THE INFLUENCE OF VARYING DEGREES OF HYPERTHYROIDISM ON SEMEN PRODUCTION IN THE DOMESTIC FOWL

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THESIS

This is to certify that the

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THE INFLUENCE OF VARYING DEGREES OF HYPERTHYROIDISM ON SEMEN PRODUCTION IN THE DOMESTIC FOWL

By

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INTRODUCTION

It is well-established that the thyroid gland has an important function in life processes. Thyroid removal will prevent maturation changes such as the metamorphosis of tadpoles as well as the growth and sexual development of higher animals. It will also depress many productive processes such as egg production in chickens and milk production in cows.

In adult animals thyroid deficiency often results in decreased fertility. In recent years a great deal of work has been done in animals that were rendered hypothyroid either by means of thyroidectomy or administration of the goitrogenic drugs. Most authors are in agreement that the decrease in metabolism observed in the hypothyroid state is accompanied by a depression of growth, milk production, sexual activity and many of the general body processes. These functions can be improved, on the other hand, by the administration of thyroid substance.

Modern investigations of thyroid function had their inception with the observations of Gull (1874) and Reverdin (1882) in women with spontaneous atrophy of this gland. The discovery of iodine in the thyroid by Bauman (1895) lead to the use of this element in the correction of certain types of thyroid abnormalities. Since that time a very extensive literature has been developed on both the clinical aspects and the fundamental nature of the

physiology of the thyroid gland.

A new impetus to thyroid investigations was produced by the discovery of the goitrogenic effects of sulfaguanadine (MacKenzie, MacKenzie and McCollum, 1941), and the series including thiourea and thiouracil (MacKenzie and MacKenzie, 1943; Aswood et al, 1943). With the development of thyroidally active iodinated casein by Reineke and Turner (1943), it became possible to vary the level of thyroid function of domestic animals at will, simply by the suitable administration of a goitrogen to produce the hypothyroid state or thyroprotein to create the desired level of hyperthyroidism. So far as the domestic animals are concerned the principal emphasis thus far has been placed on the study of such functions as milk production, egg production and growth. Little attention has been given to the possible relationships of the thyroid hormone and the reproductive functions until quite recently. The investigations to be reported represent a study of the effect of different degrees of hyperthyroidism on semen production in the domestic fowl. This work is a continuation of the investigations started by Martinez (1947).

Relationship of the Thyroid to Fertility in Domestic Animals

Many clinical reports are available that indicate that hypothyroidism in man is accompanied by reproductive disorders. In the male, deficient spermatogenesis and a decline in libido are often observed. In the female,

irregular menstrual cycles and lowered fertility accompany this condition. Many cases have been reported in which such conditions are corrected by thyroid therapy. It is only during the last few years that any attention has been given to the role of the thyroid in the reproductive processes of domestic animals. This work has been confined principally to cattle, goats and birds.

Relationship of the Thyroid to Reproduction in Cattle:

Petersen, Spielman, Pomeroy and Boyd reported that when a male Jersey was thyroidectomized at four months of age a complete suppression of libido occurred. However, semen that was obtained by rectal manipulation of the ampulla was used successfully for the insemination of cows. Oral administration of desiccated thyroid restored normal activity and sexual behavior. With the development of thyroprotein, Reineke and Turner (1943) and Turner (1943) suggested the possible use of this product to correct breeding deficiencies in bulls that become deficient in breeding performance due to subnormal thyroid activity. Reineke (1946) reported the effects of feeding thyroprotein to 14 bulls which had unsatisfactory breeding records. Definite improvement in vigor and libido was observed in 10, the time required for an observable effect to occur ranging from 7 to 40 days. Definite evidence of improvement in the conception records was observed in four cases.

Results of thyroidectomy on reproduction in the fe-

male bovine were reported by Spielman et al (1945). Four thyroidectomized cows failed to manifest the normal physical signs of estrous. By rectal palpation it was established, however, that normal ovulatory cycles were occurring. Artificial insemination of the cows with semen from a thyroidectomized bull resulted in conceptions in all of the animals. It would appear therefore that the most significant effect of hypothyroidism on the reproductive functions is a pronounced decrease in sex interest in both the male and female. However, even though the thyroidectomized bull employed in these cases produced viable semen the data presented were too limited to judge the intensity of spermatogenesis.

Relationship of the Thyroid to Reproduction in Sheep and Goats: It is well known that sheep and goats are seasonal breeders, exhibiting little or no sexual activity during the summertime and resuming their breeding cycles in the Fall. Berliner and Warburton (1937) reported that Shropshire rams produced semen of poor quality during the hot months of summer but only the poorer Hampshires declined noticeably during this period. Hampshire rams that were thyroidectomized in the Fall showed a deficiency of sperm production similar to that observed in intact Shropshires during the Summer. Sperm production was improved in both conditions by the injection of thyroxine. Turner, Mixner and Reineke (1943) reported definite improvement in the semen production of a ram which had good sex drive but

was deficient in spermatogenesis. A male Toggenburg goat that showed a definite lack of sex interest was fed thyroprotein at the rate of one gram daily. This animal showed a considerable increase in sexual vigor and settled five out of six females that were bred to him.

A quite extensive study on the relationship of seasonal temperature changes and the thyroid to spermatogenesis in rams was reported by Bogart and Mayer (1946 a, b). The administration of thyroxine or thyroprotein largely prevented the usual Summer decline in spermatogenesis of rams. Changes in semen quality similar to those resulting from high environmental temperatures were induced during the breeding season by the administration of thiouracil. These changes were also counteracted by the administration of thyroprotein.

Relationship of the Thyroid to Reproduction in Fowls:

In recent years a considerable amount of work has been done on the possible relationship of the thyroid to reproduction in both hens and cocks. However, there have been large differences in the conditions of management and the dosage of thyroidal substance used in the various laboratories. Inasmuch as the level of dosage of this type of substance is quite critical, the variance in results from several of the laboratories may be explained upon this basis.

Hens: In a very famous investigation Crew (1925)

reported the rejuvenation of seven hens, 5 to 8 years of age when they were fed desiccated thyroid daily for six months. The principal changes were the development of new plumage characteristic of younger fowls, improvement of the head furnishings and an increase in egg production. Some improvement in the head furnishings and the egg production of hens was reported by Greenwood and Blyth (1942) following treatment with thyroid substance. With the development of synthetic thyroprotein, Turner, Irwin and Reineke (1945), and Turner, Kempster, Hall and Reineke (1945) reported an increase in the egg production of White Leghorn hens when thyroprotein was fed continuously at the level of 10 gm. per 100 lbs of feed. The greatest effect observed was a tendency toward maintenance of the intensity of egg production during the hot months of summer.

A similar maintenance of egg production in these same birds when continued on thyroprotein feeding in their fourth and fifth laying years has been reported by Turner, Kempster and Hall (1946) and Kempster and Turner (1947).

Cole and Hutt (1927) reported that desiccated thyroid when fed at the rate of 59 mgs. per pound of live weight for a period of six weeks had no effect on egg production. The difference between this and results cited earlier can probably be explained by differences

in dosage.

That the thyroid secretion is involved in egg production in the hen is also indicated by the reports of Winchester (1940) and Blivaiss and Domm (1942). In the first report egg production was reduced markedly by thyroidectomy and in the second the thyroidectomized hens were not known to lay any eggs up to 8 months of age. Egg production was restored (Winchester, 1940) following the injection of thyroxine.

Effect of Thyroid Therapy on Testes and Sperm Production: Crew (1925) reported the rejuvenation of five cocks, 5 to 8 years of age that were fed desiccated thyroid. This report is based principally on the physical appearance of the birds, however.

Jaap (1933) observed that when .25 to 1.0 gram of desiccated thyroid was fed daily to Mallard drakes during late Winter and early Spring both the size of the testes and spermatogenesis increased much more than in normal drakes subjected to increased light exposure alone. Aron and Benoit (1934) also observed sexual stimulation of immature male ducks when they were fed thyroid tissue or were injected with thyroxine.

When fed a large dose of desiccated thyroid (100 mg. daily) white Leghorn cockerels showed an increase in the rate of seasonal decline in amount of semen produced (Titus and Burrows, 1940). On the other hand, Hayes

(1948) observed that during the period from January 12 to March 21 the seasonal decline in fertility of cocks could be prevented either by the administration of small doses of thyroxine or the use of supplementary lighting.

Martinez (1948) observed a definite increase in semen volume, sperm concentration and total number of sperm produced by male fowls that were fed thyroprotein at the rate of 18 gms. per 100 pounds of feed. Lower dosage levels were ineffective.

