for the first

Table 3. Composition of the experimental ration

Ingredient	Kg
Wheat middlings	50
Ground yellow corn	560
Ground oats	50
Soybean meal, 45% protein	100
Dehydrated alfalfa meal, 17% protein	50
Meat and bone scraps, 50% protein	50
Menhaden fish meal, 60% protein	50
Dried whey	25
Brewers dried yeast	20
Iodized salt	4
Dicalcium phosphate	10
Ground limestone	25
Vitamin and antibiotic mix[a]	5
Mineral mix[b]	1
Total	1000
Calculated analysis:	
Crude protein, %	17.5
Productive energy, Cal/kg	1980
Crude fat, %	3.6
Crude fiber, %	4.5
Calorie:protein ratio	113
Calcium	2.2
Phosphorus	0.9

[a]Supplied per kg of ration: Vitamin A, 9,900 USP units; Vitamin D_3, 2,248 I.C.U.; Riboflavin, 8.8 mg; D-calcium pantothenate, 22.0 mg; Niacin, 55 mg; Choline chloride, 1,320 mg; Vitamin B_{12}, 26.4 mcg; Vitamin E, 33 units; Menadione sodium bisulfite, 4.4 mg; ethoxyquin, 137.5 mg; Folic acid, 2.2 mg and zinc bacitracin, 55 mg.

[b]Supplies as per kg of ration: Manganous oxide (56% Mn), 95 mg; Zinc oxide (80% Zn), 64 mg; Copper sulpate (25.2% Cu) 33 mg.

year experiment for future histological evaluation of spermatogenesis. The procedure used to determine the spermatozoal concentrations in the left testicle and ductus deferens was as follows: After the ductus deferens was tightly tied with a thread at the junction with the epididymus and at the entrance to the cloaca, the entire reproductive tract was dissected free of surrounding tissue. Then the ductus deferens was dissected from the testicle at the junction with the epididymus and at the external entrance with the cloaca (Figs. 1 and 2). The left ductus deferens was weighed on a piece of tared wax paper on a Mettler balance to the nearest one hundredth of a gram and minced under refrigerated saline.

The two testes were weighed individually exactly as described before for the ductus deferens. Later, the left testicle was dissected free of its tunic, reweighed, minced under refrigerated saline, and placed in fifty milliliters of cold 0.9 percent physiological saline solution. The minced tissues were transferred individually and quantitatively to a semimicro Waring blendor. A pinch of Dow antifoam and additional refrigerated saline was added to each individual organ preparation to bring the total volume up to 150 milliliters and the preparation was then homogenized for three minutes. Before the readings were made, the homogenates were diluted further with refrigerated physiological saline to obtain concentrations of spermatozoa, such that 50 to 500 spermatozoa were counted within the grid of the hemacytometer (Figs. 3 and 4).

The tissue homogenates were stored for 4 to 48 hours at 5° C. with one or two drops of 100 mg percent merthiolate solution to prevent bacterial destruction of the sperm until the spermatozoal enumeration of

Fig. 1. Schematic diagram of reproductive tract in the abdominal cavity with sites of ligation indicated.

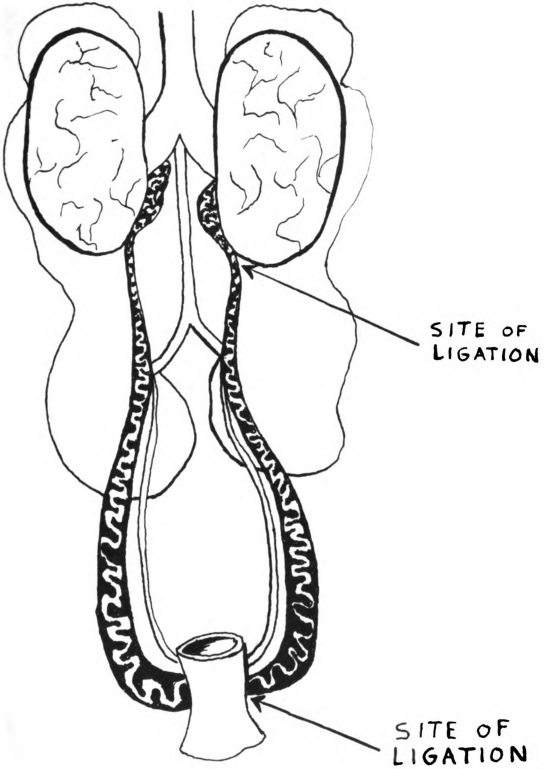
SITE OF
LIGATION
SITE OF
LIGATION

Fig. 2. Schematic diagram of left side of the reproductive tract indicating the sites of ligation before sectioning into testicle and ductus deferens.

Fig. 3. Miscroscopic view of a ductus deferens homogenate as seen under phase contrast illumination (690X).

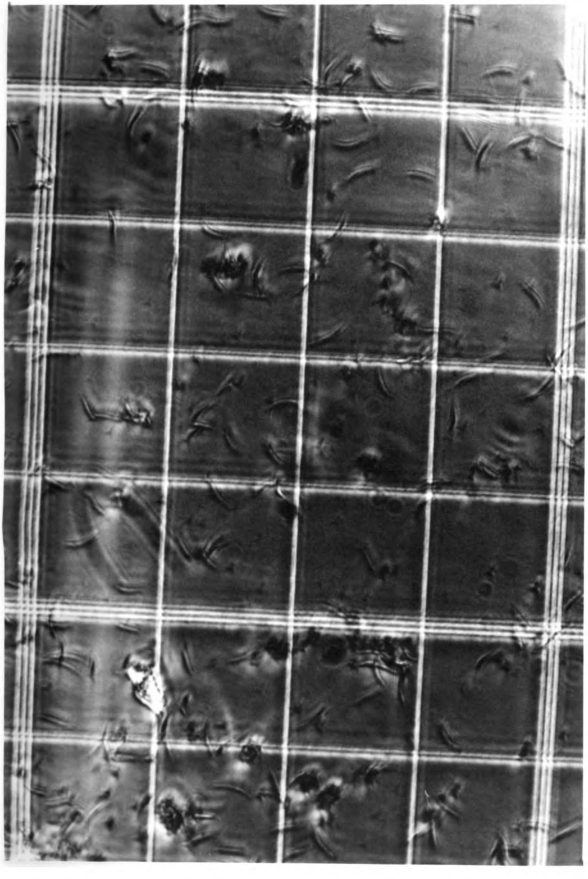

Fig. 4. Microscopic view of a testicular homogenate as seen under the phase contrast illumination (690X).

each homogenate was estimated by two observers in duplicate, using a phase contrast illumination of the spermatozoa in an A.O. Spencer Bright-Line Hemacytometer. Based upon comparison of their morphology with those in testicular histological sections, spermatids counted in testicular and ductus deferens homogenates were believed to represent only stages 7, 8, 9 and 10 of the cycle of the seminiferous epithelium according to Clermont (1958).

C. FSH Bioassay

The pituitaries were pooled by treatment groups, weighed and homogenized with normal isotonic saline solution. The solution obtained was used for FSH activity determination. The method used for FSH activity was that of human chorionic gonadotropin augmentation of Steelman and Pohley (1953) as modified by Parlow and Reichert (1963) and described in Appendix Table IV. Each assay consisted of injecting six twenty-day old female rats with the solution from one and one-half tom pituitaries in saline solution at the rate of 0.5 ml twice a day for three consecutive days. The immature female rats were of the Sprague-Dawley strain (Hormone Assay Laboratories, Chicago, Ill.), weighing approximately seventy grams each. The rats were maintained in a temperature controlled room (25 $\pm$ 1° C.) with 14 hours of light and received a commercial diet of Blox pellets (Allied Mills, Chicago, Ill.) which along with water, was available at all times. At the end of the three-day treatment period, the female rats were killed with ether. Their ovaries were removed, trimmed and weighed to the nearest one-tenth of a milligram and an analysis of variance and Duncan's multiple range test were run according to the methods of Steel and Torrie (1960).

