UNDERNUTRITION OF GROWING MALE CHICKENS AND ITS RELATIONSHIP TO SEXUAL DEVELOPMENT AND REPRODUCTIVE PERFORMANCE

By

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INTRODUCTION

A major economic loss in the poultry industry is the large number of infertile eggs among those eggs produced for hatching purposes. Many studies have been undertaken to establish production procedures to minimize infertility, especially in females. Male fertility rapidly declines with age causing some breeder males to be replaced in mid-season.

It is common practice to raise breeder cockerels together with breeder pullets since their requirements have been assumed to be the same. However, very little research has been undertaken to study the requirements of cockerels for optimum reproduction performance.

Restriction of nutrients to reduce growth in pullets has been practiced for many years. The purpose of this restriction is to delay sexual maturity, reduce mortality, and increase initial egg size with more uniform size during the laying period. This has been accomplished with no permanent effect on fertility.

The purpose of this research was to study the effect of one type of feed restriction, low dietary protein levels during the growing period, upon development of the reproductive system of cockerels as indicated by subsequent reproductive performance and morphology of the testes.

LITERATURE REVIEW

Maynard (1947) stated that in emphasizing the general measurements for rapid growth and increase in weight and size, optimum conditions for individual organ development are often overlooked. Since the time of this statement, research has tended to study individual factor effects for optimum performance. By isolating the various components of different production measurements, the interrelationships existing between many environmental, nutritional, and physiological factors have been illucidated.

Male chickens usually produce semen throughout the year, but many workers have observed seasonal variation. Semen volume and sperm concentration were found to vary seasonally in cockerels (Burrows and Titus, 1939), but cocks did not exhibit this variation. Wheeler and Andrews (1943) noted seasonal variation in semen quality and quantity, with a decrease in summer months. Heywang (1944) reported that hot weather reduced fertility and hatchability and attributed the seasonal variation primarily to the effects of high environmental temperature. Average maximum temperatures of 101.8° to 106.8°F caused a decrease in copulations with a decline in semen quality and quantity. Boone and Huston (1963) found a non-significant decrease in semen volume, sperm concentration and number of sperm per ejaculate when White Plymouth Rock males were subjected to ambient temperatures of 102.5°F and 104°F for 2 to 3½ hours.

Photoperiodism is well understood in females of most fowl species, but much information concerning the effect of light on reproduction has been applied to the male chicken from wild species and from experiments with female chickens. Callenbach et al. (1943) stated that the work with light occurred before 1900 and was concerned with increasing egg production with artificial light. Bissonnette (1936) found that many animals experienced photoperiodicity effects on the reproductive system. The stimulus acts through the optic nerve via the hypothalmus to the anterior pituitary to stimulate secretion of gonadotropins. Parker et al. (1942) showed a close relationship between length of day and semen production during the winter and spring. Benoit and Ott (1944) found that orange and red light produced maximum testicular stimulus in drakes with intact hypophyseal stalks.

With as little as one hour light per day, sexually mature male fowl produced satisfactory volumes of viable semen (Parker and McCluskey, 1964). Earlier, Asmundson et al. (1946) had found that an intensity of 2 foot candles with 13 hours of daily light produced a maximum response in turkey hens as measured by average number of lighted days required before ovulation began. It was found that no light retarded egg production and bright light intensity induced production of smaller eggs than with dim light. Hays (1954) presented an interesting review on fertility and reproduction of the male as influenced by light. His studies revealed that old males responded to 30 days of all-night light, but this treatment had little effect on cockerel fertilizing capacity. More than 30 days of all night light showed a detrimental effect to fertility in both.

Parker and McCluskey (1965) obtained semen from cockerels as early as 12 weeks of age when the cockerels were exposed to 13 hours of daily light. They found that those males receiving higher levels of light during the growing period achieved better body weight than those receiving less daily light. This effect from the growing period was not manifested in the adult males.

With the practice of restrictive feeding, more interest has been shown in research concerning the effects of the various nutritive components. This interest has been particularly noted concerning reproduction in poultry. The reasons proposed for restrictive feeding include delay of sexual maturity, to provide more hatching size eggs, more efficient use of nutrients or increase in egg size (Milby and Sherwood, 1956).

Lutwak-Mann (1958) stated that there had been few studies on reproduction in male birds and in those reported, statistical data was not satisfactory. Nutrition affects the functional development of the male reproductive system by influencing normal metabolic processes or by influencing the reproductive endocrine balance. The effects of inanition in the rat were reduced output of gonadotropins and secondary testicular atrophy (Moore and Samuels, 1931). Parker et al. (1942) found that various restrictions of the basal diet fed New Hampshire males reduced semen volume, number of sperm at each collection, and male fertilizing capacity. They attributed this to diminution of testosterone production due to inanition. Parker and McSpadden (1943) found that general inanition caused testicular atrophy in the cockerel. Ching et al (1951) found that underdevelopment of the testes and secondary sex glands, due to inanition,

was overcome when the males were placed on an adequate diet. Mann and Lutwak-Mann (1951) restored the secretory function of inanition-atrophied organs of male chickens with injections of androgens or chorionic gonadotropins which stimulate Leydig cell secretion of androgens. Erschoff (1952) pointed out that nutritional deficiencies may cause lowered secretion of gonadotropins from the anterior pituitary which brings on gonadal dysfunction.

Vitamin deficiencies as related to male reproduction have been studied to a limited extent. Burrows and Titus (1938), studying 22 cockerels (7½ months of age) from November to June, found that vitamin A deficient males gave greater semen volume than those males fed a diet containing 1900 I.U./100 g feed. Semen volume increased from 0.45 cc the first week of the trial to 1.75 cc in the twentieth week. There were no sperm counts made but the authors stated that based on artificial insemination of hens, the males were fertile. Paredes and Garcia (1959) found no effect on semen volume when diets deficient in vitamin A were fed. However, they found watery semen in vitamin A deficient males when compared to controls which were fed 1 million I.U. of vitamin A/kg of feed. After ten weeks of study, motility of sperm was low and sperm counts averaged 0.63 billion per cc for the deficient males and 2.13 billion per cc for the controls. Fertility of the deficient males eventually declined to zero, but viable sperm were produced within two weeks after the males were placed on a diet containing 30,000 I.U. of vitamin A. These workers stated that semen alteration was the first sign of a vitamin A deficiency.

Buckner *et al.* (1951) studied the effects of dietary

vitamin D levels (0, 2,000, and 20,000 A.O.A.C. units per gram of feed) on New Hampshire cockerels. Those cockerels fed no vitamin D consumed less feed and drank more water than those fed 2,000 or 20,000 A.O.A.C. units per gram of feed. The gains in weight and linear dimensions of combs, wattles and gonads between six weeks and 18 weeks of age were directly related to vitamin D level. Haque et al. (1949) stated that vitamin D was necessary for full androgenic stimulation. Their studies also revealed that deficiencies of folic acid, choline, and biotin resulted in greater testes weights (based on body weight) than controls, but body weights of the deficient males were less, indicating that body size and testes size increased disproportionately. Herrick et al. (1952) attributed testicular atrophy in fowl fed a vitamin E deficient diet to a decrease in pituitary gonadotropin secretion. In all articles reporting vitamin deficiency studies, the authors emphasized that the specific vitamin studied was the limiting deficiency rather than general inanition.

Hilton (1961), in studying starlings, reported that cholesterol, a precursor to steroid hormone synthesis, was present in inverse proportion to the production of gonadal steroid hormones which reflected the degree of reproductive activity of the starlings that were under study.

Boyer et al. (1942) found that calcium deficiency caused sterility in rats. Phosphorus deficiency inhibited testicular development in immature rats and stopped spermatogenesis in adult rats. In the same study, lack of dietary manganese caused testicular degeneration and absence of libido.

Parker and Arscott (1964) found that dietary energy restriction in the adult male chicken resulted in reduced testes and body size, and less semen volume and fertilizing capacity.