Effect of Hypothyroidism on Testes and Sperm Production: Following thyroidectomy of male chickens and ducks, Benoit and Aron (1934) reported delayed testicular growth in young birds of both species. In older birds thyroidectomy resulted in a pronounced decrease in size of the testes. Thyroidectomy also reduced considerably the testicular growth in immature ducks exposed to the stimulating action of supplemental lighting (Benoit, 1936). Spermatogenesis was affected similarly (Benoit, 1937a), and development of the penis of thyroidectomized ducks was inferior (Benoit, 1937b, 1937c). Greenwood and Chu (1939) and Blivaiss and Domm (1942) reported that when Brown Leghorn cockerels were thyroidectomized there was a pronounced reduction in the weight and histological development of the testes as well as deficient sperm production.

Shaffner and Andrews (1948) fed rations containing 2/10 and 5/10 per cent thiouracil in order to sup-

press thyroid function. Neither sperm concentration, total number of spermatozoa or methylene blue reduction time was affected by the feeding of thiouracil. Initial motility was lowered considerably at the 5/10 per cent level. Sperm survival time at 4^{o} C. was decreased by both dosages. Actual tests of fertility were made by inseminating hens with semen from the control and experimental birds. Both levels of thiouracil caused a significant reduction in fertility of the males.

With the foregoing work as a background it seemed of considerable interest to carry on further investigations on the possibility of influencing the intensity of spermatogenesis in adult male chickens by properly regulated dosages of thyroprotein. With this in mind, an attempt has been made to establish the effects of a relatively low dosage level that would be expected to stimulate spermatogenesis to some extent, and also to determine whether excessive levels given under the same conditions would cause inhibitory effects.

EXPERIMENTAL PROCEDURE

Birds and Material

Fifteen 8-month-old Rhode Island Red roosters obtained from the Poultry Department of Michigan State College, were selected for the experiment from a group of about 28, after a preliminary training period of 3 months. They were placed in individual cages on October 18, 1947 and fed a basal diet which was compounded as shown in the accompanying table:

690 lbs.	Corn Meal
400 lbs.	Ground Oats
300 lbs.	Bran
200 lbs.	Middlings
60 lbs.	17% dehydrated alfalfa meal
60 lbs.	Meat scraps
40 lbs.	Dry milk
50 lbs.	Fish meal
50 lbs.	Soybean meal
100 lbs.	Oyster shell flour
30 lbs.	Steamed bone meal
12 lbs.	Salt
8 lbs.	Fish liver oil (400 A., 2000 D.)
2000 lbs.	

During the preliminary period semen was collected from the roosters three times a week, by the method of Burrows and Quinn (1937). After this preliminary period that extended for three months, in order to allow the roosters to mature and give uniform samples, the roosters were distributed into three groups of 5 birds each on the basis of the average semen production.

One other category of birds used consisted of 8 old cocks, about 21 months old, selected from a group

of 12, on the same basis as for the young birds; these old roosters formed two groups of four birds each, and they received the ration described above.

The room temperature was maintained at about 65°F. during the period of the experiment, that is, from October till March, and the birds received artificial light 24 hours a day throughout the experimental period.

Our experiment started on January 9 and lasted until March 18 with the young birds; with the older ones, we finished on February 27.

From January 28 each group received a different treatment. The drug we used, Thyroprotein, a highly active iodinated protein (Protamone), was supplied by Cerophyl Laboratories, Inc., Kansas City, Missouri. This drug was given to the birds together with the feed, being carefully mixed in it, having in account the small amounts used and required to stimulate feather growth and egg production as reported by Turner, Irwin and Reineke (1943) and to improve semen production as reported by Martinez (1947).

Thyroprotein was added to the ration of the experimental birds as indicated below:

Young roosters:

Group A - Normal control: this group received only the basal mash, throughout the period of experiment;

Group B - Received thyroprotein at the level of 18 grams

per 100 pounds of feed, that is, 0.04% throughout the period;

Group C - Received thyroprotein at the rate of 36 grams per 100 pounds of mash, or 0.08%. This group in the two last weeks of the experiment received four times the level of group B, or 72 grams per 100 pounds of feed.

Old roosters

Group D - Normal control: treated in the same way as the normal young roosters;

Group E - Received thyroprotein at the level of 0.04%, throughout the period of experiment.

All birds received the mash "ad libitum"; the food was supplied twice a day and they had running tap water at all times.

General plan

Semen was collected from the birds three times a week, with the purpose of determining the possible variations in semen volume and sperm concentration under the influence of thyroprotein.

To collect the samples we used the method of Burrows and Quinn (1937). After the birds were well trained they produced quite constant amounts of semen. The samples were collected at intervals of 48 hours as recommended by Parker, McKenzie and Kempster (1942).

a. Semen volume

The volume was determined in a 1 ml. tuberculin

syringe and the measurements were made as accurately as possible, giving us readings in hundredths ml. We collected samples every week, throughout the period of experiment, two or three times a week. An interval of forty-eight hours was observed between the collections.

b. Sperm concentration

To determine the sperm concentration we employed the hematocytometer used in counting red blood cells. To kill the sperm in order to avoid troubles in counting, we added a few drops of alcohol to the Tyrode's solution used to dilute the semen; we have used too, with advantage, the following solution for dilution:

Methylene blue	- 30 ml.
KOH 2%	- 60 ml.
Acetic acid	- .5 ml.

The dilution of the semen was 1:200, that is the undiluted semen was taken up to the 0.5 mark in the mixing pipette for red blood cell counts and the dilute solution was taken up to the 101 mark.

From each sample we did two and sometimes three counts and the averages obtained were taken as our values for sperm concentration per cubic millimeter.

c. Total number of sperm

The total number of sperm in the ejaculate is based on the concentration per cubic millimeter and the volume; knowing these data we just multiply concentration by volume in order to get the total number of sperm.

EXPERIMENTAL RESULTS

We collected control semen samples for this experiment from January 9 to January 28, 1948, from all groups of birds.

On January 28 we started giving to the young roosters thyroprotein as follows: to group B we gave 0.04% in the feed, throughout the period of experiment, that is, until March 18: to group C we gave 0.08% in the feed until March 1st, when we started giving twice this dose (0.16% in the feed), for the rest of the period.

In the old roosters we had just two groups: group D that was the normal control and group E, receiving thyroprotein at the 0.04% level. The period of experiment for these two groups (D and E) started on January 28 and lasted until February 27.

All the birds were in good health throughout the period of experiment and no variation in semen production due to sickness or any other factor was observed.

Our results will refer to the groups of young and old roosters separately.

Semen Volume

Young roosters - In this category we had three different groups, each receiving a different treatment during the period of experiment.

Group A - This group was the normal control in this category and did not receive any thyroprotein throughout the

period of experiment.

The averages obtained during this period were between 0.40 and 0.68 ml. This group was quite constant in giving samples. The highest volume observed was 0.85 and the lowest was 0.22 ml. The greater part of the data fell between 0.50 and 0.70 ml. The results obtained from this group are shown in table I, and a comparison with the other groups will be given in table IV.

TABLE I - The Semen Volume of Control Roosters.

SEMEN VOLUME in ml.

Date	Birds					
	1	2	3	4	5	Average
Jan. 9	0.50	0.80	0.75	0.50	0.83	0.68
14	0.45	0.64	0.58	0.20	0.61	0.50
16	0.37	0.39	0.80	0.55	0.44	0.51
19	0.57	0.56	0.70	0.71	0.85	0.68
23	0.52	0.66	0.46	0.38	0.60	0.52
26	0.41	0.66	0.70	0.65	0.43	0.57
28	0.60	0.67	0.63	0.74	0.84	0.69
30	0.42	0.67	0.63	0.66	0.48	0.57
Feb. 2	0.57	0.49	0.71	0.55	0.58	0.58
4	0.42	0.77	0.66	0.67	0.63	0.63
9	0.30	0.70	0.63	0.59	0.45	0.54
11	0.23	0.61	0.22	0.32	0.58	0.39
13	0.39	0.55	0.41	0.57	0.66	0.52
16	0.38	0.60	0.45	0.55	0.60	0.52
18	0.40	0.42	0.50	0.55	0.66	0.43
20	0.26	0.60	0.35	0.38	0.39	0.40
25	0.27	0.60	0.40	0.50	0.40	0.43
27	0.30	0.50	0.58	0.51	0.45	0.47
Mar. 1	0.40	0.60	0.40	0.50	0.50	0.48
3	0.38	0.50	0.45	0.50	0.60	0.48
5	0.49	0.55	0.47	0.31	0.49	0.46
8	0.55	0.55	0.55	0.45	0.55	0.53
10	0.25	0.75	0.45	0.50	0.50	0.49
18	0.46	0.74	0.43	0.45	0.47	0.51

Group B - This group received the lowest dose of thyroprotein we have used in this experiment - 0.04% of thyroprotein in the feed. Feeding was begun on January 28 and continued for 50 days until March 18. The volume from this group was not increased when compared with the preliminary period (between January 9 to January 27), but the averages during the period of experiment were more constant and the semen voluem level was maintained just about the same throughout the period and at a more constant and higher level on the average than in Group A. The highest value was 0.95 ml. and the lowest was 0.38 ml.; most of the samples were between 0.50 and 0.70 ml. The data obtained from this group are shown in table II and a comparison will be made with the other two groups in table IV.