D. FSH-RF Assay

This experiment consisted of a 2 X 4 factorial plan with orthogonal contrasts (Steel and Torrie, 1960). The contrasts were planned as follows for mean comparisons. Contrast one consisted of cortical extract versus all others. Contrast two, hypothalamic extract from treatment group A versus hypothalamic extract of treatment groups B and C. And the final contrast number three was hypothalamic extract of treatment group B versus hypothalamic extract in treatment group C (Table 4).

Table 4. Contrasts used for comparing the means

Contrast	Treatment Representation
C_1	Cortical extract versus hypothalamic extract of the three treatment groups
C_2	Hypothalamic extract of treatment group A versus hypothalamic extract of groups B and C
C_3	Hypothalamic extract of treatment group B versus hypothalamic extract of group C

At the end of the experimental period, the toms were decapitated and their hypothalami and anterior pituitaries removed. The anterior pituitaries were assayed for FSH activity as described before in this thesis. The hypothalami were placed in ice cold 0.1N HCl (0.2 ml per hypothalamus) and stored at -20° C. At the time of removing the hypothalami, a similar volume of cerebral cortex was also removed, but only from those toms that had received treatment A. The cortex was treated in exactly the same manner as all the hypothalami used in the

experiment. The following week the tissues (hypothalami and cortex) were homogenized separately and by treatment groups in chilled 0.1N HCl and centrifuged at 12,000 g. for forty minutes at 4° C. just prior to use. The supernatant was decanted and placed in a protein-free medium 199 (Difco Laboratories, Detroit, Michigan). The pH was brought up to 7.4 by titration with 1N NaOH solution using a pH meter with glass electrodes.

For incubation purposes, adult Sprague-Dawley male rats were decapitated and their pituitaries rapidly removed. The anterior pituitary was separated from the posterior lobe and cut in half. Each half was placed in a different flask containing three milliliters of medium 199. Eight halves were placed in each flask giving a total of four pituitaries per flask. Following this procedure, the flasks were placed for incubation in a Dubnoff metabolic shaker (60 cycles per minute) under constant flow of 95 percent oxygen and five percent carbon dioxide at a temperature of $37^{\circ} \pm 0.5^{\circ}$ C. for a period of four hours.

The pituitaries were preincubated for a period of thirty minutes after which the medium was discarded to avoid any spontaneous release of FSH. The discarded medium was replaced by two milliliters of fresh medium 199 and either one milliliter of hypothalamic or cortical extract per flask. The incubation consisted of four groups with four flasks per group representing the hypothalamic extracts of treatment groups A, B and C as well as the cortical extract of group A.

At the end of the incubation period the medium was separated from the anterior pituitaries, frozen and stored at -20° C. until assayed.

The FSH content of the medium was bioassayed by means of a four point bioassay with two doses of standard (FSH-NIH-S3) and two doses of the experimental unknown. The difference between doses was two-fold, administering two milliliters of the incubated media per assay rat (Sprague-Dawley strain female rats with an average weight of 60 grams) as a high dose and one milliliter for the low dose. The standard FSH used consisted of 70 and 140 micrograms per rat for the low and high dose, respectively. A total of four rats were used per dose level or a total of 32 rats.

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RESULTS AND DISCUSSION

A. Effect of Feed Restriction During the Breeding Season

1. Feed consumption --

Feed restriction alone has been shown to influence reproduction; therefore, it would be of interest to know if possible differences are the result of feed restriction, periods of feed withheld or both; hence, the importance of knowing the amount of feed intake per tom during the experiment.

The experimental results of the first year showed that feed consumption varied little between treatments A (control) and B (24-hr. feed withheld). However, both treatment A and B differed widely from treatment C (36-hr. feed withheld). The same trend was observed during the second year when there were twice as many toms and more observations (Table 5). An analysis of covariance and Dunnett's t-tests (Ostle, 1963; Li, 1964) showed no significant differences between treatments A and B ($P > 0.05$), but there was a highly significant difference ($P < 0.01$) between groups A and C for both experimental periods. The trends in feed consumption for each treatment during the first and second year can be observed in Figures 5 and 6, respectively.

Apparently a 24-hour feed restriction period twice a week was compensated for by overeating between fasting periods up to a point where there was no real change in total feed intake per tom. However, two 36-hour fasting periods per week were evidently so severe that