Arscott and Parker (1963) fed 16.9, 10.7 and 6.9% protein level diets over a period of 33 weeks to dubbed SCWL cockerels. These were housed at eight months of age and were maintained in individual cages. The decreased protein level had no adverse effect on semen volume or fertilizing capacity of the semen based on artificial insemination results. Actually, the males fed a protein level of 6.9% had significantly higher fertility than those fed the other two protein levels. Dietary protein level had no effect on hatchability of fertile eggs. The authors calculated amino acid content for the three diets and concluded that protein quality within the amino acid levels encountered in the experiment was of limited importance from the standpoint of reproductive efficiency. They also reported that males on the higher protein diet ate more feed and gained more weight than those on the lower dietary protein levels.

In feeding trials with SCWL pullets, Waldroup and Harms (1962) compared diets which varied as to protein and energy content. The protein content ranged from 9 to 25% with energy values of 700, 940, and 1180 kilocalories of productive energy per pound of feed. Sexual maturity was delayed when either energy or protein was fed at lower levels than controls during the growing period (8 to 21 weeks). Their findings agreed with the results of studies with high energy-low fiber diets (Couch et al., 1957). Dietary protein or energy level did not affect the total rate of egg production, but pullets reared on

the lower levels of protein or energy produced more eggs after 32 weeks of age. The restricted diets during the growing period did not influence egg weights. The authors proposed feeding lower levels of protein as a means of delaying sexual maturity when a delay is desired, or 16.0% protein with 940 kilocalories of productive energy per pound of feed for commercial grower diets. Harms and Waldroup (1963b) found that low levels of dietary protein reduced the length of the laying cycle (clutch size). They suggested that the reduced laying cycle was due to the lack of hormone production, release, or target organ sensitivity.

Based on the work at the Florida Experiment Station with pullets and Parker's work with protein restriction in mature males, Wilson et al. (1965) applied the principle of feeding low protein diets to White Leghorn males during the growing period. Dietary protein levels of 16.0, 9.0, 6.75, and 4.5% were fed in two different experiments. The diets were fed to the cockerels beginning at nine weeks of age in the first experiment and seven weeks of age in the second. In both experiments, the cockerels were placed on a 17.0% protein breeder diet at 23 weeks of age and fed until the males were 37 weeks of age. Body growth and sexual maturity were depressed in those males receiving the low protein diets. There was apparently no permanent testicular damage due to dietary protein levels as all males were giving semen with comparable sperm scores within seven weeks after being placed on the 17.0% protein recovery diet. By 37 weeks of age, the low dietary protein groups had higher sperm concentration and heavier testes than those males fed higher levels of protein. The males fed intermediate protein levels gave better

fertility than those receiving 16.0% or 4.5% protein diets. Hatchability of fertile eggs was not affected by protein levels. This concurred with the findings of Arscott and Parker (1963). Wilson and his associates suggested that diets in the range of 7 to 9% protein during the growing period might be more desirable for cockerels which are to be used for reproductive purposes.

Harms and Waldroup (1963a) added methionine hydroxy analogue calcium (MHAC) to corn-soybean meal diets formulated with different levels of protein to study the effect on production in pullets. They found that there was a change from a methionine deficiency at the 13.0% protein level to a lysine deficiency when 11.0% protein was fed. This was due to the substitution of corn for soybean meal in the lower protein diet. Adding MHAC, lysine, or both, improved production in pullets, but the response never exceeded the production obtained in the control 17.0% layer diet.

A discussion of lysine deficiency as it affected pullet reproductive development was presented by Singsen et al. (1964). Feeding the deficient diet for 12 weeks or longer from day of hatch delayed age at 50.0% production an average of 17.7 days. Lysine deficiency did not effect adult mortality, body weight, average egg size or hatchability of fertile eggs. Ovary and comb weights were reduced due to the treatment. This may have the same effect if applied to cockerels.

There are several methods available to measure the effect of various treatments on reproduction in male chickens. Sperm concentration, motility, abnormalities and semen volume along with results of fertilization of hens are among the standard criteria

considered. Matching the fertilizing capacity of the sperm from one male with the sperm of another (competitive fertility) has been attempted in a number of studies.

Dunn (1927) stated that the original idea of competition between sperm for ova fertilization came from cross pollination experiments with corn. The corn plant had an affinity for its own pollen when pollen from another plant was introduced. The question whether sperm from closely related males was more successful in fertilizing an ova than sperm from distant relations instigated the first investigations into selective, or competitive, fertilization. Although Dunn's procedures lacked the refinements that are available today, his data did indicate that there was competition between sperm.

Even though their data were not significant, Curtis and Lambert (1929) stated that they noted the presence of selective fertilization in studies utilizing Single Comb White Leghorns (SCWL), White Plymouth Rocks (WPR) and Rhode Island Reds (RIR). Bonnier and Trulsson (1939), in a study using RIR and SCWL males with RIR females, found that RIR semen yielded more offspring than semen from SCWL males.

Parker et al. (1942) inseminated New Hampshire (NH) hens with equal volume mixtures of semen from SCWL, Barred Plymouth Rock (BPR), and NH males. They found that when a hen produced two or more chicks, the chicks were sired by two different males. Five of 15 hens produced chicks sired by three different males. Offspring from the BPR and NH males were about equal in number, but the SCWL males sired significantly fewer chicks. This agreed with the work of Bonnier and Trulsson (1939). Parker et al. (1942) suggested that competition between sperm is a matter of chance providing there is compatability between sperm and ova.

Ferrand and Bohren (1948) reported data that did not support the theory of Dunn (1927). Pooled semen from BPR and WPR males was inseminated into WPR hens. Since the BPR sired more offspring, they stated that this ruled out selective preference of ova for closely related sperm. However, they pooled equal volumes of semen and the BPR had higher concentrations of sperm, which may have influenced competition.

Parker et al. (1942), Bonnier and Trulsson (1939), Curtis and Lambert(1929) and Dunn (1927) all used color of plummage for early recognition of the sire's offspring. However, Allen and Champion (1955) used this method for identification as well as the dominant factor, rose comb. In their studies, homozygosity for color characteristics and the rose comb genes were proven by test mating with a minimum of eight offspring for each test. They used NH, WPR, Rose Comb White Wyandotte (RCWW) and SCWL breeds of poultry. Equal numbers of sperm were used in their test, whereas, other workers had artificially inseminated equal volumes of semen (Parker et al., 1942; Bohren et al., 1945; Ferrand and Bohren, 1948; Bonnier and Trulsson, 1939) or practiced consecutive or alternating matings of the different males (Dunn, 1927; Curtis and Lambert, 1929). Semen quality was determined within one hour of the time of insemination to affirm sperm concentration. Their results revealed no incompatability between SCWL sperm and NH ova as reported by Parker et al. (1942) and Bonnier and Trulsson (1939). They did not find that selective fertility or preference of ova for closely related sperm existed. However, they did state that there was discrimination against weakness and poor adaption to the female

oviduct. This conclusion was due to the very few offspring sired by the RCWW males in the competitive fertility trials. They attributed selective fertilization to certain semen possessing lower incidence of abnormal sperm and better motility than semen of other males.

In competitive fertility studies, homozygous rose comb males provide an excellent means for distinguishing the sire of the offspring. Mixing semen of rose comb males with semen of single comb males and inseminating single comb hens was proven by Allen and Champion (1955) to allow easy identification of offspring. Homozygotes (RR) and heterozygotes (Rr) are indistinguishable, but both differ phenotypically from single comb (rr) chicks. For competitive trials it is necessary to test by mating the chickens to insure homozygosity. Selection practices to improve production introduced the single comb gene into breeds having the rose comb factor (Crawford and Smyth, 1964a).

Ponsignon (1951) and Cochez (1951) obtained poor fertility from matings of RR males to RR females. Crawford and Merritt (1963) reported that low fertility was characteristic of RR males, but heterozygous and single comb males exhibit normal fertility. Their work with artificial insemination eliminated behavioral mating patterns as a cause for low fertility. Crawford and Smyth (1964b) attributed most of the low fertility of the RR males to poor survival of sperm in the female tract. But, fertility within the duration of the fertility period was found to be lower in the RR males as compared to the Rr and rr males.

Crawford and Smyth (1964c) compared the quality of semen

and seminal plasma of males with RR, Rr, and rr genotypes in an attempt to identify the causitive factor for low fertility in the RR males. Seminal plasma from the low fertility homozygous males was replaced by seminal plasma from the normal fertility single comb males. No apparent deficiency of seminal plasma fluid from the RR males was indicated as a cause for the low fertility.