TABLE II - The effect of thyroprotein on semen production of young roosters when given as 0.04% of the ration

Date	SEMEN VOLUME in ml.					
	Birds					
	9	10	11	12	13	Average
Jan. 9	0.95	0.67	0.43	0.45	0.65	0.63
14	0.76	0.70	0.62	0.55	0.67	0.66
16	0.57	0.48	0.48	0.45	0.40	0.48
19	0.75	0.60	0.70	0.67	0.46	0.64
23	0.65	0.50	0.45	0.61	0.57	0.55
26	0.38	0.61	0.40	0.95	0.47	0.56
28*	0.65	0.42	0.46	0.61	0.65	0.56
30	0.56	0.57	0.62	0.69	0.75	0.64
Feb. 2	0.55	0.57	0.50	0.60	0.85	0.61
4	0.67	0.66	0.50	0.77	0.58	0.64
9	0.59	0.75	0.40	0.64	0.38	0.55
11	0.65	0.60	0.41	0.46	0.52	0.53
13	0.46	0.44	0.40	0.51	0.44	0.45
16	0.70	0.70	0.47	0.60	0.60	0.61
18	0.68	0.70	0.48	0.65	0.70	0.64
20	0.65	0.68	0.55	0.60	0.65	0.62
25	0.70	0.65	0.50	0.65	0.70	0.64
27	0.72	0.56	0.60	0.45	0.78	0.62
Mar. 1	0.60	0.55	0.61	0.50	0.75	0.60
3	0.70	0.67	0.45	0.57	0.60	0.60
5	0.41	0.59	0.38	0.30	0.75	0.49
8	0.60	0.85	0.53	0.40	0.65	0.61
10	0.60	0.70	0.58	0.30	0.65	0.56
18	0.40	0.85	0.45	0.25	0.80	0.55

*Started receiving 0.04% of thyroprotein.

Group C - This group received two different doses of thyroprotein throughout the period of experiment; from January 28 until February 29 it received 0.08% of thyroprotein in the feed; from March 1 until the end of the experimental perion (March 18) it received twice this dose, that is, 0.16% of thyroprotein.

The semen volume in this group was not so constant

as in the two others. By the end of the experiment the average volume had gone down about 50%.

The highest volume checked was 1.37 ml. and the lowest volume was 0.18 ml. The data in the first half of the experiment were about the same as the other two groups; between 0.40 and 0.60 ml. Later the volumes declined rather markedly. In table III are shown the volumes obtained from group C and a comparison with the two other groups will be given in table IV.

TABLE III - The effect of thyroprotein on semen production of young roosters when given as 0.08 and 0.16 per cent of the ration.

SEMEN VOLUME in ml.

Date	Birds					
	20	21	22	23	24	Average
Jan. 9	0.60	0.65	0.95	0.35	0.66	**0.64**
14	0.55	0.60	0.57	0.40	0.95	0.61
16	0.30	0.45	0.56	0.39	0.77	0.59
19	0.41	0.48	0.79	0.48	0.91	0.61
23	0.40	0.24	0.46	0.39	0.60	0.42
26	0.42	0.40	0.44	0.70	1.37	0.67
28*	0.43	0.27	0.42	0.39	1.07	0.52
30	0.62	0.61	0.62	0.65	0.84	0.67
Feb. 2	0.57	0.38	0.38	0.38	0.55	0.54
4	0.57	0.45	0.43	0.47	0.65	0.51
9	0.60	0.35	0.43	0.47	0.65	0.51
11	0.62	0.40	0.32	0.44	0.70	0.50
13	0.35	0.31	0.30	0.29	0.45	0.34
16	0.68	0.25	0.45	0.40	0.65	0.48
18	0.45	0.50	0.24	0.45	0.60	0.45
20	0.52	0.32	0.22	0.20	0.64	0.38
25	0.50	0.40	0.20	0.25	0.50	0.37
27	0.53	0.40	0.36	0.38	0.30	0.39
Mar. 1**	0.52	0.35	0.30	0.45	0.30	0.39
3	0.50	0.40	0.40	0.38	0.36	0.41
5	0.45	0.35	0.45	0.38	0.32	0.39
8	0.50	0.40	0.40	0.30	0.20	0.36
10	0.59	0.47	0.25	0.36	0.18	0.37
18	0.45	0.35	0.40	0.40	0.18	0.36

*Started receiving 0.08% of thyroprotein.
** Started receiving 0.16% of thyroprotein.

Table IV - Summarizes the effect of different levels of thyroprotein on semen volume of the three groups of young birds. These data are the average obtained from those groups shown in the three first tables.

The semen volume of the first three groups is com-

pared in figure I. Group B, receiving thyroprotein as 0.04 per cent of the feed, showed more constant and higher average values than the control group. The semen volume of group C was less constant and showed a marked decline when the dose of thyroprotein was increased to 0.16 per cent of the feed.

TABLE IV - Summary of the effect of different levels of thyroprotein on semen production of young roosters.

SEMEN VOLUME in ml.			
	Groups		
Date	A	B	C
Jan. 9	0.68	0.63	0.64
14	0.50	0.66	0.61
16	0.51	0.48	0.59
19	0.68	0.64	0.61
23	0.52	0.55	0.42
26	0.57	0.56	0.67
28	0.69	0.56*	0.52**
30	0.57	0.64	0.67
Feb. 2	0.58	0.61	0.54
4	0.63	0.64	0.51
9	0.54	0.55	0.56
11	0.59	0.53	0.50
13	0.52	0.45	0.34
16	0.52	0.61	0.48
18	0.43	0.64	0.45
20	0.40	0.62	0.38
25	0.43	0.64	0.37
27	0.47	0.62	0.39
Mar. 1	0.48	0.60	0.39***
3	0.48	0.60	0.41
5	0.46	0.49	0.39
8	0.53	0.61	0.36
10	0.49	0.56	0.37
18	0.51	0.55	0.36

*Started receiving thyroprotein 0.04 per cent in the ration
**Received 0.08 per cent of thyroprotein until February 29
***The dose of thyroprotein was increased to 0.16 per cent.

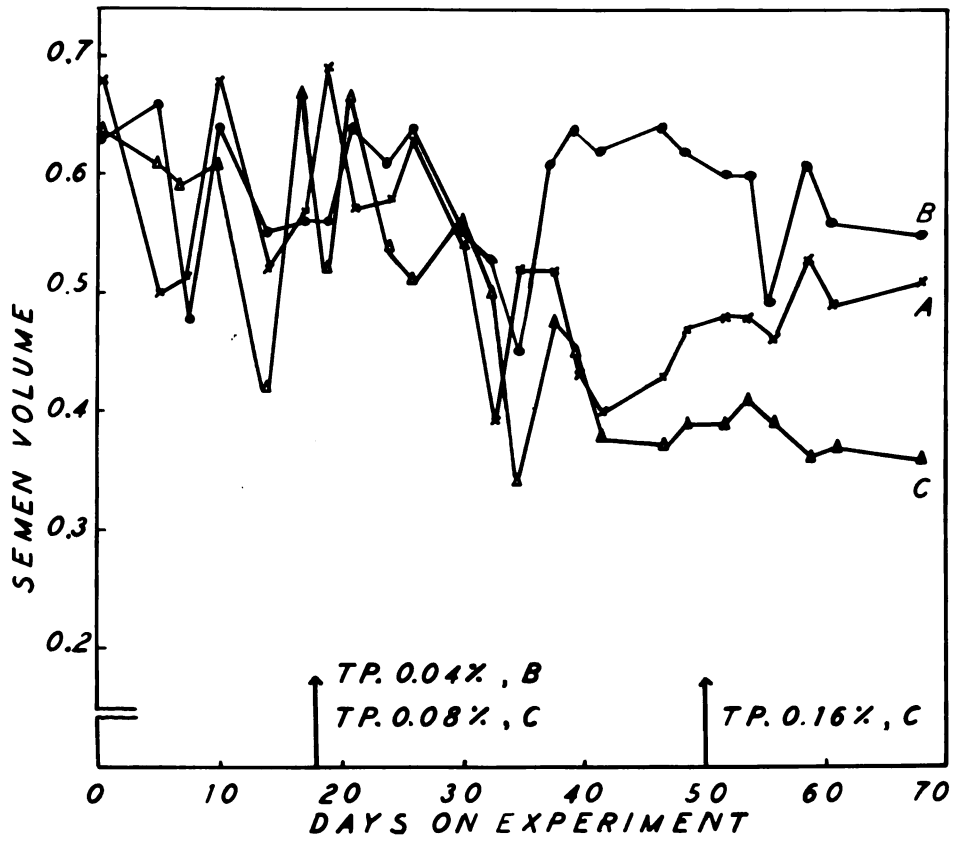


Figure I - The effect of thyroprotein on semen volume of young roosters when given as 0.04, 0.08 and 0.16 per cent of the feed.