Weekly Feed Consumption Per Tom During The Year 1966

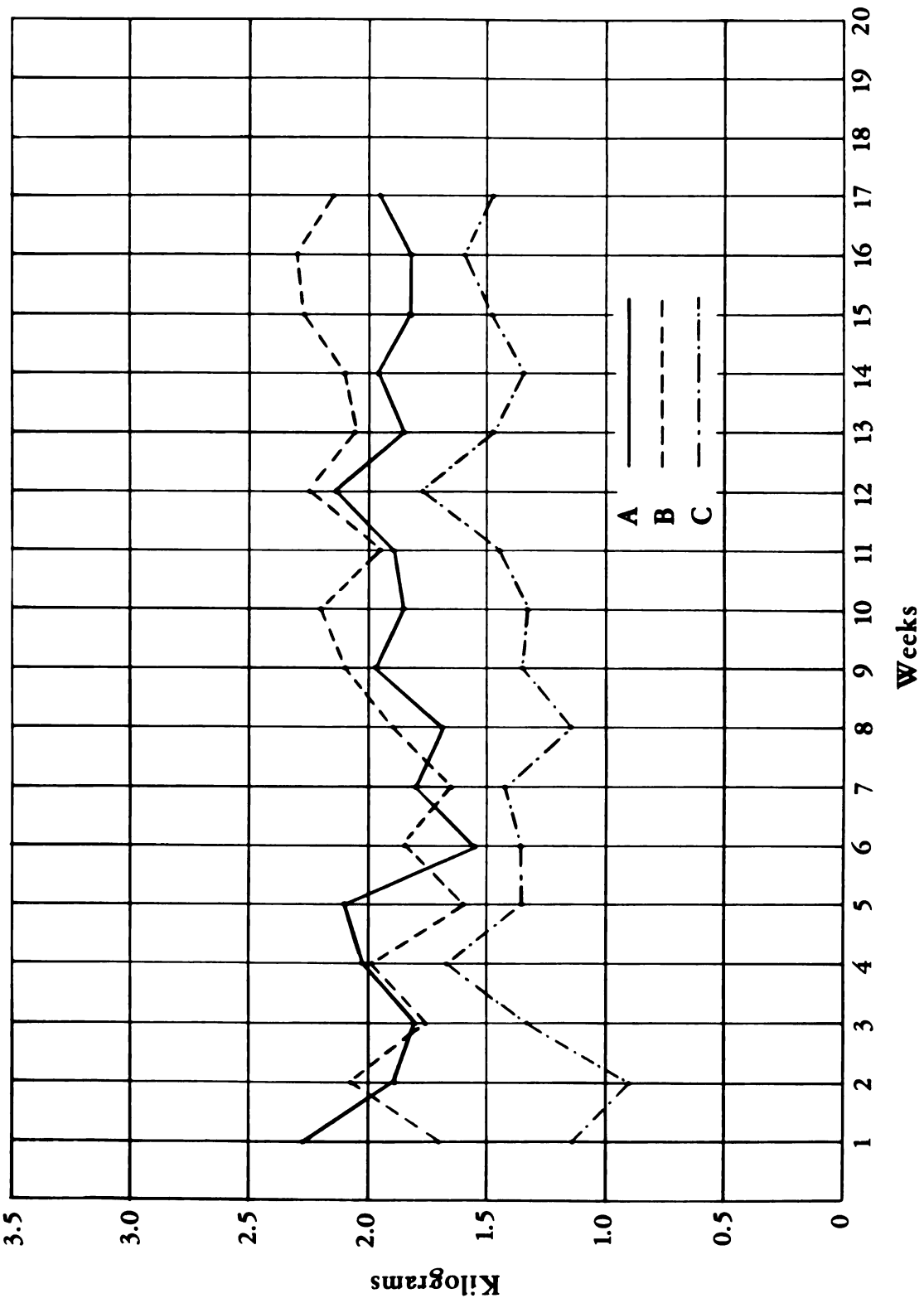

Fig. 5 Weekly Feed Consumption Per Tom During The Year 1966

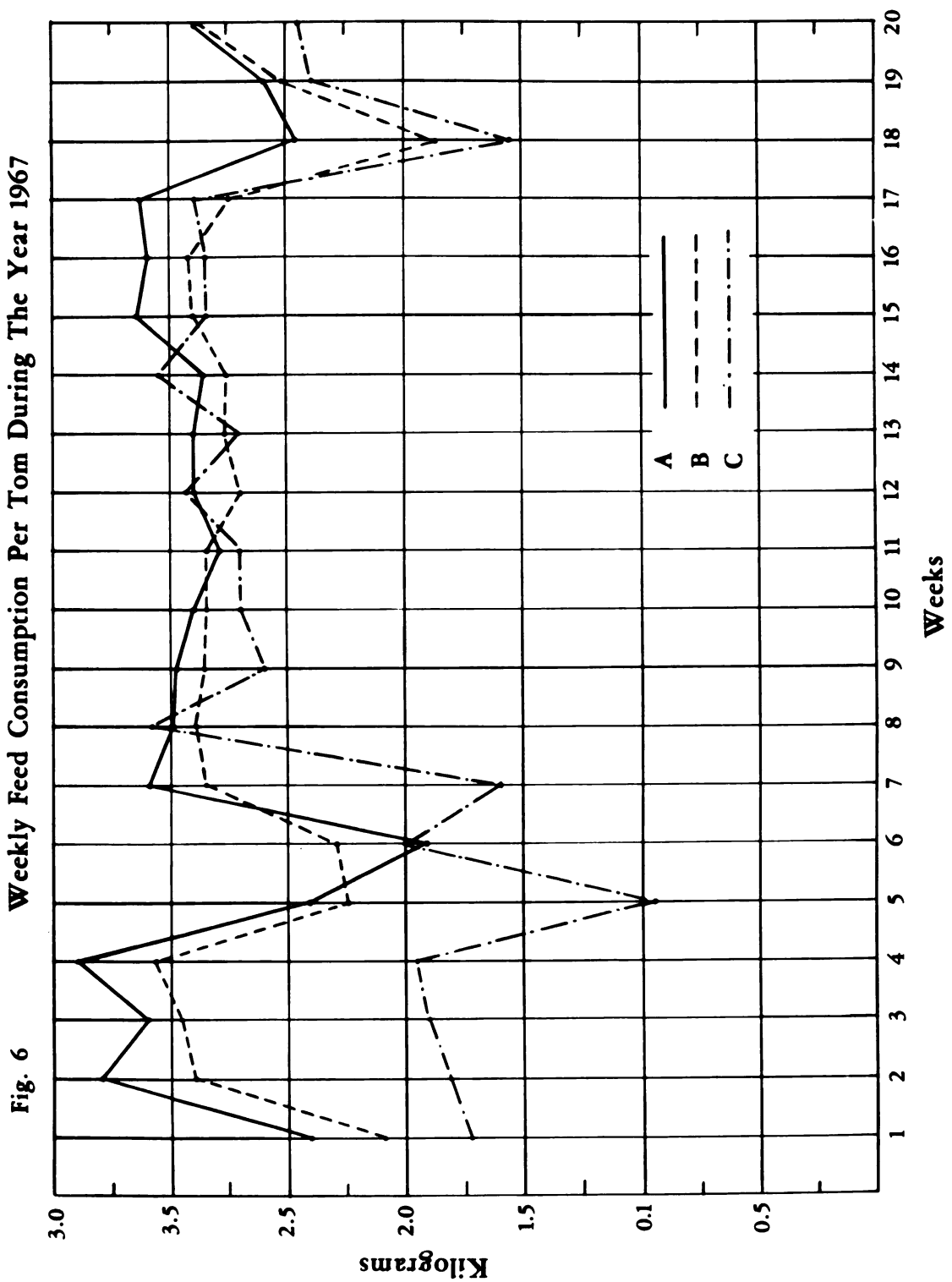
Weekly Feed Consumption Per Tom During The Year 1967
Kilograms
3.0
3.5
2.5
2.0
1.5
0.1
0.5
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
Weeks
A
B
C

Fig. 6

Table 5. Effect of feed being withheld twice weekly on feed consumption[a,b]

Treatment	Time feed withheld (hrs.)	Feed intake per tom per week (kg)		Total feed intake per tom (kg)	
		1966	1967	1966	1967
A	0	2.682	2.867	45.594	57.332
B	24	2.773	2.696	47.141	53.918
C	36	1.955**	2.399**	33.235**	47.977**

[a]17 weeks for 1966.

[b]20 weeks for 1967.

**($P < 0.01$) significantly different from A and B.

compensatory overeating could not achieve levels of feed intake similar to those of full-fed toms. If we considered the total number of hours in which the toms were out of feed, and interpret them as follows: Treatment A = 7/7 or ad libitum feeding; treatment B = 5/7 and treatment C = 4/7 ad libitum feeding. We can conclude from our results that our toms showed a capacity to compensate by overeating if feeding time was restricted by 2/7 (28.6%) that of ad libitum fed toms. However, the toms could not compensate if restricted to 3/7 (42.9%) ad libitum fed toms. Based on these results, if actual feed restriction is to be studied, consideration should be made to restrict the toms in excess of that of treatment B.

2. Body weight --

The nutritional status of an individual can be measured to a certain extent by its changes in body weight. Therefore, body weight was measured in the experimental animals as a parameter of the effects

of periods of feed restriction upon the physiological state of the body.

The differences obtained in body weight do not agree statistically between years as far as treatments A and B are concerned (Table 6). In the first year of the experiment no significant differences ($P > 0.05$) between treatment groups A and B resulted; however, during the second year a highly significant statistical difference ($P < 0.01$) was observed between the three groups. Nevertheless, the two-year data agree in that both groups A and B were heavier at the end of the experimental period than group C ($P < 0.05$) and ($P < 0.01$) during 1966 and 1967, respectively. Possibly these differences were influenced by the original weights of the toms. As shown in Table 6, the weights of the toms at the beginning of the first year were heavier than those in 1967. This suggests that the fasting periods may have affected feed utilization even though the total intakes were similar for both treatments. Perhaps the amount of feed utilized per tom might have been sufficient in 1966 to maintain body weight, but it was suboptimal in 1967 for maximal growth and gain in body weight.

3. Semen volume --

The main purpose of this experiment was to determine the effects of withholding the feed twice per week upon semen volume.

The average semen volume collected per tom during each semen collection of the two experimental periods is summarized in Table 7. The results showed higher volumes of semen for toms of treatment B than for those of treatment groups A and C during both experimental periods. However, in 1966, no statistical significance ($P > 0.05$) was demonstrable among groups. This was not the case for the 1967 data where the

Table 6. Effect of feed being withheld twice weekly on body weight[a]

	Treatment and years					
	A (0 hrs.)		B (24 hrs.)		C (36 hrs.)	
	1966	1967	1966	1967	1966	1967
	(kg)	(kg)	(kg)	(kg)	(kg)	(kg)
Initial weight	16.180	13.450	16.180	13.500	15.140	13.490
Final weight	16.020	17.360	16.910	16.200	13.420	14.270
Weight difference	-0.160	+3.910	+0.730	+2.700**	-1.720*	+0.780**

[a]Standard error for each final weight mean, (1966) = ± 1.8; (1967) = ± .491.