CHAPTER I

PROTEIN RESTRICTION

EXPERIMENT I

Wilson et al. (1965) suggested the possibility of improved reproductive performance when cockerels were fed protein levels of 7-9% during the growing period. This work and the studies of Arscott and Parker (1963) raised questions concerning testicular development during the treatment period and semen quality over an extended period. The following experiment was designed with four protein levels and examined the problems presented by previous research.

Procedure

This experiment was divided into two phases; the first dealt with the testes growth as related to dietary protein and as a measure of sexual maturity. The second phase dealt with the effects of the dietary protein levels on semen production and quality. The two phases were designed to relate body size and testes development with semen production and fertility.

Single Comb White Leghorn cockerel chicks that had been brooded on a litter floor under infra red lamps from day of hatch (Dec. 10) to seven weeks of age were utilized in both phases. They were subjected to natural daylight and received a 21.0% protein starter diet (Table 1) for this period. At seven weeks of age, 160

Table 1. Composition of starter diet.

Ingredients	(%)
Yellow corn	54.95
Animal fat	4.35
Soybean meal (50% protein)	36.45
Ground limestone	1.58
Dicalcium phosphate (34% Ca + 18% P)	1.00
Oat hulls	0.37
Iodized salt	0.40
Micro-ingredients*	0.90
% Calcium	1.00
% Phosphorus	0.60
% Protein	21.00
Productive Energy (Kilocalories/kg. diet)	2068.00

*Supplies per kg. of diet: 6930 I.U. vitamin A; 1320 I.U. vitamin D_3; 770 mg. choline; 40 mg. niacin; 4.4 mg. riboflavin; 20 mg. calcium pantothenate; 22 mcg. vitamin B_{12}; 20 mg. iron; 2 mg. copper; 198 mcg. cobalt; 11 mg. iodine; 400 mcg. zinc; 220 mg. $MnSO_4$; and 12.5 mg. ethoxyquin.

cockerels were randomized into 16 pens in growing batteries. Four pens of ten birds each were fed dietary protein levels of 16.0, 9.0, 6.75, and 4.5%. The 6.75% and 4.5% protein diets were made by diluting the 9.0% (Table 2) basal formulation with glucose monohydrate[1]. Proper additions of limestone, defluorinated phosphate, and vitamin supplementation were provided to make up deficiencies created by the dilution. At 21 weeks of age, all cockerels were changed to a 17.0% protein layer diet (Table 3). One male per replication from each treatment was weighed, sacrificed, and the testes removed and weighed, at three week intervals beginning at nine weeks of age and ending at 36 weeks of age.

In the second phase of the experiment, 72 cockerels were randomized into individual wire layer cages (8" x 18" x 18"). Three replications of six cockerels each were fed the four dietary protein levels. When the cockerels were 21 weeks of age all groups were placed on a 17.0% protein layer diet.

Body weight was measured at two week intervals beginning at seven weeks of age and ending at 37 weeks of age. Each male was ejaculated weekly by the massage technique and the semen evaluated as to motility and concentration. Scoring ranged from 0-5, with five being maximum movement and concentration (Allen and Champion, 1955). Sperm counts were made every four weeks by a modification of the procedure described by Allen and Champion (1955) with A. O. Spencer Hemocytometers following dilution of the semen 1:200 in red blood cell pipettes. At 24 and 31 weeks of age three SCWL hens per

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[1]Brand name Cerelose.

Table 2. Composition of basal diets.

Ingredients	16% protein diet	9% protein diet
	%	%
Yellow corn	49.3	67.8
Ground oats	16.7	22.0
Soybean meal (50% protein)	18.8	----
Animal fat	3.8	0.4
Oat hulls	5.5	3.7
Alfalfa meal (17% protein)	2.0	2.0
Ground limestone	0.9	0.9
Defluorinated phosphate (34% Ca + 18% P)	2.0	2.2
Iodized salt	0.4	0.4
Micro-ingredients*	0.6	0.6

*Supplied per kg. of diet: 39.6 mg niacin; 4.4 mg. riboflavin; 19.8 mg. calcium pantothenate; 770.0 mg. choline; 19.8 mg. iron; 2.0 mg. copper; 220.0 mg. $MnSO_4$; 125.4 mg. ethoxyquin (Santoquin, Monsanto Chemical Co., St. Louis); 22.0 mcg. vitamin B_{12}; 99.0 mcg. zinc; 6930 I.U. vitamin A; and 1320 I.C.U. vitamin D_3.

Table 3. Composition of 17% protein layer diet.

Ingredient	%
Yellow corn meal	67.73
Soybean meal (50% protein)	20.60
Alfalfa meal (20% protein)	3.00
Ground limestone	5.81
Defluorinated phosphate	1.96
Iodized salt	0.40
Micro-ingredients*	0.50
% Protein	17.00
Productive energy (Kilocalories/kg. diet)	2017.00

*Supplied per kg. of diet: 4,400 I.U. vitamin A; 500 mg. choline; 13.2 mg. niacin; 1,540 I.C.U. vitamin D_3; 4.4 mg. riboflavin; 8.8 mg. calcium pantothenate; 13.2 mcg. vitamin B_{12}; 12.5 mg. ethoxyquin; and 220 mg. $MnSO_4$.

male were artificially inseminated to determine fertilizing capacity of the males. Eggs were examined for fertility by candling at one week of incubation followed by a macroscopic examination of the germinal disc of those eggs considered infertile. Hatchability was not determined since it had been previously shown that dietary protein level had no effect on hatchability of fertile eggs (Wilson et al., 1965; Arscott and Parker 1963). The results of the experiment were statistically analyzed using analysis of variance according to Snedecor (1956). All statements of probability are based on the results of these analyses.

Results and Discussion

Body weights of cockerels fed 4.75% dietary protein gained only 300 g from seven weeks to 21 weeks of age (Figure 1). The males fed the other three dietary protein levels gained weight more rapidly with 200 g separating the males of each level at 21 weeks.

Body weights of the cockerels fed the lower dietary protein increased rapidly after being placed on the recovery diet, however, they never attained the level of those fed 16.0% protein. At 45 weeks of age, body weight of males fed 4.5% protein exceeded that of the males fed the intermediate protein levels but the difference was not significant. This compensatory gain in weight is in agreement with the findings of Wilson et al. (1965) and has been discussed by Wilson and Osbourn (1960).

Mortality during this study was 16.0, 7.0, 7.0, 7.0% for the groups which received dietary protein levels of 16.0, 9.0, 6.75, and 4.5%, respectively. This indicated that low dietary protein fed under these conditions during the growing period did not increase

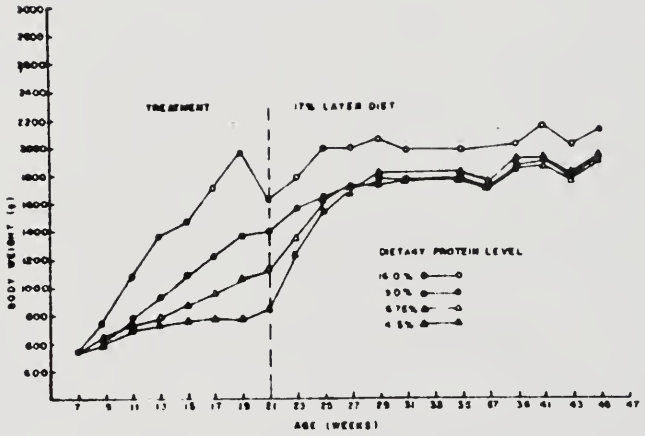

Figure 1. Growth rate of cockerels fed various dietary protein levels during the growing period and 17.0% protein layer diet after 21 weeks of age (Exp. 1).

mortality. The abnormal number of losses in the 16.0% group was attributed to large comb and body size with activity limited by cage size. The males had not been dubbed and the large combs interfered with their normal eating and drinking from the small openings provided in the laying cages. The condition resembled one aspect of blue comb disease having cyanotic comb and wattles (Fisher et al. 1961). This factor is probably part of the reason for the erratic number of males producing sperm in the 16.0% protein group mentioned by Wilson et al. (1965).