Old roosters

In this category of birds we worked with two groups: group D, the normal control, did not receive any treatment, and group E received thyroprotein at the level of 0.04% in the feed. Group D - This group did not receive any treatment throughout the period of experiment which started on January 9 and went on until February 27. The highest semen volume checked was 0.80 ml. and the lowest was 0.20 ml. Most of the values were between 0.30 ml. and 0.55 ml. The results obtained from this group are shown in table V and a comparison with group E will be shown in table VII.

Table V - Semen volume of control roosters.

SEMEN VOLUME in ml.					
Date	Birds				
	41	42	43	45	Average
Jan. 9	0.40	0.50	0.75	0.40	0.51
14	0.27	0.18	0.37	0.43	0.31
16	0.25	0.64	0.35	0.43	0.42
19	0.22	0.26	0.56	0.48	0.38
23	0.24	0.37	0.48	0.45	0.38
26	0.55	0.49	0.27	0.80	0.53
28	0.39	0.50	0.45	0.57	0.48
30	0.48	0.55	0.44	0.66	0.53
Feb. 2	0.28	0.38	0.32	0.63	0.40
4	0.61	0.38	0.24	0.40	0.41
9	0.29	0.45	0.41	0.60	0.44
11	0.26	0.40	0.45	0.51	0.40
13	0.32	0.20	0.16	0.55	0.31
16	0.50	0.32	0.30	0.65	0.44
18	0.55	0.25	0.20	0.57	0.39
20	0.50	0.30	0.20	0.50	0.38
25	0.38	0.30	0.20	0.55	0.36
27	0.32	0.40	0.22	0.50	0.36

Group E - This group received thyroprotein at the dose of 0.04%. Feeding was begun on January 28 and lasted for 30 days until February 27. The highest volume checked was 0.85 ml. and the lowest was 0.18 ml. The averages were between 0.30 and 0.62 ml. and most of the volumes checked were between 0.30 and 0.55 ml.

In table VI are shown the individual volumes obtained in this group. A comparison with group D will be made in table VII.

TABLE VI - The effect of thyroprotein on semen production of old roosters when given as 0.04 per cent of the ration.

SEMEN VOLUME in ml.

Date	Birds				
	36	37	38	39	Average
Jan. 9	0.46	0.48	0.75	0.23	0.48
14	0.25	---	0.58	---	---
16	0.28	0.33	0.40	0.18	0.30
19	0.48	0.37	0.46	0.25	0.39
23	0.34	0.39	0.56	0.21	0.37
26	0.37	0.22	0.78	0.42	0.45
28*	0.40	0.57	0.70	0.31	0.50
30	0.47	0.57	0.75	0.32	0.53
Feb. 2	0.27	0.40	0.58	0.41	0.41
4	0.31	0.64	0.84	0.47	0.56
9	0.30	0.30	0.55	0.27	0.35
11	0.35	0.35	0.79	0.68	0.62
13	0.25	0.57	0.33	0.23	0.34
16	0.38	0.27	0.70	0.30	0.41
18	0.30	0.40	0.71	0.40	0.45
20	0.22	0.55	0.56	0.50	0.46
25	0.25	0.45	0.61	0.55	0.46
27	0.29	0.57	0.81	0.65	0.58

*Started receiving 0.04% of thyroprotein in the feed.

Table VII - In this table are summarized the averages of semen volumes obtained from groups D and E. Group D did not receive any treatment and group E received 0.04% of thyroprotein in the feed, during 30 days.

A graph will show more clearly the variations in the semen volume in these two groups. (Figure II). The average values of group E, that received thyroprotein as 0.04% of the feed were increased near the end of the experiment, when compared with the control group D.

TABLE VII - Summary of semen volume of old roosters.

SEMEN VOLUME in cc.

Date	Groups D	Groups E
Jan. 9	0.51	0.48
14	0.31	---
16	0.42	0.30
19	0.38	0.39
23	0.38	0.37
26	0.53	0.45
28*	0.48	0.50
30	0.53	0.53
Feb. 2	0.40	0.41
4	0.41	0.56
9	0.44	0.35
11	0.40	0.62
13	0.31	0.34
16	0.44	0.41
18	0.39	0.45
20	0.38	0.46
25	0.36	0.58
27	0.36	0.58

*Group E started receiving 0.04% of thyroprotein in the feed.

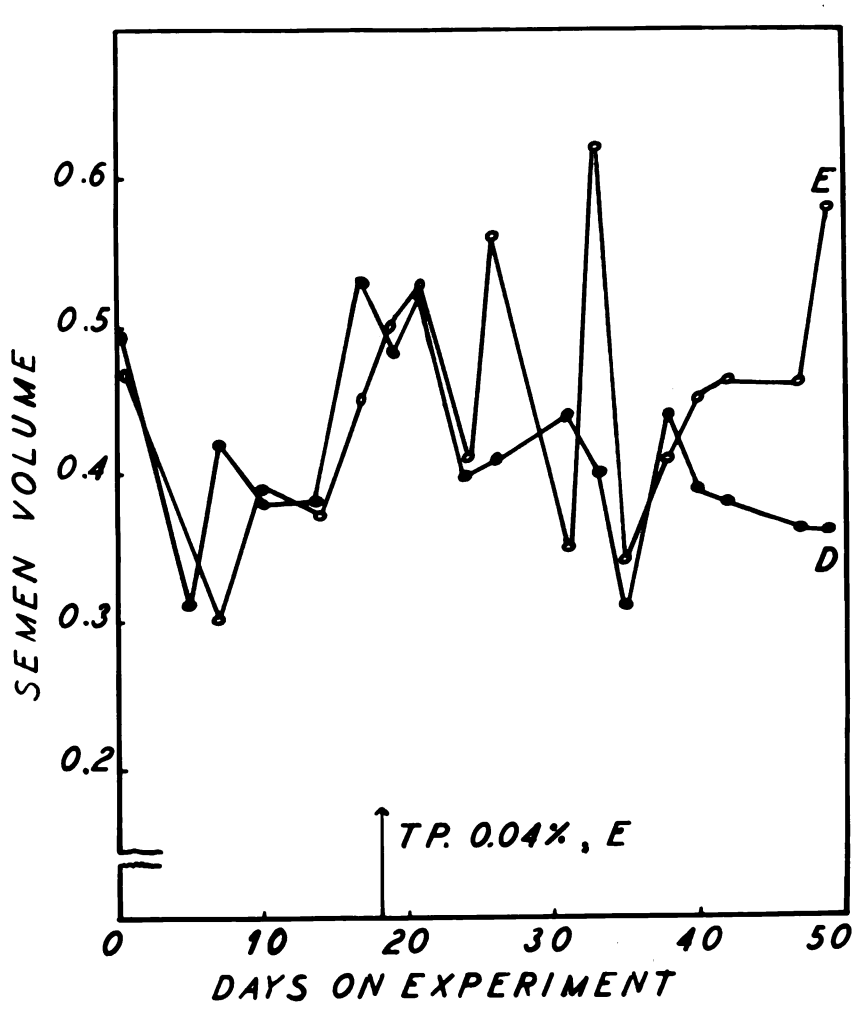


Figure II - The effect of thyroprotein when given as 0.04% of the feed, on semen volume of old roosters.

Sperm Concentration

Young roosters

Group A - This group was our normal control and did not receive any treatment during the period of experiment. Sperm concentration was checked every week from January 23 until March 18. The data obtained in this group were quite constant, the highest average value being 2,200,000 sperm per cubic millimeter and the lowest average value being 1,854,000 sperm per cubic millimeter. The highest individual value was 2,390,000 and the lowest was 1,670,000 sperm per cubic millimeter.

The results obtained from this group are seen in table VIII. In table XI a comparison will be made with the other two groups - B and C.

TABLE VIII - The sperm concentration of the control group of young roosters.

NUMBER OF SPERM IN MILLIONS PER cu. mm.