*($P < 0.05$) significantly different from A and B.

**($P < 0.01$) significantly different from A.

Table 7. Effect of feed being withheld twice weekly prior to semen collection on the volume of semen collected[a]

	Yield per collection in ml.			
Treatment	Number of observations	1966	Number of observations	1967
A (0 hrs.)	170	0.33	400	0.33
B (24 hrs.)	170	0.41	400	0.40**
C (36 hrs.)	170	0.33	400	0.32

[a]Standard error of each mean yield for 1966 = ± 0.010; for 1967 = 0.003.

**($P < 0.01$) significantly different from A and C.

statistical analysis showed a highly significant difference ($P < 0.01$) favoring treatment group B. Further, the first year numerical values were similar to those obtained for the second year. The agreement of our numerical values in both years strongly indicates that the lack of statistical significance in the first experiment can be attributed to the small number of toms per treatment.

4. Number of spermatozoa per cubic millimeter --

Knowledge of the effect of feed withholding upon seminal volume would be of little value if the effects of such treatment upon the cellular or fluid fractions of semen were not known. Hence, a study was made to determine if there were any effects upon the spermatozoal concentration of the semen obtained under such conditions.

The results obtained during the first year varied statistically from those obtained during 1967 (Table 8). The values obtained in 1966 show that treatment group B had nine percent more sperms per cubic milliliter than treatment group A and 12 percent more than treatment group C. However, these figures were not statistically different ($P > 0.05$) among treatment groups. In 1967, treatment group B had two percent more spermatozoa than treatment group A and twenty percent more than treatment group C. The value obtained for treatment group C in 1967 varied significantly ($P < 0.05$) from treatment groups B and A. Regardless of the statistical differences, the numerical differences for the three treatments were alike for the two years; treatment group B always had the highest concentration and treatment group C the lowest. These results indicate that the observed increase in seminal volume of treatment group B over treatment groups A and C was not due to seminal fluid alone, but was also due to an increase in spermatozoa production.

Table 8. Effect of feed being withheld twice weekly on spermatozoal concentration

Treatment	Number of spermatozoa in thousands per mm^3 ± standard error of the mean			
	Number of observations	1966	Number of observations	1967
A (0 hrs.)	85	9,700 ± 179	200	9,200 ± 144
B (24 hrs.)	85	10,600 ± 179	200	9,400 ± 144
C (36 hrs.)	85	9,300 ± 179	200	7,500 ± 144*

*($P < 0.05$) significantly different from A and B.

5. Spermatocrit values --

The second evaluation of the semen fractions can be obtained by spermatocrit values. These, therefore, were used as another criteria for semen evaluation.

The results obtained by this method of evaluation of spermatozoa concentration agreed with the results obtained in evaluating the number of spermatozoa per cubic millimeter. The spermatocrit values were not statistically different ($P > 0.05$) between treatments for the first year. However, the results obtained during 1967 showed a significant difference ($P < 0.05$) among treatment groups (Table 9). Once again treatment B had the highest concentration of spermatozoa, while treatment C had the lowest concentration. Therefore, under our experimental conditions, both hemacytometer evaluation and spermatocrit technique resulted in higher spermatozoa concentration for treatment group B. These results further corroborate the findings that treatment B stimulated seminal volume output by increasing the number of spermatozoa ejaculated as well as possibly increasing the volume of seminal fluid.

Table 9. Effect of feed being withheld twice weekly upon spermatocrit values and motility scores

Treatment	Percent of spermatozoa		Motility Scores	
	1966	1967	1966	1967
A (0 hrs.)	25.1 ± .791	20.8 ± .496*	4.6 ± .114	3.7 ± .087
B (24 hrs.)	28.5 ± .791	22.6 ± .496*	4.4 ± .114	3.6 ± .087
C (36 hrs.)	29.7 ± .791	19.4 ± .496*	4.3 ± .114	3.4 ± .087

*($P < 0.05$) A, B and C significantly different from each other.

6. Motility --

Although motility is not a good evaluation of fertilizing capacity in fowl, it is presumably true that immotile spermatozoa are infertile. Hence the importance of measuring the influence of feeding treatment upon motility of the spermatozoa.

No statistical differences were detected among treatment groups ($P > 0.05$) as shown in Table 9.

7. Fertility and hatchability --

The final tests for spermatozoal vitality and fertilizing capacity are detection of egg fertility and hatchability.

The effects of feed restriction upon fertility and hatchability showed a repeatable trend from 1966 to 1967 as can be seen in Table 10. The numerical differences showed a tendency of decreased fertility and hatchability as the fasting hours were increased. This trend was repeatable, but no statistical difference was detected ($P > 0.05$) among treatment groups. However, if spermatozoal motility is accepted as an indication of fertility, even though no statistical differences were found in fertility and hatchability, the numerical trends tend to

agree with the motility results of 1967 showing an adverse effect as the fasting time increased.

Table 10. Effects of feed being withheld twice weekly upon fertility and hatchability, expressed as percentage[a,b]

	Fertility		Hatchability[c]	
Treatment	1966	1967	1966	1967
A (0 hrs.)	91.5 ± 2.6	84.1 ± 4.1	64.1 ± 3.9	58.0 ± 4.1
B (24 hrs.)	87.0 ± 2.6	82.6 ± 4.1	61.2 ± 3.9	57.2 ± 4.1
C (36 hrs.)	86.7 ± 2.6	80.0 ± 4.1	60.5 ± 3.9	56.7 ± 4.1

[a]Given as treatment means ± standard error of the mean.

[b]12 hatches.

[c]Percent hatch of fertile eggs.

8. Blood constituents --

In mammals, differential blood cell counts can be used as tests for detecting stress; however, this test in poultry has not been very reliable (Wolford and Ringer, 1962).

Fasting the toms was not shown to affect the differential counts ($P > 0.05$) nor the hematocrit determinations, as shown in Table 11. The lack of response of blood corpuscle to fasting periods indicates that the blood cell producing tissues are either quite refractory to this type of stimulus or else that the stress was not strong enough to produce a response.

In the chicken, it has been shown that feed deprivation produces a decrease in blood glucose concentration (Sturkie, 1965). No reports of similar studies with turkeys could be found.

Table 11. Average values in per cent of blood cells at the end of the last feed deprivation period[a]

Group	Hematocrits	Heterophils	Lymphocytes	Monocytes	Eosinophils	Basophils
A	47.80	52.60	43.80	1.30	0.80	1.50
B	48.75	52.00	43.20	1.40	0.20	3.20
C	47.50	49.70	45.60	0.80	0.20	3.70

[a]Blood cell differential determinations were made only during 1966.

In the present experiments, the blood glucose concentration did not show a significant change ($P > 0.05$) among treatment groups when determined after a fasting period. On the other hand, blood cholesterol concentration did show a highly significant increase ($P < 0.01$) in group C when compared to the other two treatment groups (Table 12).

Table 12. Average values of the three different treatments in blood glucose and cholesterol concentration at the end of the experimental period $\pm$ the standard error of the mean

Treatment	Number of observations	Cholesterol mg. %	Glucose mg. %
A	5	160.4 $\pm$ 10.1	324.2 $\pm$ 18.3
B	5	139.2 $\pm$ 10.1	302.8 $\pm$ 18.3
C	5	214.7 $\pm$ 10.1**	359.5 $\pm$ 18.3

**($P < 0.01$) highly significantly different from A and B.

The above results suggest that the turkey tom is quite insensible to blood glucose concentration changes when subjected to fasting periods of 24 to 36 hours. These data do not agree with the results obtained with chickens (Sturkie, 1965) but do agree with those obtained in mammals (Dukes, 1955).