Testes growth was inhibited with dietary protein levels of 6.75 and 4.5% (Figure 2). When the diet was changed to 17.0% protein, the testes in those males grew rapidly and by 33 weeks of age cockerels fed low dietary protein approached or exceeded all other males in testes size. When testes size was plotted as percent of body weight there was essentially no change in the response. This indicated that as body size increased, testes size increased in the same proportion. A maximum testes size of 1.5% of body weight was obtained at 33 weeks of age in those cockerels fed 6.75% dietary protein.

Sexual maturity was delayed in all three low protein groups (Figure 3). Some individual males fed intermediate protein levels during the growing period reached sexual maturity earlier than those fed the highest and lowest levels of dietary protein. This was considered to be individual variation rather than an effect of the diet. The delay in sexual maturity was attributed to the low dietary protein level as suggested by Arscott and Parker (1963) and Wilson et al. (1965). By 25 weeks of age (four weeks on the recovery diet), 90.0% of all males were giving semen.

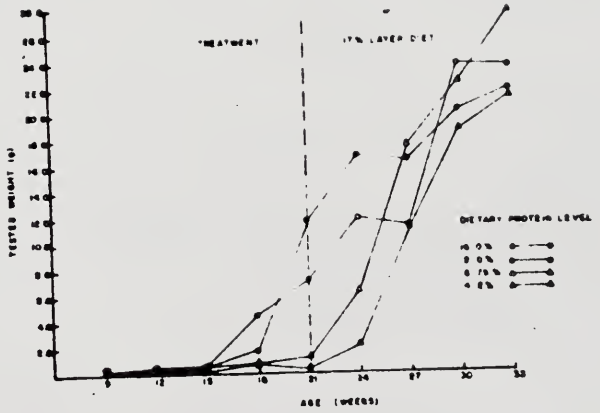

Figure 2. Testes weight of cockerels fed various dietary protein levels during the growing period and then 17.0% protein after 21 weeks of age (Exp. 1).

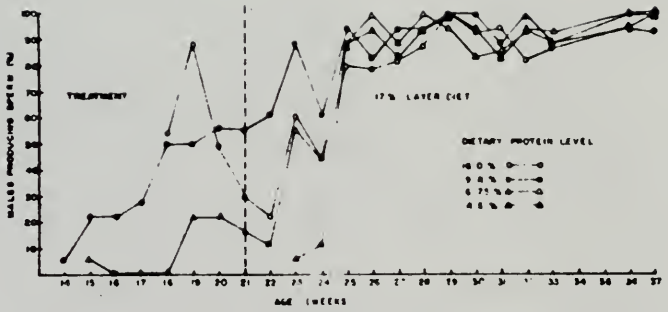

Figure 3. Sexual maturity determined by sperm production of cockerels fed low levels of dietary protein during the growing period (Exp. 1).

By 29 weeks of age (eight weeks on recovery diet), males fed 4.5% dietary protein had peaked higher than other treatment groups in sperm concentration by 0.75 billion per cc (Figure 4). This indicated that there was no permanent damage to the reproductive system due to low dietary protein levels. There was an immediate decline in sperm concentration in all treatments after this period which was assumed to be due to the confined condition previously described. The larger males were most severely affected and a few individuals in all treatment groups were affected.

The semen scores were relatively high (Figure 5) in males producing semen. It should be pointed out that semen scores for the lowest dietary protein group exceeded all others once they began producing semen.

Fertility of eggs following matings with caged SCWL hens at 24 and 31 weeks of age (three and ten weeks respectively, after being placed on the 17.0% protein layer diet) is indicated in Table 4. Even though males fed high dietary protein during the growing period showed higher fertility at 31 weeks of age, those fed lower levels had improved as indicated by gains in fertility over the 24 week results. This is in agreement with the findings of Wilson et al. (1965).

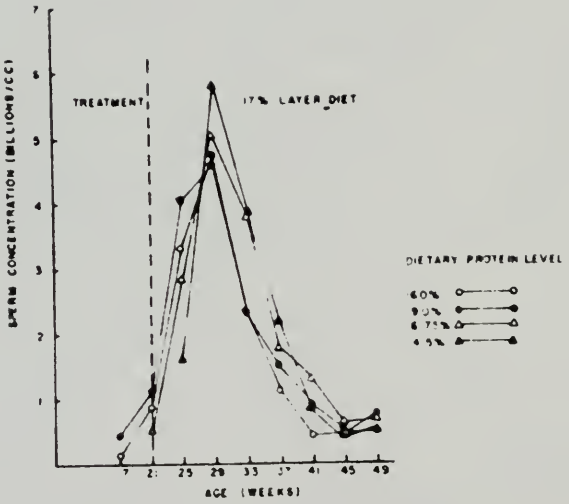

Figure 4. Sperm concentration of cockerels fed low levels of dietary protein during the growing period (Exp. 1).

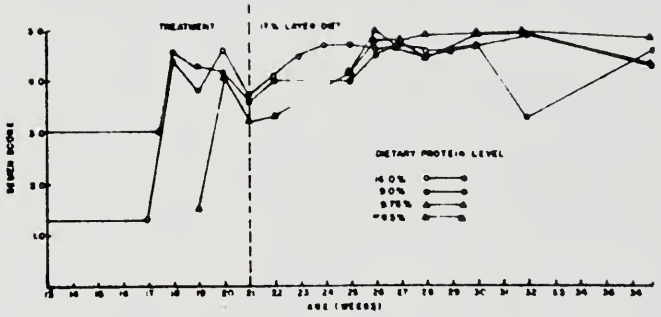

Figure 5. Semen scores of cockerels fed low levels of dietary protein during the growing period (Exp. 1).

0 = No motility discernable.

1 = 1-20% of the spermatozoa exhibiting slight undulating movement. Few spermatozoa.

2 = 20-40% of the spermatozoa showing undulatory movement. No waves or eddies formed. Many sperm, but some inactive.

3 = 40-60% of the spermatozoa showing progressive motility. Vigorous motion. Slow waves and eddies produced. A few inactive sperm.

4 = 60-80% of the spermatozoa showing progressive motility. Waves and eddies very rapid in movement.

5 = 80-100% of the spermatozoa in vigorous and progressive movement. Extremely rapid formation of eddies and movement.

Table 4. Fertility of males following treatment with various dietary protein levels during the growing period.

Protein Level %		Percent Fertility			
		Av. of Males Giving Semen		Av. of All Males	
		24 wks.	31 wks.	24 wks.	31 wks.
16.0	*(8)	44.8	(13) 86.1	32.1	82.3
9.0	(14)	60.5	(16) 81.2	52.7	72.6
6.75	(8)	33.8	(14) 86.0	21.2	74.4
4.5	(1)	25.0	(15) 72.9	5.6	68.6

*Figure in parentheses indicates number of cockerels giving semen from a total of 18 males.

EXPERIMENT II

In previous studies on the effect of low dietary protein, feed consumption and sources of protein had not been considered. The following experiment was designed to study the effects of equal levels of protein from corn and soybean meal and corn only with lysine as the limiting amino acid. Histological studies of testes, competitive fertility, feed consumption, and longevity of protein effect were also examined in this study.

Procedure

This experiment was divided into two phases; the first dealt with histological study of testes, testes weight, and body weight as a measure of sexual maturity in relation to dietary protein levels. The second phase dealt with the effects of dietary protein levels on semen production, quality, and longevity of semen quality.

Both phases utilized SCWL cockerels (hatched in Feb.) reared in the same manner and fed the same starting diet described in Experiment 1. In phase 1, 200 cockerels were randomized into 20 groups of ten males each at seven weeks of age. They were placed in raised wire floor growing batteries for the remainder of the experiment. Light consisted of natural daylight supplemented with artificial light to assure at least 13 hours of light per day.

Four pens received each of the five experimental diets as indicated in Table 5. All diets were maintained isocaloric at 2200 kilocalories of productive energy per kg of feed. Protein levels

Table 5. Composition of experimental diets.