Date	Birds					
	1	2	3	4	5	Average
Jan. 23	1.820	1.780	1.760	1.980	1.930	1.854
26	2.030	2.070	2.150	2.110	2.200	2.112
30	1.670	2.180	2.000	1.980	1.820	1.930
Feb. 4	2.040	2.250	2.180	2.220	2.170	2.172
13	2.160	2.020	2.060	2.390	1.920	2.110
20	2.030	2.010	1.890	2.210	1.980	2.024
27	1.930	2.020	2.190	2.320	1.870	2.066
Mar. 8	2.090	2.290	2.040	2.280	2.250	2.190
10	2.030	2.300	2.060	2.330	2.280	2.200
18	2.010	2.260	2.180	2.210	2.090	2.150

Group B - This group received 0.04% of thyroprotein in the feed throughout the period of experiment, from January 28 to March 18. Two weeks after receiving thyroprotein the birds showed a little improvement in sperm concentration as can be seen in table IX. The highest value for this group was 2,590,000 sperm per cubic millimeter and the lowest was 1,790,000 sperm per cubic millimeter. The highest average value was 2,390,000 and the lowest was 1,888,000 sperm per cubic millimeter.

The data concerning this group are given in table IX and the averages are put together in table XI, for comparison.

TABLE IX - The effect of 0.04 per cent of thyroprotein on sperm concentration in young roosters.

NUMBER OF SPERM IN MILLIONS PER cu. mm.

Date	Birds					
	9	10	11	12	13	Average
Jan. 23	1.860	1.900	1.790	1.940	1.950	1.888
26	2.020	2.060	2.110	2.180	2.130	2.100
30	1.890	2.220	2.410	2.320	2.040	2.176
Feb. 4	2.160	2.670	2.180	2.310	1.860	2.236
13	1.970	2.140	2.440	2.550	1.910	2.202
20	2.210	2.380	2.310	2.380	2.250	2.306
27	2.110	2.280	2.570	2.110	2.050	2.224
Mar. 8	2.100	2.250	2.500	2.450	2.200	2.300
10	2.200	2.300	2.590	2.490	2.370	2.390
18	2.210	2.250	2.450	2.430	2.410	2.350

Group B received 0.04% of thyroprotein in the feed; feeding was begun on January 28 and continued for 50 days.

Group C - This group received two different levels of thyroprotein, during the period of experiment. From January 28 until February 29 they received 0.08% of thyroprotein in the feed; from March 1 until the end of the experiment they had twice this dose, that is, 0.16% of thyroprotein.

The highest average value was 2,108,000 sperm per cubic millimeter and the lowest was 1,374,000 sperm per cubic millimeter. The highest individual value was 2,500,000 and the lowest was 1,310.000 sperm per cubic millimeter.

There was a marked decrease in sperm concentration at the end of the experiment due probably to the quite heavy dosage of thyroprotein.

The data obtained from this group are shown in table X and the averages given in table XI for compari son with the other groups.

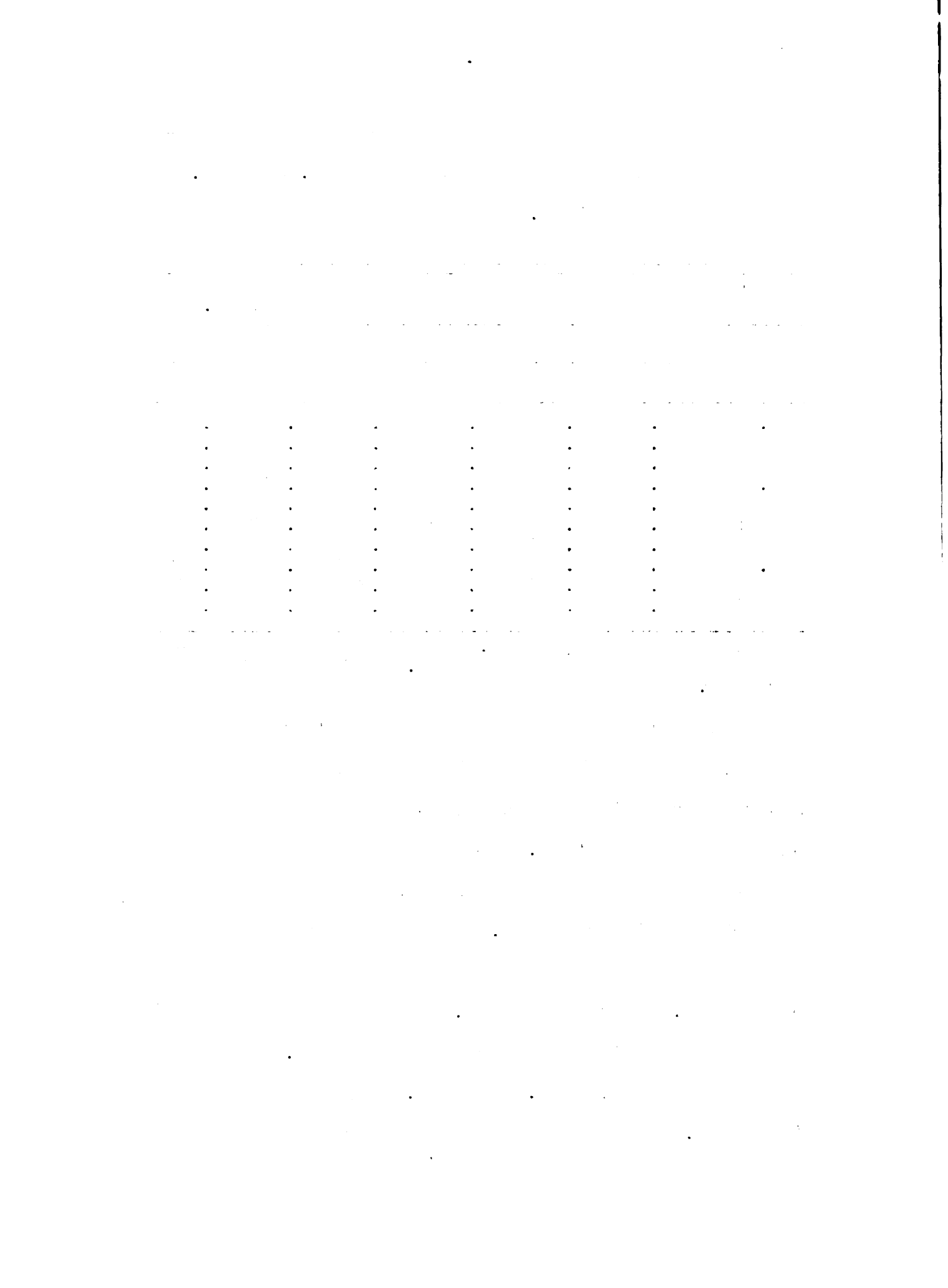
TABLE X - The effect of thyroprotein on sperm concentration of young roosters when given as 0.08 and 0.16 per cent of the ration.

Date	NUMBER OF SPERM IN MILLIONS PER cu. mm. Birds					
Jan. 23	1.680	1.970	1.750	1.730	2.040	1.834
26	1.990	2.030	1.960	2.170	2.210	2.072
30	1.890	1.850	2.500	2.330	1.950	2.104
Feb. 4	1.180	2.170	2.290	2.450	2.310	2.080
13	1.820	1.930	1.360	1.770	1.890	1.754
20	1.830	2.050	1.800	1.960	1.980	1.880
27	1.990	1.750	1.570	1.820	1.880	1.802
Mar. 8	1.600	1.560	1.530	1.690	1.770	1.630
10	1.700	1.650	1.570	1.690	1.590	1.640
18	1.390	1.360	1.440	1.370	1.310	1.374

Received thyroprotein as 0.08% of the feed from January 28 until February 29; and 0.16% from March 1 to March 18.

Table XI will show a summary of the average values obtained from the birds in the three groups and it shows the effect of different dosages of thyroprotein on sperm concentration.

A graph of this data will show these variations more clearly (Figure III). Spermatogenesis was influenced by the administration of thyroprotein at different levels. When given as 0.04% of the feed (group B) sperm concentration was increased markedly. When given at higher levels, as 0.08% and 0.16% of the feed (group C), a marked decline in sperm concentration was



observed, due probably to the depressing effect of excessive thyroprotein on the gonads. Sperm concentration of the control group (group A), was quite constant throughout the experimental period.

TABLE XI - Summary showing the effect of different levels of thyroprotein on sperm concentration of young roosters.

NUMBER OF SPERM IN MILLIONS PER cu. mm.

Date	Groups		
	A	B	C
Jan. 23	1.854	1.888	1.834
26	2.112	2.100	2.072
30	1.930	2.176	2.104
Feb. 4	2.172	2.236	2.080
13	2.110	2.202	1.754
20	2.024	2.306	1.880
27	2.066	2.224	1.802
Mar. 8	2.190	2.300	1.630
10	2.200	2.390	1.640
18	2.150	2.350	1.374

Group B received 0.04% of thyroprotein in the feed from January 28 to March 18.
Group C received 0.08% of thyroprotein from January 28 until February 29; and 0.16% from March 1 to March 18.