9. Organs and glands --

Since differential blood cell counts are not a very reliable test for stress, total adrenal cholesterol determinations were used for this purpose.

The total cholesterol concentration in the adrenals expressed as milligrams per 100 grams of wet weight tissue increased as feed deprivation increased in severity. The values obtained in milligrams

per 100 grams of wet adrenals were as follows: Treatment A = 3,697; treatment B = 4,018 and treatment C = 4,130. Since the values were obtained from pooled adrenals, no replication was possible and, therefore, the data were not analyzed statistically. The amount of cholesterol in the adrenals shows the same pattern as blood cholesterol when related to the treatment received.

A possible explanation for the increased amount of cholesterol in the adrenals is an increased synthesis of glucocorticoids as a response to stress. This increase, and subsequent release, of glucocorticoids is possibly required by the tom for gluconeogenesis during the fasting periods. If true, this theory helps to explain the absence of a blood glucose concentration difference from that found in chickens. A possible explanation is that the chickens were fasted only once before the glucose measurement while the toms were repeatedly exposed to periods of fasting prior to the glucose determination and, therefore, the possibility of adaptation is suggested by the enlarged adrenals and lighter thyroids.

Cholesterol depletion tests have been used for testing adrenal stimulation from either exogenous or endogenous ACTH by several researchers (Sayers et al., 1944; Howard and Constable, 1958; Siegle and Beane, 1961). However, these tests have been used with either very acute stressors or direct ACTH injection. In the first of these cases, the general adaptation syndrome might have been carried to the last phase or depletion stage, which was obviously not the case of the experimental toms discussed in this thesis. Evidence for this is that in 1967, both treatment groups made weight gains over the initial weight.

With respect to adrenal weight comparisons among treatment groups, both restricted groups had a trend toward heavier adrenal weights, but these differences were not statistically significant ($P > 0.05$) (Table 13).

Since the gastrointestinal tract of the turkey has the capacity of retaining large amounts of ingesta, the thought occurred that, after periodic periods of feed withholding, the toms might overeat prior to such periods and cause an enlargement of the tract. Another possibility was a decrease in size of the tract due to a constant restriction of feed.

In the present experiment, the gastrointestinal tract showed a tendency to decrease in weight as the fasting regime became more severe; however, no statistical differences ($P > 0.05$) were shown among treatment means (Table 13).

No significant differences ($P > 0.05$) were found with respect to testicular or ductus deferens weights among treatment groups (Table 13).

Thyroid weight was significantly depressed in treatment C ($P < 0.05$) when compared to both treatment groups A and B but there was no significant difference ($P > 0.05$) in thyroid weight between treatment groups A and B (Table 13). Therefore, as far as treatment group B is concerned, the data agree with that reported in the literature, that is, restricted feed intake has no effect on the thyroid weight as far as poultry is concerned (Premachandra, 1962; Mellen et al., 1954). However, treatment group C did have smaller thyroids and showed cholesteremia which is a sign of hypothyroidism (Reineke et al., 1946; Benoit and Bogdonovitch, 1937; Clegg et al., 1959). This sign of hypothyroidism is in agreement with the results obtained by Premachandra

Table 13. Effect of feed deprivation on certain gland and organ weights given as averages ± mean standard errors[a]

Treatments	Adrenals 1966	Thyroids 1966	G.I.T. 1966	Testes		Ductus deferens
				1966	1967	1967
	(mg)	(mg)	(gms)	(gms)	(gms)	(gms)
A	588 ± 51.1	364 ± 23.2	367 ± 16.9	32.8 ± 2.1	35.4 ± 2.3	2.28 ± 1.6
B	681 ± 51.1	367 ± 23.2	348 ± 16.9	39.0 ± 2.1	36.3 ± 2.3	2.38 ± 1.6
C	660 ± 51.1	286 ± 23.2	336 ± 16.9	30.6 ± 2.1	37.9 ± 2.3	2.32 ± 1.6

[a]1966 = 5 observations per treatment.
1967 = 10 observations per treatment.

(1962) and those of Mellen et al. (1954) in which restricted caloric intake produced lower metabolic rate and diminished thyroid function.

In mammals, Anand (1961) stated that thyroid control and body temperature regulation appeared to be integrated with a third controlling system, that of feed intake regulation. Brobeck (1960) also showed a close relationship between feed intake and both the environmental and body temperature. This triple relationship, temperature-ingesta-thyroid function, raises the possibility of a heat expenditure control through thyroid function regulated by the level of caloric intake. Several authors have supported the idea of a thyroid-temperature relationship by showing that cold exposure increases TSH secretion, while warmth reduces TSH secretion (D'Angelo, 1960 and 1960b; Bottari, 1957; Knigge, 1960; Kuschinsky, 1935). Therefore, it is possible that chronic restriction of feed intake depresses secretion of TSH and thereby prevents normal thyroid growth. It has been reported that certain mammals under nutritional stress, have a decreased thyroid function (Stephens, 1940; Reichlin, 1957; D'Angelo, 1951). Thus, under chronic stress the depressed function of the thyroids may be due to nutritional pseudo-hypophysectomy (decreased TSH) with a reduction in weight logically following.

10. The relationship between testicular size and spermatozoal output --

The tests used for evaluating testicular function are tedious and time-consuming. If it could be shown that there is a high positive correlation between testicular weight and spermatozoal output, an easier procedure could be devised. All that would be required for such

a test, would be to treat the turkeys, weigh their testes and compare them as measurements of treatment effect.

Spermatozoal output per ejaculation of all treatments for the two years as well as testes weight were analyzed and their partial correlations calculated (Table 14). The correlation values were not pooled together by years since treatment group A which was the control group had values statistically significantly different ($P < 0.05$) between years. This was so even after a possible "outlier" tom was discarded due to extremely small testes and sperm outputs. In this case the correlation value of .970 was lowered to 0.659 but still was statistically significantly different ($P < 0.05$) from the corresponding value for 1967.

Table 14. Correlation values between testicular size and spermatozoal output during 1966 and 1967

Treatment	1966	1967
A	0.970[a]**	0.177
B	0.083	0.271
C	0.102	-0.159

[a]Recalculation of this value due to a possible outlier with extremely small testes and decreased spermatozoal output give a figure of 0.659.

**Highly significant difference from zero.

All other correlation values were tested against zero and none of the values proved to be statistically ($P > 0.05$) different from zero. Therefore, there seems to be no significant correlation between

testicular weight and spermatozoa output when semen was collected from the toms twice a week.

11. Spermatogenesis and sperm reserves --

Since measurements of semen volume, spermatocrits and sperm counts showed an increase of activity in the testicular tissue of treatment group, B, further studies were performed to determine the total potential of semen production in the different treatment groups per gram of testicle and the amount of semen present in the ductus deferens.

Differences among the mean values for number of spermatozoa in testes and ductus deferens for treatment groups B and C were highly significantly ($P < 0.01$) greater than that for treatment group A. These differences indicate an increased rate of spermatogenesis when the toms were deprived of feed twice a week prior to semen collection for periods of 24 or 36 hours (Table 15).

As reported previously in part D, the number of spermatozoa obtained per collection was lowest for treatment group C. However, in the macerated testes and ductus deferens, the number of spermatozoa from treatment group C was much higher than from treatment group A ($P < 0.01$); while no significant difference ($P > 0.05$) was found between treatment groups B and C. A possible explanation is that there was a greater reabsorption of spermatozoa from the excurrent ducts for treatment group C due to the prolonged periods of fast before semen collection.