Ingredients	Diet %				
	1	2	3	4	5
Yellow corn	71.00	35.50	17.75	89.00	44.50
Soybean meal (50%)	19.20	9.60	4.80	-----	-----
Iodized salt	0.25	0.25	0.25	0.25	0.25
Micro-ingredients*	0.50	0.50	0.50	0.50	0.50
Ground limestone	1.30	0.43	-----	0.71	0.27
Defluorinated phosphate	1.83	2.68	3.10	2.52	2.94
Animal fat	2.20	3.75	4.51	0.57	-----
Cerelose	-----	42.61	63.60	-----	50.83
Sand	3.22	4.74	5.49	6.45	0.72
% Protein	16.0	8.0 B**	4.0 B	8.0 U***	4.0 U
Productive Energy (Kilocalories/kg. of diet)	2200	2200	2200	2200	2200
% Calcium	1.05	1.05	1.05	1.05	1.05
% Phosphorus	0.65	0.65	0.65	0.65	0.65

*Supplied per kg. of diet: 39.6 mg. niacin; 4.4 mg. riboflavin; 19.8 mg. calcium pantothenate; 770.0 mg. choline; 19.8 mg. iron; 2.0 mg. copper; 220.0 mg. $MnSO_4$; 125.4 mg. ethoxyquin (Santoquin, Monsanto Chemical Co., St. Louis); 22.0 mcg. vitamin B_{12}; 99.0 mcg. zinc; 6930 I.U. vitamin A; and 1320 I.C.U. vitamin D_3.

**B = Balanced - Diets formulated from corn and soybean meal protein sources

***U = Unbalanced - Diets formulated from corn as protein source.

consisted of 16.0, 8.0, and 4.0%. Two different formulations were used for each of the lower protein levels. One was derived by varying the levels of corn and soybean meal while the other was formulated by diluting the 16.0% diet with glucose monohydrate. Proper additions of limestone, defluorinated phosphate, and vitamin supplementation were provided to correct dilution deficiencies as reported in the previous experiment.

In both phases, the experimental diets were fed until the cockerels were 21 weeks of age at which time they were placed on a 17.0% protein layer diet (Table 2). All diets were fed ad libitum.

Body weight and testes weight was obtained by sacrificing one male from each replication every two weeks from nine through 21 weeks of age, then every four weeks thereafter. Histological sections were made of each excised testis; stained with Eosin-Hemotoxylin stain (Humason, 1962); then examined for abnormalities and stage of spermatogenesis. In the second phase, at seven weeks of age, three replications of five cockerels each were randomized into individual wire male cages 16" x 18" x 24" and fed the five experimental diets.

Body weight was measured at bi-weekly intervals from seven through 33 weeks of age and then at 52 weeks. Each male was massaged by the technique of Burrows and Quinn (1937) in an attempt to collect semen beginning at nine weeks of age. The males were ejaculated every two weeks until 33 weeks of age and then at four week intervals until the end of the study.

Sperm concentration of each male was examined every two weeks in the manner described in Experiment 1, and was recorded as

billion per cc. Sperm concentrations were measured microscopically until the males were 45 weeks of age and then a Model F Coulter Counter was used for sperm concentration determinations. The instrument was calibrated to agree with hemocytometer-microscopic counts. Settings on the Model F Coulter Counter were: Attenuation - .707; Sensitivity - 2; Threshhold - 10.0; using a 100 micron apperture. The counter operates on the principals outlined by Gregg and Steidley (1965) and the procedures utilized in this study were modifications of those employed by Segal and Laurence (1964).

Fertility was measured by artificial insemination of three SCWL caged hens with semen from each male when the males were 17, 21, 25, and 38 weeks of age. Competitive fertility studies were intended as a measure for fertilizing capacity, as described by Allen and Champion (1955) but due to the low ratio of rose comb to single comb offspring, the data is presented in Chapter II.

Feed consumption was measured bi-weekly in phase 2 from nine weeks through 29 weeks of age. Feed consumption was calculated for each two week period on an individual male basis.

The results of the experiment were statistically analyzed using correlation and analysis of variance according to Snedecor (1956) with differences between treatment means determined by Duncan's multiple range tests (1955). All statements of probability are based on results of these analyses.

Results and Discussion

Body weights of those males sacrificed for excision of testes (Figure 6), increased in size in proportion to the body weight of the males weighed in phase 2 (Figure 7). Cockerels fed

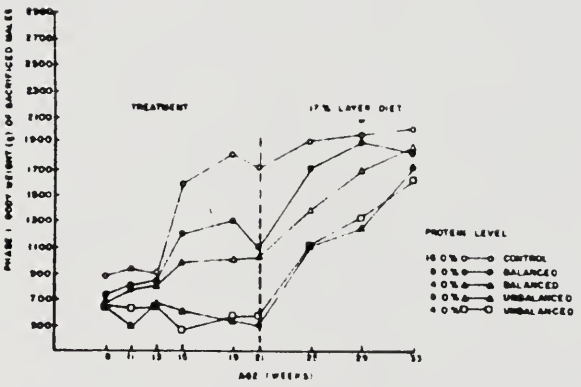

Figure 6. Growth rate of males fed various dietary protein levels during the growing period (Phase 1, Exp. 2).

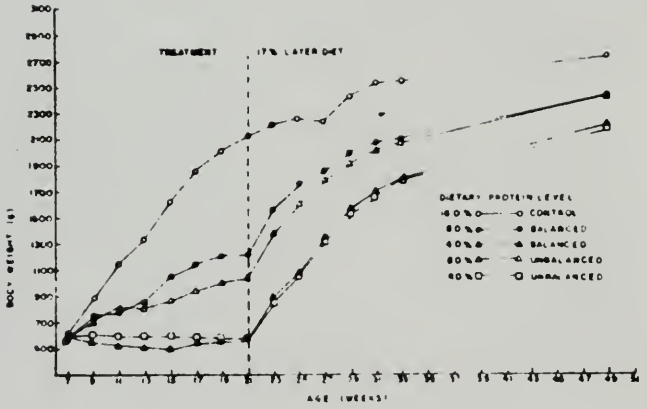

Figure 7. Growth rate of males fed various dietary protein levels during the growing period (Phase 2, Exp. 2).

low levels of dietary protein did not gain weight as rapidly as those fed 16.0% dietary protein during the growing period (Figure 7). Growth was accelerated once the males were placed on the 17.0% layer recovery diet. Males fed 16.0% protein lost body weight at five to six weeks after being placed on the recovery diet. Males fed 8.0 and 4.0% protein formulations weighed less at 52 weeks of age than males fed 16.0% protein. It was noted that an immediate decrease in feed consumption occurred in the 16.0% protein group after being placed on the layer diet (Figure 8). Decrease in body growth was attributed to the decline in feed consumption. Since increased calcium was the only ingredient change from the 16.0% protein grower diet to the recovery diet, it is proposed that the 3.0% calcium level caused the temporary decrease in feed consumption.

The 8.0 and 4.0% protein diets were formulated to provide diets with balanced and unbalanced amino acid content for each protein level. Table 6 contains the amino acid content of the treatment diets expressed as a percentage of the National Research Council's (NRC, 1960) recommendation for the chick. It is realized that the amino acid requirements for the growing chick are lower than the listed NRC requirements, but a similar relationship exists, and a complete list of requirements for the growing chick is not available.

A differential weight of approximately 900 g existed at 21 weeks of age between the males fed the control diet and those fed the 8.0% balanced diet (Figure 7). Singsen et al. (1964) found a 400 g weight difference at 21 weeks of age between controls and broiler

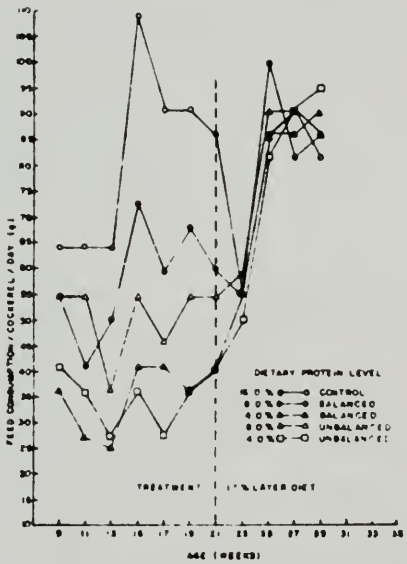

Figure 8. Feed consumption of cockerels fed various dietary protein levels during the growing period (Phase 2, Exp. 2).

Table 6. Dietary amino acid content as percent of NRC chick requirement.