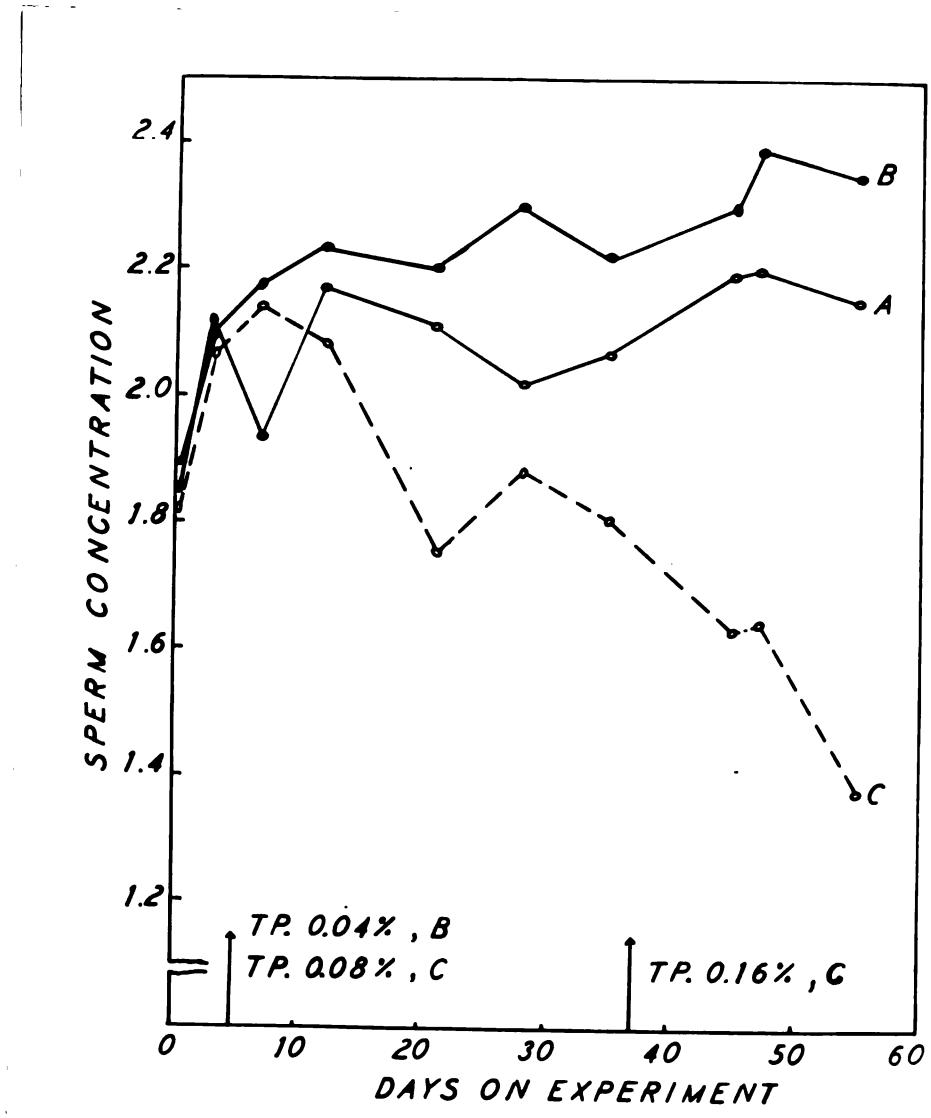


Figure III - The effect of thyroprotein on sperm concentration of young roosters when given as 0.04, 0.08 and 0.16 per cent of the feed.

Old Roosters

Group D - This was the control group and did not receive any treatment during the period of experiment.

The data obtained are shown in table XII and the averages are given in table XIV with group E.

The highest individual sperm concentration value for this group was 2,280,000 and the lowest was 800,000 sperm per cubic millimeter. The highest average value was 2,057,000 and the lowest was 1,547,000 sperm per cubic millimeter.

TABLE XII - The sperm concentration of the control group of Old roosters.

SPERM CONCENTRATION IN MILLIONS

Date	Birds				
	41	42	43	45	Average
Jan. 19	2.150	1.950	1.810	1.930	1.960
23	2.020	2.000	1.810	2.060	1.972
26	1.970	2.070	1.910	2.120	2.017
28	0.800	2.280	1.940	1.430	1.612
Feb. 2	0.910	1.810	1.900	1.570	1.547
9	2.030	2.050	1.950	2.100	2.033
13	1.800	2.120	2.050	2.260	2.057
18	1.630	2.100	1.970	2.120	1.955
27	2.060	2.050	1.740	1.810	1.907

Group E - Feeding this group with thyroprotein began on January 28 and lasted until February 27, at the level of 0.04% in the feed.

The highest individual value for this group was 2,360,000 sperm per cubic millimeter and the lowest was

1,520,000 sperm per cubic millimeter. The highest average value was 2,145,000 and the lowest was 1,775,000 sperm per cubic millimeter.

The data obtained from this group are shown in table XIII and the averages in table XIV with group D.

TABLE XIII - The effect of thyroprotein on sperm concentration of old roosters when given as 0.04 per cent of the feed.

SPERM CONCENTRATION IN MILLIONS

Date	Birds				
	36	37	38	39	Average
Jan. 19	2.090	1.990	1.580	1.910	1.892
23	2.200	2.080	1.610	2.140	2.007
26	2.090	2.010	1.590	2.060	1.937
28*	1.690	2.050	1.650	1.710	1.775
Feb. 2	1.780	2.280	1.960	2.000	2.005
9	1.660	2.150	1.700	2.210	1.930
13	1.520	2.360	2.090	2.120	2.022
18	1.820	2.010	1.900	2.100	1.957
27	2.030	2.150	2.040	2.360	2.145

*Started receiving thyroprotein as 0.04% of the feed.

Table XIV shows the average values obtained from groups D and E, group D being the normal control and E receiving 0.04% of thyroprotein for about 30 days.

The variations in sperm concentration of these two groups is shown more clearly in graphic form in figure IV. Sperm concentration was not influenced in this category of birds as it was in young roosters. The average values from the control group and the group

receiving thyroprotein as 0.04% of the feed, were about the same.

TABLE XIV - Summary of the sperm concentration of old roosters.

	SPERM CONCENTRATION IN MILLIONS	
	Groups	
Date	D	E
Jan. 19	1.960	1.892
23	1.972	2.007
26	2.017	1.937
28	1.612	1.775
Feb. 2	1.547	2.005
9	2.033	1.930
13	2.057	2.022
18	1.955	1.957
27	1.907	2.145

Group E received 0.04% of thyroprotein in the feed, from January 28 to the end of the experiment.

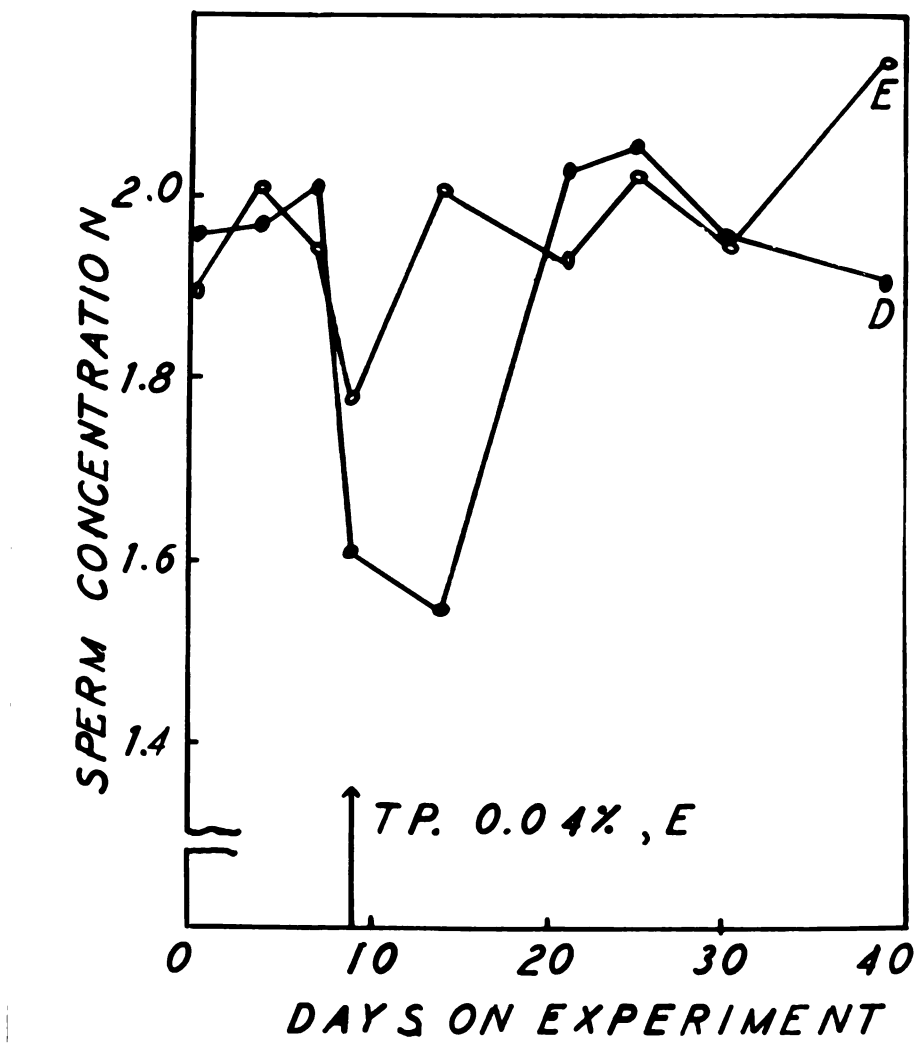


Figure IV - The effect of thyroprotein when given as 0.04% of the feed on sperm concentration of old roosters.