12. Follicle stimulating hormone content of the pituitaries --

That there was an increase in semen volume and spermatogenesis in treatment group B suggests the possibility that the restriction

Table 15. Effect of feed deprivation upon spermatogenesis and sperm reserves[a]

Treatment	Number of observations	Millions of spermatozoa in ductus deferens[b]	Millions of spermatozoa per gram of testicle[c]	Millions of of spermatozoa in the two testes[d]
A	10	8,291	229	75,083
B	10	15,239	365	120,157
C	10	14,746	352	121,564

[a]Testes include epididymides.

[b]Total count in left organ X2.

[c]Calculated using the total weight of left testicle without its tunica.

[d]Total number of spermatozoa per gram of left testicle times the total weight of the two testes without their tunicas.

periods were acting as pituitary "stimulants" and produced an increase of FSH synthesis which in turn was responsible for the increased spermatogenic activity of the testes.

The FSH content of the pituitaries, expressed as the average of six rat ovarian weights per treatment group was as follows: Treatment group A, 148 $\pm$ 3.5 mg; treatment group B, 167 $\pm$ 3.5 mg and treatment group C, 167 $\pm$ 3.5 mg. In preliminary studies, ovarian weights of similar rats treated with similar doses of mature male turkey pituitaries showed ovarian weights extremely close to those obtained in treatment group A.

An analysis of variance was performed, followed by Dunnett's t-test for treatment means according to the methods of Steel and Torrie (1960). The results of the statistical analysis show that both treatment groups B and C had a significantly higher ($P < 0.05$) content of FSH in the pituitary than treatment group A. These differences are in accordance with the results observed in the testicular homogenates as reported in the previous section.

During the course of the experiment, it was observed that treatment group C showed decreased physical activity, with less strutting. Fighting between these toms became practically nil when compared to the other two treatment groups. This suggests a possible decrease in androgen levels which could in turn be a result of decreased LH release. This observation and speculation could possibly explain the difference in results between sperm output of treatment group C and the spermatozoal counts in the testicular homogenates. It has been stated by Turner (1960) that the germ cells require LH or androgen to develop beyond the

stage of secondary spermatocytes, but not further with large numbers of secondary spermatocytes being reabsorbed.

13. Presence and content of a follicle stimulating hormone releasing factor (FRF) --

An increase in pituitary FSH does not necessarily reflect an increase in FSH synthesis and release because pituitary content is a function of each. Consequently, further evidence was necessary to demonstrate that the observed increase in FSH was due to increased FSH synthesis as well as to increased FSH release. Due to the fact that there is no evidence for a FRF in turkeys, or for that matter in aves, an experimental design had to be chosen that would not only detect the possible existence of FRF, but also would detect differences in concentration of such a releasing factor if there was one.

The results suggest that follicle stimulating hormone releasing factor is present in the turkey hypothalamus. The difference in FSH releasing capacity between hypothalamic extracts and cerebral cortex extracts was statistically significant ($P < 0.05$) as shown in Table 16. Further, analysis of variance also showed a dose response difference ($P < 0.01$) between the high and low dose of hypothalamic extracts added to incubation media. The results showed that groups B and C had a significantly ($P < 0.05$) greater concentration of FRF than group A, but there was no significant difference between treatment groups B and C ($P > 0.05$). The detection of levels of neurosecretory stimulating material for FSH corroborates the existence of a FRF in the turkey.

The increase in FRF content in the hypothalamic extract of the fasted group supports the findings of increased FSH activity in the

Table 16. Results obtained on the presence of FRF and its concentration in the hypothalamic extracts of the different treatment groups, represented as rat ovarian weights in milligrams

Dose of incubated media	HE-A[a] (0 hrs.)	HE-B (24 hrs.)	HE-C (36 hrs.)	CE-A (0 hrs.)	Overall dose average
Low (1 ml)	88.0	86.3	104.3	74.0	88.1
High (2 ml)	108.8	158.5	136.5	111.5	128.8[c]
Overall treatment average	98.4[b]	122.4	120.4	94.0[d]	

[a]HE = Hypothalamic extract
CE = Cortical extract

[b]Significantly different ($P < 0.05$) from treatment groups B and C.

[c]Highly significantly different ($P < 0.01$) between high and low doses.

[d]Significantly different ($P < 0.05$) from the hypothalamic extracts.

anterior pituitary of the same treatment groups as previously reported in this thesis. The increment in spermatogenesis observed in the same groups can, therefore, explain the increase in semen volume observed in treatment group B as a possible result of increased FRF synthesis stimulated by the fasting periods. The increase in FRF may have been responsible for the increased reproductive activity through FSH stimulation of spermatogenesis.

14. Escherichia coli contamination

Since the toms on treatment groups B and C had been without feed for a period of 24 or 36 hours, the possibility of a decreased amount of feces and, therefore, reduced bacterial contamination of semen was contemplated. For the purpose of checking this possibility Escherichia coli counts were made. Escherichia coli was chosen due to its normal presence in great numbers in the intestines of the turkey.

The total number of Escherichia coli counted per milliliter of semen varied tremendously from zero to 112,000,000 organisms with an overall average of 9,917,046 organisms per milliliter. No statistical difference ($P > 0.05$) or numerical trend was observed among treatment groups.

B. Effect of Light Pattern on Time of Oviposition

1. First experiment --

In order to change the time of artificial insemination from that normally used by turkey breeders, it was necessary to alter the light pattern in an attempt to alter oviposition time.

Turkey hens were exposed to 15 hours of artificial light per day (8 p.m. until 12 noon). The eggs were collected three times daily,

at the time the lights were turned on (8:00 p.m.), 12 hours later (8:00 a.m.) and 15 minutes before the lights went off (11:45 a.m.). The number of eggs gathered per collection was recorded and each egg was marked using a wax pencil with the hen's number and the time of collection. The period of collection and recording of data was January 14 to April 30, 1966. The total number of eggs laid during this experimental period was 3,636 of which 333 were laid during darkness. The distribution of those eggs laid when the lights were off was as follows: January, none; February, 63; March, 153 and April, 117. When expressed as percentages as in Table 17, the data are: January, 0 percent; February, 4.4 percent; March, 11.6 percent and April, 17.6 percent of the total eggs laid in the respective months. The data from this experiment show that there is a trend for an increased number of eggs to be produced during darkness as the season progresses. However, the entire amount of eggs that were laid when the lights were off was only 9.2 percent (Table 17) of the total.

Table 17. Effect of light pattern upon oviposition time during the year of 1966 (16 L:8D)[a]

	Number of eggs laid		Percent of eggs laid	
Month	Dark period	Light period	Dark period	Light period
Jan.	0	223	0.0	100.0
Feb.	63	1368	4.4	95.6
Mar.	153	1163	11.6	88.4
Apr.	117	549	17.6	82.4
Total	333	3303	9.2	90.8

[a]Lights on at 8:00 p.m. and off at 12 noon.

The above results indicate that it is possible to reverse the oviposition time in the majority of the hens by light manipulation.

2. Second experiment --

Exactly the same procedure was used as in the first year. The lighting schedule remained the same. The total number of eggs recorded during the 20-week period (January 16 to June 5) was 2,947 out of which 312 were laid during darkness. The distribution of eggs laid during darkness was as follows: January, 2; February, 41; March, 124; April, 92 and May, 54. If we express the number of eggs laid during darkness as a percentage of the total eggs laid during the month, the data are as follows: January, 0.4 percent; February, 6.4 percent; March, 15.0 percent; April, 13.9 percent and May 9.9 percent (Table 18).