Protein Level	Arg	Hist	Gly	Phen	Meth & Cyst	Leu	Lys	Threo	Tryp	Valine	Iso Leu
16%	73.0	180.0	73.0	76.0	69.0	156.0	72.0	145.0	235.0	137.0	112.5
8% B*	36.0	90.0	37.0	39.0	33.0	76.0	36.0	73.3	120.0	68.0	56.3
4% B	18.0	47.0	18.0	20.0	18.0	39.0	18.0	120.0	60.0	34.0	137.3
8% U**	27.0	66.7	24.0	28.0	35.0	89.0	16.0	47.0	200.0	51.0	60.8
4% U	15.0	33.3	13.0	14.0	20.0	44.0	9.0	23.0	110.0	25.0	30.4

*B = Balanced - Diets formulated from corn and soybean as protein sources.

**U = Unbalanced - Diets formulated from corn as protein source.

pullets fed a complicated lysine deficient diet from 0-21 weeks. Harms et al. (1964) compared amino acid content of Singsen's diet with the diet used in studies at Florida Agricultural Experiment Station and concluded that lysine was the first limiting amino acid in both diets. In each report it was found that feeding these diets ad libitum resulted in reduced growth and delayed sexual maturity of pullets. Based on thses findings and comparison of amino acid content of the experimental diets in this study (Table 6), males fed the 4.0% balanced formulation should have weighed more at 21 weeks of age than males fed 8.0% unbalanced protein diet, since lysine is the first limiting amino acid in the latter formulation and methionine the first limiting amino acid in 4.0% protein balanced diet.

However, the males fed 8.0% unbalanced dietary protein weighed approximately 450 g more at 21 weeks of age than those fed 4.0% balanced dietary protein. It is suggested that under the conditions of this study, the reduced body weight was caused by a protein deficiency per se rather than attributed to the action of the first limiting amino acid. The 8.0% balanced protein diet with corn and soybean meal had methionine as the first limiting amino acid, yet the males fed this diet weighed approximately 400 g less than the males fed the control diet.

Mortality during the study period was 2, 3, 3, 0, and 5 for males fed dietary protein levels of 16.0, 8.0 and 4.0% (balanced), and 8.0 and 4.0% (unbalanced), respectively. Deaths were not greater at any particular time period of the experiment and were not attributed to treatment.

Testes weights (Figure 9) varied greatly between individ-

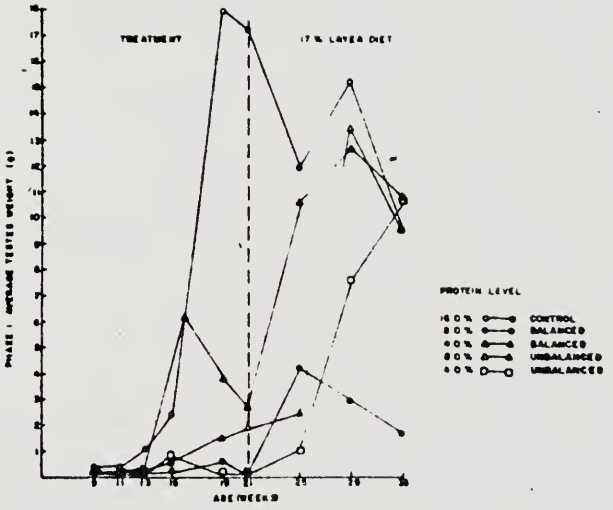

Figure 9. Average testes weights of males fed various dietary protein levels during the growing period (Phase 1, Exp. 2).

uals which is in agreement with the findings of Hogue and Schnetzler (1937). By 19 weeks of age, testes weight of the males fed 16.0% dietary protein during the growing period greatly exceeded that of all other treatment groups. After 21 weeks of age (age placed on recovery diet), all males fed the lower protein levels during the growing period gave an immediate testicular growth response. However, the group fed 4.0% balanced protein prior to 21 weeks of age did not develop testes weight comparable to the males of the other treatment groups. No explanation can be given for this observation.

Histological studies of testicular development of males sacrificed in phase 1, indicated that males fed low dietary levels of protein were in an arrested state of spermatogenesis. Males fed dietary protein levels of 16.0, and the 8.0% balanced during the growing period had testicular development in agreement with the findings of Hogue and Schnetzler (1937) as to seminiferous tubules and stages of mitosis at 13 weeks of age. Those workers found that BPR males fed a starting diet during a 20 week study varied greatly as to individual testicular development. At 14 weeks of age, the males examined had not developed past the primary spermatocyte stage, but by 16 weeks of age spermatogenesis was almost complete. At 18 weeks of age, spermatids and spermatozoa were found.

Males (phase 1) fed the 8.0% unbalanced protein diet were slightly delayed in development. Those fed both formulations of the 4.0% protein diet did not develop past the primary spermatocyte stage until after being placed on the recovery diet at 21 weeks of age.

Analysis of variance indicated that the decline in semen volume between 49 and 61 weeks of age was statistically significant ($p < .05$), but there was no interaction between time and dietary protein levels (Table 7). No significant change was found in number of sperm per ejaculate from 49 to 61 weeks of age. Males fed 4.0% balanced dietary protein level had significantly ($p < .05$) more sperm per ejaculate than males fed the other dietary protein levels.

A few individual males fed 16.0 and 8.0% protein levels produced semen at 13 and 15 weeks of age. Once males began producing semen, production was consistent for the remainder of the test. Males fed lower levels of balanced or unbalanced dietary protein (Figure 10), did not produce semen until 23 weeks of age (two weeks after being placed on recovery diet). The cyanosis condition noted in the first experiment was not a problem in this study. This was attributed to the large cages and dubbed combs which allowed normal access to feed and water.

Sperm concentration (all males included) was not affected by feeding low dietary protein during the growing period (Figure 11). At 61 weeks of age all males were producing high sperm concentrations. Sperm concentration of males fed 4.0% and 8.0% balanced protein during the growing period were still increasing at 61 weeks of age (40 weeks on the 17.0% protein layer diet), but those males fed the control diet and the unbalanced protein levels were less consistent in increased concentration.

Table 7. Dietary protein level during the growing period and subsequent semen volume, sperm concentration and total sperm per ejaculate.

Dietary Protein (%)	Semen Volume (cc)		Sperm Concentration (billion/cc)		Sperm per ejaculate (billion)		
	Week of age		Week of age		Week of age		
	49	61	49	61	49	61	Av.
16.0	.47	.42	6.13	8.41	2.88	3.53	3.20
8.0 B*	.44	.39	6.43	8.61	2.83	3.36	3.10
4.0 B	.56	.50	6.90	9.42	3.86	4.71	4.26***
8.0 U**	.49	.41	6.33	8.43	3.0	3.46	3.22
4.0 U	.48	.37	6.63	7.06	3.18	2.61	2.87

*B = Balanced - Diets formulated from corn and soybean as protein sources.

**U = Unbalanced - Diets formulated from corn as protein source.

***Significantly different ($p<.05$).

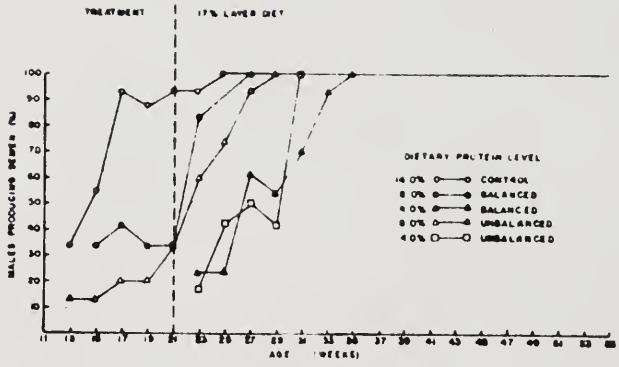

Figure 10. Sexual maturity determined by semen production of cockerels fed low levels of dietary protein during the growing period (Exp. 2).

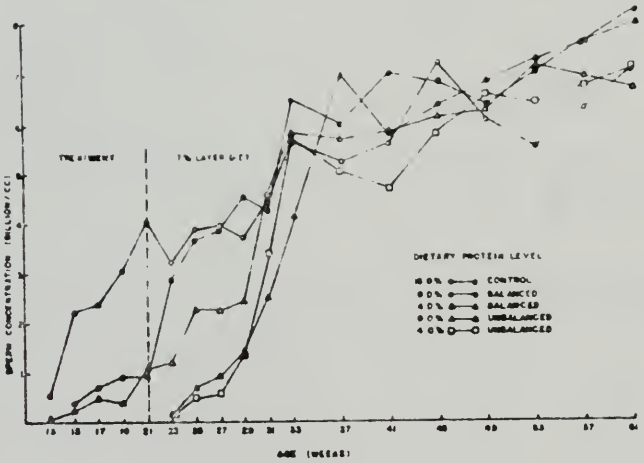

Figure 11. Sperm concentration of cockerels fed various dietary protein levels during the growing period (Exp. 2).