Total Number of Sperm

Group A - The data obtained from this group are shown in table XV and the average values will be put together with groups B and C in table XVIII.

The highest individual value was 1,732 millions of sperm and the lowest was 501 millions of sperm per ejaculation. The highest average value was 1,368 millions of sperm and the lowest was 809 millions of sperm.

TABLE XV - The total number of sperm of the control group of young roosters.

TOTAL NUMBER OF SPERM IN MILLIONS

Date	Birds 1	2	3	4	5
Jan. 23	946.43	1,174.80	809.60	752.40	1,338.00
26	832.30	1,336.20	1,505.00	1,371.50	946.00
30	701.40	1,460.60	1,260.00	1,306.80	857.60
Feb. 4	856.80	1,732.50	1,438.80	1,487.40	1,267.10
13	842.40	1,111.00	844.60	1,362.30	1,267.20
20	527.80	1,260.00	661.50	839.80	772.20
27	576.00	1,010.00	1,270.20	1,182.20	841.50
Mar. 8	1,147.50	1,259.50	1,122.00	1,026.00	1,237.50
10	501.50	1,725.00	927.00	1,165.00	1,140.00
18	924.60	1,642.80	937.40	994.50	982.30

Group B - This group receiving 0.04% of thyroprotein throughout the experimental period showed a quite consistent improvement in spermatogenesis.

The highest individual value was 2,071 millions of sperm and the lowest was 607 millions of sperm per

ejaculation. The average values were: 1,431 millions of sperm for the highest and 930 millions of sperm for the lowest one.

Table XVI shows the data obtained from this group and in table XVII will be given the average values with groups A and C.

TABLE XVI - The effect of thyroprotein on total number of sperm of young roosters when given as 0.04% of the feed.

Date	TOTAL NUMBER OF SPERM IN MILLIONS				
	Birds				
	9	10	11	12	13
Jan. 23	1,209.00	950.00	805.50	1,183.40	1,111.50
26	767.60	1,256.60	844.00	2,071.00	1,001.10
30	1,058.40	1,265.40	1,494.20	1,600.80	1,530.00
Feb. 4	1,447.20	1,762.20	1,090.00	1,778.70	1,078.80
13	906.20	941.60	976.00	1,300.50	840.40
20	1,436.50	1,618.40	1,270.50	1,428.00	1,468.50
27	1,519.20	1,276.80	1,542.00	949.50	1,599.00
Mar. 8	1,260.00	1,912.50	1,325.00	980.00	1,430.00
10	1,320.00	1,610.00	1,508.20	747.00	1,540.50
18	884.00	1,912.50	1,102.50	607.50	1,928.00

Thyroprotein feeding was begun on January 28 and continued to the end of the experimental period.

Group C - The data obtained from this group that received higher levels of thyroprotein than group B, are shown in table XVII and the average values will be put together with groups A and B, in table XVIII, for comparison.

The highest individual value was 3,027 millions of

sperm per ejaculation and the lowest was 235 millions of sperm per ejaculation. The highest average value was 1,409 millions of sperm and the lowest was 549 millions of sperm.

TABLE XVII - The effect of thyroprotein on total number of sperm of young roosters when given as 0.08 and 0.16 per cent of the feed.

TOTAL NUMBER OF SPERM IN MILLIONS

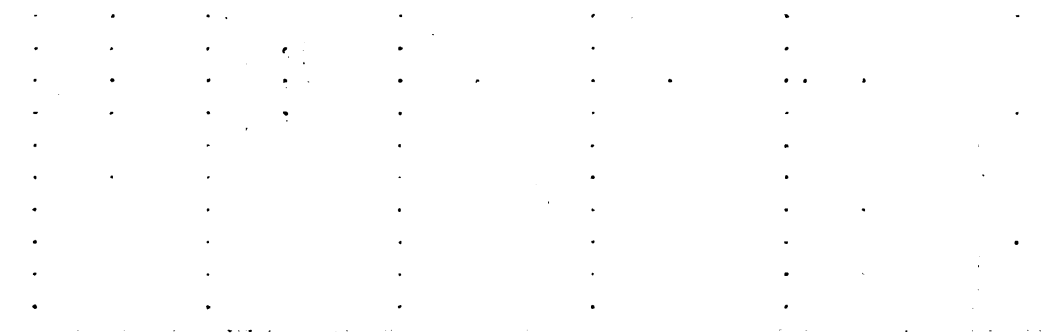
Date	Birds				
	20	21	22	23	24
Jan. 23	672.00	472.80	805.00	674.70	1,224.00
26	835.80	812.00	862.40	1,510.00	3,027.70
30	1,171.80	1,128.50	1,550.00	1,514.50	1,638.00
Feb. 4	672.60	976.50	984.70	1,151.50	1,501.50
13	637.00	598.30	408.00	513.00	850.50
20	951.60	656.00	396.00	392.00	1,267.20
27	1,054.70	700.00	565.20	691.60	564.00
Mar. 8	800.00	624.00	612.00	507.00	354.00
10	1,003.00	775.50	392.50	608.40	286.20
18	625.50	476.00	576.00	548.00	235.80

Received thyroprotein as 0.08% of the feed from January 28 until February 29; and 0.16% from March 1 to March 18.

Table XVIII shows a summary of the average values obtained from the birds in these three groups and it shows the effect of different dosages of thyroprotein on the total number of sperm.

A graph of these data will show these variations in total number of sperm more clearly. (Figure V).

The effect of varying levels of thyroprotein on semen



production is better seen in the total number of sperm. It depends on volume and sperm concentration. In group B, that received 0.04% of thyroprotein in the feed we observed a quite consistent increase in the total number of sperm; in group C, receiving higher doses of thyroprotein, we observed a rather marked decline in the total number of sperm. In group A, the control group, we had quite constant values for the total number of sperm.

TABLE XVIII - Summary showing the effect of different levels of thyroprotein on total number of sperm of young roosters.

Date	TOTAL NUMBER OF SPERM IN MILLIONS		
	Groups		
	A	B	C
Jan. 23	964.08	1,038.40	770.28
26	1,203.84	1,176.00	1,388.24
30	1,100.10	1,392.64	1,409.68
Feb. 4	1,368.36	1,431.04	1,060.80
13	1,097.20	990.90	596.36
20	809.60	1,429.72	731.12
27	971.02	1,378.88	702.78
Mar. 8	1,160.70	1,403.00	586.80
10	1,078.00	1,338.40	606.80
18	1,096.50	1,292.50	549.60

Group B received 0.04% of thyroprotein in the feed from January 28 to March 18. Group C received 0.08 of thyroprotein from January 28 until February 29; and 0.16% from March 1 to March 18.