Table 18. Effect of light pattern upon oviposition time during the year of 1967 (16L:8D)[a]

	Number of eggs laid		Percent of eggs laid	
Month	Dark period	Light period	Dark period	Light period
Jan.	1	273	0.4	99.6
Feb.	41	596	6.4	93.6
Mar.	124	703	15.0	85.0
Apr.	92	569	13.9	86.1
May	54	494	9.9	90.1
Total	312	2635	10.6	89.4

[a]Lights on at 8 p.m. and off at 12 noon.

During this experiment, there was an initial trend of progressive increase in the number of eggs laid in the period of darkness and

a second trend during April and May of decreasing numbers of eggs produced during darkness. However, the total number of eggs laid during the dark periods only amounted to 10.6 percent of the total. These data confirm those of the first experiment, that is, that the time of oviposition can be controlled by changing the light pattern. The 9.2 and 10.6 percent of eggs laid in darkness do not mean a failure of having successfully changed the oviposition pattern, since it has been reported that even under a regime such as that used commercially, turkey hens laid 5.5 percent of their eggs during darkness (Woodward et al., 1963).

The difference in trends on the percentage of eggs laid in darkness between years can be explained as follows: It has been shown that the average interval between successive eggs of a sequence in turkey hens varies from 25 to 27 hours depending on the strain of turkeys (Wolford et al., 1964). This sequence time is larger than a 24-hour day and since the turkey ovulates approximately 15 to 30 minutes after oviposition (Wolford et al., 1964), there is a lag time accumulation leading to later ovipositions with each succeeding egg until the end of the sequence. Hence, as the production cycle progresses, there is an increase of later eggs and, therefore, more eggs are laid in darkness. Further, it is common knowledge that after the first weeks of lay a number of turkey hens go out of production for some time and if they do not become broody, they may come back into production. Generally, these hens are the poor producers and will, therefore, have shorter sequences. If what has been reported in the case of chickens by Fraps (1955) is also true for turkeys, the lag time of the terminal oviposition decreases as the sequence length

increases from 2 to 7 or 8 eggs and the lag time approaches zero or even may fall below zero as the sequence length increases. This phenomena could explain the reason for two trends during the year of 1967. As the poor producing hens went out of production during April and May, the number of eggs laid during darkness also decreased.

CONCLUSIONS

Two years of experimental data have indicated that withholding feed prior to semen collection twice a week for periods of 24 hours produced an increase in semen volume without altering feed consumption or body weight gains. However, withholding of feed prior to semen collection twice a week for periods of 36 hours did not increase the amount of semen collected but did depress body weight significantly. The increase in semen volume was not of seminal fluid alone since two evaluations of seminal spermatozoa concentrations (sperm counts and spermatocrits) showed either an increase over the other treatment groups or no statistical difference. Therefore, it can be concluded that, under the experimental conditions described in this thesis, withholding feed twice a week for 24 hours produced an increase in total semen volume. Further, this increase was not of seminal fluid alone which indicates that increased spermatogenesis occurred.

Although spermatozoa motility appeared to be decreased as the time feed was withheld increased, the difference was not significant. Both fertility and hatchability decreased after withholding feed, coincidental with the motility changes. These differences make the practice of feed withholding prior to semen collection questionable, although neither of these characteristics was significantly affected by feeding treatment. Further research on the effects of feed withholding upon fertility and hatchability are highly desirable.

The data strongly suggest that the increase in semen volume shown by withholding feed for 24 hours prior to semen collection was due to an increase in follicle stimulating hormone and follicle stimulating hormone releasing factor activity. This increased activity seemed to be triggered by the withholding period per se and was not due to decreased feed intake.

Spermatozoal output at twice-a-week ejaculation and testicular size of the toms were not significantly correlated. Thus, in toms collected twice a week no evaluation of function could be made through the measurement of testicular weight.

The total number of spermatozoa in the testes-epididymides structures and ductus deferens were evaluated. The results showed increased rates of spermatogenesis in the two groups in which feed was withheld. This trend coincided with similar trends both semen volume and total number of spermatozoa collected in those birds where feed was withheld for 24 hours, but this did not hold for toms where feed was withheld for 36 hours. Possible explanations are: (1) LH activity was not increased parallel to that of FSH; (2) nutritional stress and/or the length of the withholding period per se was/were responsible for an increased spermatozoal reabsorption; (3) a combination of (1) and (2).

Follicle stimulating hormone releasing factor (FRF) appears to be present in the hypothalamus of the turkey toms and the level of FRF was elevated after feed restriction. This factor had heretofore not been demonstrated in avian species.

The oviposition of turkey hens was successfully altered by using artificial light from 8:00 p.m. until noon. About 9 to 10 percent

of the eggs were laid between 12 noon and 8:00 p.m. (in darkness), but even under normal artificial lighting some eggs were laid in darkness. The laying cycle can be altered sufficiently to permit the breeder to plan the light schedule in such a way that artificial insemination of the hens can be done at a convenient time.

SUMMARY

Individual seminal volume, sperm counts, spermatocrits, motility scores, testicular weights, ductus deferens weight and sperm reserves were obtained for turkey toms under three different nutritional regimes. Data were also collected on fertility, hatchability, feed consumption, body weight, follicle stimulating hormone content and follicle stimulating hormone releasing factor concentration in the hypothalami.

The time of oviposition and its relation to the light regime was also studied.

Semen volume and sperm counts were increased when feed was withheld twice a week for a period of 24 hours prior to semen collection as compared to ad libitum fed toms and those that had feed withheld 36 hours twice a week prior to semen collection.

No significant differences were detected among groups with respect to motility scores, testicular weights and ductus deferens weights. An increase in spermatogenesis over that of the ad libitum fed toms was observed in the testicular homogenates of the toms where the feed was withheld.

The follicle stimulating hormone concentration of the pituitary and the follicle stimulating hormone releasing factor found in the hypothalamic extracts were increased in those treatment groups in which the feed was withheld when compared to the ad libitum fed toms.

Feed intake was significantly depressed when the toms were fasted twice a week for periods of 36 hours prior to semen collection

when compared to those toms fed ad libitum or deprived of feed for 24 hours twice a week prior to semen collection. No statistical difference ($P > 0.05$) was detected between the last two treatment groups.

Heavier body weights were recorded for the ad libitum fed toms when compared to the two treatment groups in which feed was withheld prior to the collection of semen.

From the data collected, it appears that restricting the feed intake for 24 hours prior to the collection of semen stimulates spermatogenesis and produces higher semen volumes.

Oviposition time was changed by means of light manipulation. Using a windowless house and a light regime in which the lights came on at 8:00 p.m. and went off at 12 noon, it was possible to obtain approximately ninety percent of all eggs before noon.

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APPENDIX

APPENDIX TABLE I

Eosin solution for sperm counts

Water	500 milliters
2 per cent eosin solution	25 "
3 " " NaCl "	10 "

Mixed thoroughly and diluted in a 1:1 proportion with physiological saline for spermatozoal determinations.

APPENDIX TABLE II

A. Procedures

Procedure for cholesterol determination in blood and adrenal glands.

Reference:

Zlatkis, A., B. Zak and A. J. Boyle, 1953. A new method for the direct determination of cholesterol. J. Lab. Clin. Med. 41: 486-492.

Reagents:

1. Standard cholesterol solution (100 mg %). Dissolve 100 mg of pure, dry and ash-free cholesterol in 100 ml of 100 percent Merck glacial acetic acid.

2. Ferric chloride solution (10 mg percent). Dissolve 10 grams of ferric chloride, reagent grade in 100 ml of 100 percent Merck glacial acetic acid.

3. Color Reagent: Dilute 2.0 ml of the ferric chloride solution to 200 ml with chemically pure concentrated sulfuric acid. To prevent chloride precipitation, add 100 ml of sulfuric acid into volumetric flask. Then add the ferric chloride solution slowly while swirling flask.