SUMMARY

The body weight of cockerels fed the lower protein levels never attained those weights of the cockerels fed the 16.0% protein grower diet. Sexual maturity was delayed an average of eight weeks by feeding 9.0% or less protein. Testes weight and histological studies revealed arrested testicular development in those males fed low dietary protein levels.

Semen volume, fertility, and peak sperm concentration were increased rapidly when all males were placed on a 17.0% protein diet at 21 weeks of age. Males fed low dietary protein attained a higher sperm concentration peak which was maintained for a longer period of time than controls. Males fed 4.0% balanced protein had significantly higher numbers of sperm per ejaculate ($p < .05$) at 61 weeks than males fed the other dietary protein levels.

CHAPTER II

COMPETITIVE FERTILITY

EXPERIMENT I

Competitive fertility was intended to be used as a measure of the effect of dietary protein on subsequent sperm viability and fertilizing capacity. In the initial study, a low ratio of rose comb to single comb chicks was obtained and it was of interest to investigate the cause and possible remedial procedures.

Procedure

To identify the sire of the offspring, the homozygous rose comb gene (RR) was the means of choice. Rose Comb Brown Leghorn (RCBL) chicks were hatched (Feb.) from eggs obtained from a commercial hatchery. At hatch, it was noted that the chicks were not homozygous for the rose comb gene. The chicks with single combs were discarded at one day of age and those with the phenotypic rose comb character were banded for identification and vaccinated against Newcastle-Bronchitis. They were fed the standard experiment station diets ad libitum throughout the entire rearing period. Upon reaching sexual maturity, males were progeny tested using SCWL hens housed in individual layer cages, to determine homozygosity or heterozygosity in reference to their comb type. RR and Rr individuals show the rose comb phenotypic character, therefore, when RR males were bred to rr females all chicks exhibited the typical smooth, flat

comb which indicated an RR sire. When an Rr male and rr female are crossed the offspring will theoritically be 50% rose comb (Rr) and 50% single comb (rr), which can be distinguished at hatching.

If one chick from a male exhibited the single comb character, the male was considered heterozygous and eliminated from the study. Some males sired as many as 60 chicks with the average about 30 chicks for identification. This was considered sufficient since Allen and Champion (1955) accepted eight chicks as valid proof of homozygosity.

A sufficient number of RR males were identified to supply adequate semen for competitive fertility studies. When the males were 37 weeks of age, the first sperm competition study was initiated.

Semen from RCBL males was collected as a pooled mixture from all males. Semen from all SCWL males under the dietary protein treatment was collected into individually identified tubes. A .2 cc sample of semen from a SCWL male and .2 cc of pooled semen from the RCBL males was mixed and three caged SCWL hens were each inseminated with .05 cc of the mixed semen. The eggs were collected for ten days, incubated, and chicks identified as to sire at hatch.

Results and Discussion

Thirty-eight rose comb chicks were recorded from a total hatch of 960 chicks. Males fed dietary protein levels of 16.0, 8.0, and 4.0% (balanced), and 8.0 and 4.0% (unbalanced) produced 18, 6, 8, 6, and 0 rose comb chicks, respectively. There was no significant difference in rose comb chicks produced between treatment groups when based on percentage of total chicks hatched. One hen accounted for eight of the rose comb chicks from the males fed 16.0% protein.

However, the small percentage of rose comb offspring was not sufficient for a valid comparison.

Sperm concentrations determined prior to initiation of the competitive fertility studies indicated that the average concentration of the SCWL males was 1.4 billion per cc of semen greater than that of the RCBL males (5.88 vs. 4.48 billion per cc). Fertility for both groups of males was consistantly better than 85.0% which was considered normal with the volume and frequency of insemination (Burrows and Quinn, 1938) when unmixed semen was used. Based on fertility and sperm concentration of the RCBL males, the proportion of rose comb to single comb should have been higher with the volume to volume semen mixture.

To determine if number of sperm from each male contributed to this low ratio, the SCWL males were ranked according to sperm concentration with those below 5.5 billion per cc of semen considered low and those ranked above as having high concentrations. Analysis of variance of ranked sperm concentration and percent of single comb chicks indicated that sperm concentration influenced the small number of rose comb chicks and was significant ($p < .05$). A positive significant correlation of .528 ($p < .01$) verified this analysis. These findings do not agree with those of Sampson and Warren (1939), Parker et al. (1942), and Hutt (1929) who found no correlation between sperm concentration and fertility when concentration was 825,000 to 7,000,000 per mm^3 of semen. However, these findings agreed with the proposal by Allen and Champion (1955) that equal sperm numbers were necessary for unbiased competition between competing males.

EXPERIMENT II

Based on Experiment I, the second experiment was designed to determine the effect of number of sperm inseminated on competitive fertility of RCBL and SCWL males.

Procedure

Sperm concentration was determined on both individual RCBL and individual SCWL males. Sperm numbers were equalized between the two breeds of males by taking proportioned amounts of semen from each male as indicated by sperm concentration. Three SCWL caged hens were inseminated with .05 cc of this mixed semen.

Equal volumes of pooled SCWL semen (6.8 billion sperm/cc) and semen from individual RCBL males (range of 1.45 to 8.73 billion sperm/cc) were mixed and three caged SCWL hens per combination were artificially inseminated. Eggs were collected for two weeks and the eggs from each week were incubated separately.

Results and Discussion

One RCBL chick was hatched from a total hatch of 786 chicks. This indicated that sperm numbers had no effect on competitive fertility within the limits of this experiment.

These findings raised several questions. A possible cause of the low number of RCBL offspring could have been low viability of the RCBL sperm. This undoubtedly was not the problem since RCBL had good fertility when mated to SCWL hens. The situation seemed to indicate incompatability between the semen of the

breeds even though Parker et al. (1942) found no effect on fertility from pooling semen from males of the same breed. However, it has been proposed by Harris (1966), that pooling semen does reduce fertility in some cases.

EXPERIMENT III

To study the hypothesis of seminal plasma incompatability, the following study was designed to determine whether the factor involved was due to the seminal plasma or sperm.

Procedure

Four RCBL and four SCWL males were selected based on similar sperm concentrations. An isotonic phosphate buffer solution (Wilcox and Shaffner, 1957; and Schindler and Hurwitz, 1966) was used to wash the sperm isolated from the seminal plasma by centrifugation. RCBL and SCWL males were paired and the following treatments were applied to their semen:

1. Washed RCBL sperm pooled with washed SCWL sperm in the buffer solution.
2. Washed RCBL sperm pooled with washed SCWL sperm in SCWL seminal plasma.
3. Washed RCBL sperm pooled with washed SCWL sperm in RCBL seminal plasma.

All three treatments were applied to the semen of each pair of males and three different SCWL caged hens were inseminated from each combination. Eggs were saved for ten days and incubated.

Motility scores were determined on semen from each individual male and mixed semen from the paired males as described by Allen and Champion (1955). Determinations were made ten minutes, 30 minutes and one hour after semen collection.

Results and Discussion

A total of 158 chicks were hatched with only four rose comb chicks, the remainder were single comb indicating dominance by SCWL sperm. The four rose comb chicks occurred 1, 2, 1 for the three treatments, respectively. The findings agree with those of Crawford and Smyth (1964c) in that low fertility of RR males was not attributed to seminal plasma deficiencies.

Mixing semen from RCBL males and SCWL males had no detrimental effect on semen motility scores ten minutes, 30 minutes, or one hour after semen collection.

EXPERIMENT IV

Since our results had indicated that the low ratio of rose comb chicks was caused partly by sperm concentration, but nature of the seminal fluid had no effect, procedures for obtaining 50.0% rose comb chicks from competitive matings were investigated.