Procedure:

Use triplicate samples

A. Blood plasma:

1. Thoroughly mix thawed frozen plasma

2. Pipette 0.1 ml into (Step 3)

B. Adrenal tissue:

1. Make a 1.5 gram thawed frozen tissue in 40 ml of glacial acetic acid.
2. Transfer to 120 ml Erlenmeyer flask; shake at high speed for 1.5 hrs; filter (Whatman #1) and pipette 0.1 ml into: (Step 3)

General Steps:

3. 10 ml of glacial acetic acid in 20 ml test tube.
4. Add 7 ml of color reagent and mix immediately.
5. Use plastic stoppers and invert three times with thumb or plastic top.[1]
6. Let stand for one hour; read in spectrophotometer twenty at 560 millimicrons.
7. Read against a sample of 10 ml glacial acetic acid and seven ml color reagent.

Standard: 1. Use 0.1 ml of standard cholesterol solution and pipette to step #3 of procedures.

Calculations:

1. $$\frac{\text{O.D. unknown}}{\text{O.D. standard}} \times 0.1 \text{ ml Std.} \times \frac{100}{0.1 \text{ ml}} = \frac{\text{mg cholesterol}}{100 \text{ ml plasma}}$$

2. $$\frac{\text{O.D. unknown}}{\text{O.D. standard}} \times 0.1 \text{ mg Std.} \times \frac{60}{0.1} \times \frac{100}{\text{gm liver}} = \frac{\text{mg cholesterol}}{100 \text{ gms wet adrenal}}$$

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[1] Caution: tubes become hot.

APPENDIX TABLE III

A. Procedures - Determination of blood sugar.

References:

Method of Folin and Wu, 1920. J. Biol. Chem. 41: 367.

Reagents:

A. 10% sodium tungstate (Na_2Wo_4 H_2O)

1. In a 1 liter volumetric flask containing 600 ml of distilled water, dissolve 100 grams reagent grade, sodium tungstate.

2. Dilute to 1 liter with distilled water.

B. 2/3 N sulfuric acid (H_2SO_4)

1. Into a liter volumetric flask containing about 600 ml of distilled water, add slowly with constant stirring 19 ml of concentrated sulfuric acid.

2. Dilute to the liter mark with distilled water. Check by titration against a standard sodium hydroxide (NaOH) solution.

C. Alkaline copper reagent:

1. Dissolve 40 grams of anhydrous sodium carbonate (Na_2CO_3) in 400 ml of distilled water, and transfer to a 1 liter volumetric flask.

2. Add 7.5 grams of tartaric acid.

3. Add 4.5 grams of crystalline copper sulfate ($CuH_2O \cdot 5H_2O$) and dissolve.

4. Mix and dilute to the 1 liter mark with distilled water.

5. On standing for sometime a sediment may appear. If this happens decant or filter through a good quality filter paper. This reagent keeps for a long time. However, it

should be checked by transferring 2 ml of phosphomolybdic acid solution. The blue color of the copper solution should almost completely disappear.

D. Phosphomolybdic acid color reagent:

1. Place 35 grams of molybdic acid and 5 grams of sodium tungstate in a liter beaker.
2. Add 200 ml of a 10% sodium hydroxide (carbonate free) solution and add 200 ml of distilled water.
3. Boil vigorously for 20 to 40 minutes (until all the ammonia is disposed).
4. Cool, dilute to 350 ml and add 125 ml of 85% (concentrated) phosphoric acid.
5. Dilute to 500 ml with distilled water and mix.

E. Standard glucose solutions:

1. Stock standard (1% solution):
   a) Dissolve 1 gram of highest purity anhydrous dextrose in 50 ml of saturated benzoic acid solution.
   b) Make up the volume to 100 ml with saturated benzoic acid solution.
   c) This solution keeps indefinitely.
2. Dilute standard
   a) Transfer 2 ml of the stock 1% glucose standard to a 100 ml volumetric flask.
   b) Make up the volume with distilled water or saturated benzoic acid solution. This solution stands indefinitely and equals 200 mgs percent.

3. Saturated benzoic acid solution.

   a) Dissolve 2.5 grams of benzoic acid crystals in 500 ml of distilled water by boiling.

   b) Transfer to a 1000 ml volumetric flask and dilute to a liter rinsing the benzoic acid until the volume has been reached.

Procedures for Unknown:

Into a Folin-Wu sugar tube place:

1. 2.0 ml of 1:10 protein free blood filtrate (Filter 42, blue).
2. 2.0 ml of alkaline copper reagent.
3. Mix by lateral shaking and place into a boiling water bath for 6 minutes.
4. Remove without shaking and place in a large beaker of cold water for 2 to 3 minutes.
5. Add 2.0 ml of phosphomolybdic acid color reagent.
6. Replace in boiling water bath for 5 minutes.
7. Dilute to the 25 ml mark with distilled water.
8. Mix well by repeated inversions.
9. Allow to stand for 10 to 15 minutes.
10. Transfer a portion of the colored solution into a colorimeter tube.
11. Read in the colorimeter within the next 15 minutes, against a blank tube set at 0.

Procedure for blank:

Run a parallel determination as described above on 2.0 ml of distilled water in place of the blood filtrate. Transfer a portion of the final solution to a colorimeter tube and set the colorimeter to its 0 reading against this solution.

Procedure for standard:

Run a determination as described above but use 2.0 ml of a standard glucose solution instead of the blood filtrate. Read a portion of the final colored solution in the colorimeter against the blank tube set at 0.

Calculations (Against the standard):

$$\frac{\text{Optical density of unknown}}{\text{Optical density of standard}} \times 0.4 \times \frac{100}{0.2} = \text{mg of glucose per 100 ml of plasma}$$

OR

$$\frac{\text{Concentration of the standard}}{\text{Reading of the standard}} \times \text{reading of unknown} = \text{concentration of the unknown.}$$

Calibration Factor: Reading of unknown X Folin-Wu blood sugar factor = mgs % of blood sugar in unknown. The factor is obtained from the reading of the standard solution described above, and calculating the factor from the following formula:

$$\text{Folin-Wu sugar factor} = \frac{200}{\text{Reading of Sd - reading of blank}}$$

The calibration factor should be determined in duplicate or triplicate and the average value taken. Once determined, the calibration factor is valid indefinitely within the limits of calibration.

APPENDIX TABLE IV

A. Procedures - Follicle stimulating hormone (FSH) bioassay.

FSH Augmentation - Method of Steelman and Pohley (1953) as modified by Parlow and Reichert (1963).

References:

1. Parlow, A. F. and L. E. Reichert, Jr., 1963. Endocrinol. 74: 740.

2. Steelman, S. L. and F. M. Pohley, 1953. Endocrinol. 53: 604.

Procedure:

1. The pituitaries are rapidly removed, placed in physiological saline solution and homogenized in a glass homogenizer.

2. The different solutions are brought up to the required volume by adding physiological saline according to the desired concentration of pituitary tissue per milliliter.

3. Doses of three milliliters per rat are prepared by adding physiological saline and 50 I.U. of human chorionic gonadotropin per dose.

4. Inject 0.5 milliliters every 12 hours for three days totalling 3.0 milliliters per rat.

5. 72 hours after the first injection was made, kill the rats and perform a necropsy.

6. Remove the ovaries, dissect free of surrounding tissue and weigh to the nearest one-tenth of a milligram.

7. A FSH preparation of known potency should be run for comparison purposes.

8. Compare the results by appropriate statistical methods.

Material required:

Female rats 20 to 25 days of age.

Note: The use of human chorionic gonadotropin to augment the responses observed with FSH, results in an assay with increased sensitivity and decreases the variation due to luteinizing hormone contamination.