Since data from early literature suggested an affinity of ova for sperm from males of close relation as compared to fertilizing capacity of sperm from more distant relatives (Dunn, 1927), the next study utilized RIR hens inseminated with pooled semen from both breeds. Warren and Kilpatrick (1929) reported that if a male is replaced by another male in a mating, the second male takes over fertility of the ova with no sire over-lapping. However, they pointed out that spermatozoa lose their flagella the first day in the oviduct. Since they stated that flagella aided motility, it was proposed that the second male's sperm lost its advantage after one day. Munro (1937) and Shaffner et al. (1941) found that motility was not reliable as an index of viability, but Crawford and Smyth (1964c) found a correlation of 0.76 between motility and fertility. Nicolaides (1934) stated that the average duration of fertility from a single mating was 14.83 days with a maximum of 29 days in his studies. Nalbandov and Card (1943) reported that senescent sperm retained their ability to fertilize eggs, but embryonic mortality of ova fertilized from sperm over ten days in the oviduct died after one to five days of development.

Procedure

SCWL caged hens were inseminated with semen from SCWL males and followed at different time periods by insemination with RCBL semen. Mixed SCWL and RCBL semen was inseminated into unrelated females. The outline of the experiment was as follows:

1. RIR females inseminated with pooled SCWL semen combined with pooled RCBL semen.
2. SCWL females inseminated with pooled RCBL semen.
3. SCWL females inseminated with pooled SCWL semen -- after 20 minutes inseminated with pooled RCBL semen.
4. SCWL females inseminated with pooled SCWL semen -- after 48 hours inseminated with pooled RCBL semen.
5. SCWL females inseminated with pooled SCWL semen -- after seven days inseminated with pooled RCBL semen.

Ten hens were inseminated with .05 cc of semen from each breed in each treatment. Eggs were dated and collected daily for 14 days and incubated so as to identify the egg laid each day to determine effect of time in the oviduct on fertilizing capacity between the two breeds.

Results and Discussion

Matings with pooled semen from RCBL and SCWL males artificially inseminated into RIR females resulted in one rose comb chick from an egg laid four days after insemination. A total of 53 single comb chicks were hatched. These data indicated that ova preference of sperm from closely related males was not a major factor causing this low number of rose comb chicks being produced.

Three SCWL caged hens inseminated with pooled RCBL semen

resulted in an 87.0% fertility indicating fertilizing capacity of the sperm was good.

SCWL caged hens inseminated with pooled RCBL semen 20 minutes after being inseminated with SCWL semen produced two rose comb chicks. One hatched from an egg laid on the twelfth day and the other on the thirteenth. Therefore, no change in the ratio of rose comb to single comb offspring resulted from a 20 minute delay.

SCWL caged hens inseminated with SCWL semen and then 48 hours later inseminated with RCBL semen resulted in 70.5% single comb and 29.5% rose comb chicks. Three days after inseminating with RCBL semen, the fertile eggs laid produced 77.8% rose comb chicks. This does not quite agree with the findings of Warren and Kilpatrick (1929) who stated that the sperm from the second insemination lost its advantage after one day. However, the next day only 12.5% of the chicks were rose comb. The last six days varied greatly in the percentage of rose comb chicks. Based on these variable results, no conclusions could be made on the effect of the 48 hour delay on the rose comb to single comb chick ratio.

The last phase in which SCWL caged hens were inseminated with SCWL semen and then inseminated seven days later with semen from RCBL males resulted in 50.0% rose comb chicks the fifth, sixth and seventh day after the RCBL insemination. The third day after this insemination 75.0% of the hatch was rose comb chicks with a decline in rose comb chicks until the eighth day after the RCBL insemination when again 75.0% of the hatch had rose combs. These findings do not agree with those of Ruus (1956) who mated SCWL hens with SCWL males followed in four days by matings with RIR males.

The second and third chicks hatched from eggs laid on the day after the last mating were all purebred leghorns, but thereafter, there was a steady progressive increase in crossbreds. This phase of the experiment established a means of obtaining 50% rose comb chicks, but the use of this procedure as a means of measuring competitive fertility is questionable.

The poor fertility from the RCBL males when competing with SCWL males is consistent with data in the literature. A definite cause of this incompatability has not been established, but the use of the RR factor in competitive fertility is a valuable tool which warrants further investigation.

SUMMARY

A very high percentage of single comb offspring were observed when Single Comb White Leghorn (SCWL) hens were inseminated with mixed semen from Rose Comb Brown Leghorn (RCBL) and SCWL males.

Four experiments were conducted to study the cause and possible remedial action to overcome this low ratio of rose comb to single comb offspring.

Semen mixed in equal volumes and/or equal concentrations did not alter the previously observed ratios of offspring. Washing sperm with a buffer prior to mixing, and washing and suspending in reciprocal seminal plasma also gave the same ratio of single comb chicks.

Insemination of Rhode Island Red females with equal volumes of mixed semen resulted in the same low ratio of rose comb chicks. Insemination of SCWL females with RCBL semen 20 minutes, two days, and seven days after insemination with SCWL semen was studied. Insemination with RCBL semen seven days after insemination with SCWL semen resulted in 50.0% of each comb type on the fifth, sixth, and seventh day after insemination with RCBL semen.

CHAPTER III

SUMMARY

Two experiments were conducted to study the effects of various dietary protein levels (4.0 to 16.0%) during the growing period on the subsequent reproductive performance of Single Comb White Leghorn (SCWL) cockerels. Treatment effects were measured by growth rate, sperm concentration, semen volume, persistence, fertility, and sexual maturity including testicular histological examination.

Low levels of dietary protein reduced final body weight compared to males fed 16.0% dietary protein. Sexual maturity was delayed in males fed 9.0% protein or less and revealed an arrested testicular development by histological study.

Semen volume, sperm concentration and fertility increased when all males were placed on a 17.0% protein layer diet at 21 weeks of age. Cockerels fed low dietary protein developed higher sperm concentrations which were maintained over a longer period of time than males fed 16.0% protein during the growing period. Males fed 4.0% balanced protein during the growing period had significantly greater sperm numbers per ejaculate ($p < .05$) than those fed other dietary protein levels.

Further measurement of fertilizing capacity was attempted by allowing the SCWL cockerel sperm to compete in the female oviduct with Rose Comb Brown Leghorn (RCBL) sperm for ova fertilization.

Offspring were identified by the distinguishing characteristics of the comb at hatch.

A low ratio of rose comb to single comb offspring were observed when SCWL hens were inseminated with mixed semen from RCBL and SCWL males on an equal volume basis.

Insemination with equal sperm numbers did not alter the previously observed ratios of offspring. Washing sperm after centrifugation with a phosphate buffer prior to resuspending in reciprocal seminal plasma or buffer solution gave the same low ratio of rose comb chicks. Insemination of Rhode Island Red hens with equal volumes of mixed semen gave primarily single comb offspring. Insemination of SCWL females with RCBL semen 20 minutes, two days, and seven days after insemination with SCWL semen was studied. Insemination with RCBL semen 20 minutes after insemination with SCWL semen resulted in no increase in numbers of rose comb offspring, but RCBL insemination two days after SCWL insemination resulted in an increased ratio of rose comb to single comb chicks. Insemination with RCBL semen seven days after insemination with SCWL semen resulted in 50.0% or more rose comb chicks from the third through the eighth day after insemination with RCBL semen.

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BIOGRAPHICAL SKETCH

Jack Edenfield Jones was born October 24, 1929, in Jacksonville, Florida. In February, 1948, he was graduated from Andrew Jackson High School, Jacksonville, Florida. In June, 1951, he received the degree of Bachelor of Science in Agriculture from the University of Florida. From 1951 to 1955 Mr. Jones served in the United States Air Force in the Air Defense Command. Following his discharge he worked as a County Supervisor for the Farmers Home Administration for one and one-half years and then worked as Sanitarian with the Florida State Board of Health for five years in St. Johns County. He entered the University of Florida in September, 1962, and received the Master of Science in Agriculture degree in December, 1964. Mr. Jones has been working toward the Doctor of Philosophy degree from 1964 to the present time.

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This dissertation was prepared under the direction of the chairman of the candidate's supervisory committee and has been approved by all members of that committee. It was submitted to the Dean of the College of Agriculture and to the Graduate Council, and was approved as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

December 17, 1966

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