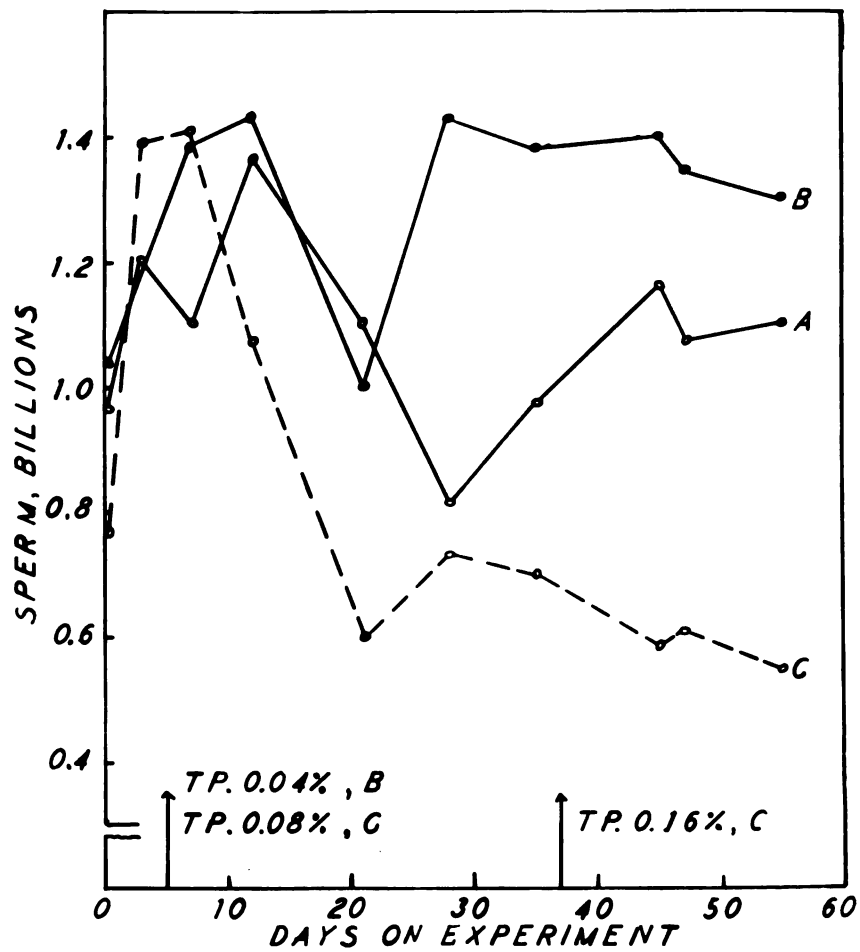


Figure V - The effect of thyroprotein on the total number of sperm of young roosters when given as 0.04, 0.08 and 016 per cent of the feed.

Old Roosters

Group D - The data from this group are given in table XIX and the average values will be given in table XXI with the averages from group E.

The highest individual value was 1,690 millions of sperm and the lowest was 254 millions of sperm per ejaculation. The highest average value was 1,069 millions of sperm and the lowest average value was 618 millions of sperm.

TABLE XIX - The total number of sperm of the control group of old roosters.

TOTAL NUMBER OF SPERM IN MILLIONS

Date	Birds			
	41	42	43	45
Jan. 19	473.00	507.00	1,013.60	926.40
23	484.80	740.00	868.80	927.00
26	1,083.50	1,014.30	515.70	1,696.00
28	312.00	1,140.00	873.00	686.40
Feb. 2	254.80	687.80	608.00	989.10
9	588.70	922.50	799.50	1,260.00
13	576.00	424.00	328.00	1,243.00
18	896.50	525.00	394.00	1,208.40
27	649.60	820.00	382.80	905.00

Group E - The data from this group are shown in table XX and the average values will be given in table XXI with group D.

The highest individual value was 1,652 millions of sperm and the lowest was 380 millions of sperm per

ejaculation.

The highest average value was 1,242 millions of sperm and the lowest was 675 millions of sperm.

TABLE XX - The effect of thyroprotein on total number of sperm when given as 0.04% of the ration in old roosters.

Date	TOTAL NUMBER OF SPERMS IN MILLIONS			
	Birds			
	36	37	38	39
Jan. 19	1,003.20	706.60	726.80	477.50
23	748.00	811.20	901.60	449.40
26	773.30	442.20	1,240.20	865.20
28	676.00	1,168.50	1,155.00	530.00
Feb. 2	480.60	912.00	1,136.80	820.00
9	498.00	645.00	935.00	596.70
13	380.00	1,345.20	689.70	487.00
18	546.00	804.00	1,349.00	840.00
27	588.70	1,225.50	1,652.40	1,534.00

Thyroprotein feeding was begun on January 28 and continued to the end of the experimental period.

Table XXI - In this table are summarized the average values of the total number of sperm from groups D and E. Group D did not receive any treatment and group E received 0.04% of thyroprotein in the feed for 30 days.

The variations in total number of sperm of these two groups are shown more clearly in figure VI. Feeding thyroprotein to old roosters for about 30 days, as 0.04% of the feed, increased somewhat the total number of sperm, as can be seen in group E, when compared with the control group (group D). In the control group we had quite constant values for the total number of sperm.

throughout the experimental period.

TABLE XXI - Summary of the total number of sperm of old roosters

Date	TOTAL NUMBER OF SPERMS IN MILLIONS	
	Groups	
	D	C
Jan. 19	744.80	737.88
23	749.36	742.59
26	1,069.01	871.65
28	773.76	887.57
Feb. 2	618.80	822.05
9	894.52	675.50
13	637.67	687.48
18	762.45	880.65
27	686.52	1,242.94

Group E received thyroprotein as 0.04% of the feed since January 28.

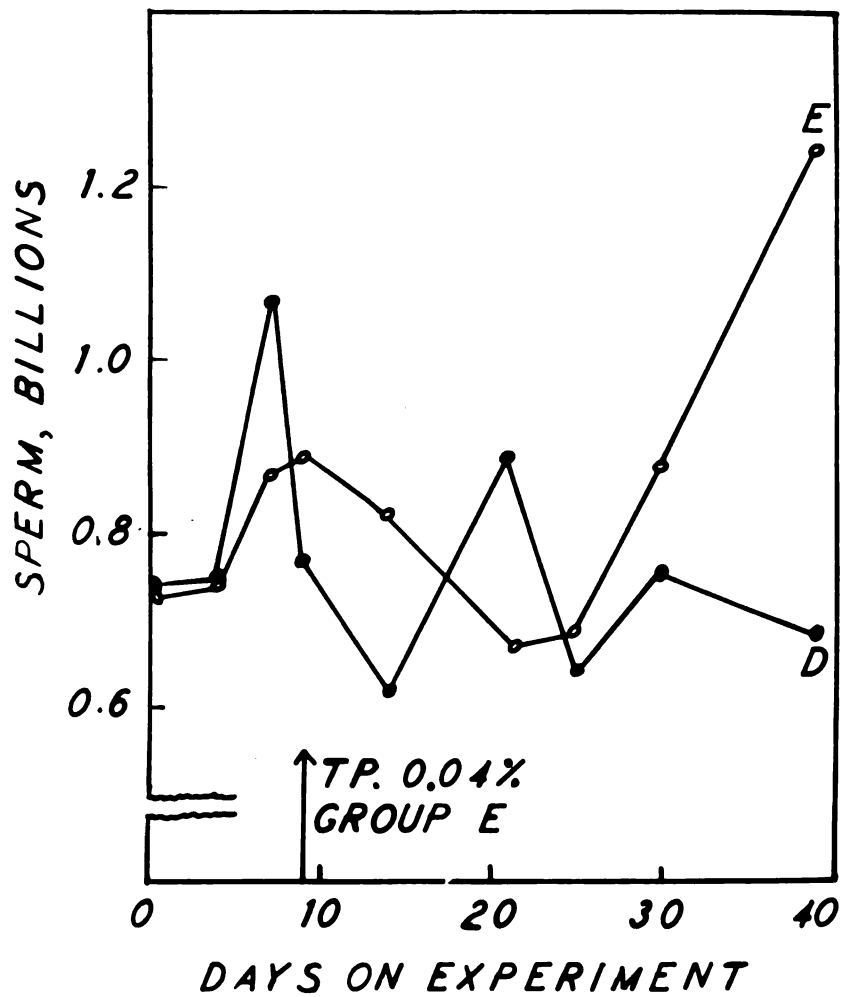


Figure VI - The effect of thyroprotein when given as 0.04% of the feed on the total number of sperm of old roosters.

DISCUSSION AND SUMMARY

In this experiment we used two categories of birds; fifteen 8-month-old Rhode Island Red roosters were divided into three groups of five each, and eight old cocks about 21-month-old were divided into two groups of four each.

All birds were placed in individual cages, receiving the same basal diet "ad libitum", supplied twice a day, had running tap water and received artificial light twenty-four hours a day. The room temperature was maintained at about 65°F. during the experimental period. Each group received a different treatment, since January 28, after a preliminary period of training.

In each category of birds we had a control group receiving only the basal diet. In young roosters we had two more groups that were fed thyroprotein at three different levels:

Group B received thyroprotein as 0.04% of the ration throughout the period of experiment;

Group C received two different levels during the experimental period: from January 28 until February 29 it received 0.08% of the ration; in the last two weeks it received twice this dose, that is, 0.16% of the ration.

In the old roosters we had a control group (group D), receiving the basal mash, and a group (group E)

receiving thyroprotein as 0.04% of the feed.

The results obtained in this experiment showed that thyroprotein when given as 0.04% of the feed is able to improve spermatogenesis in both young and old roosters. We obtained an increase in semen volume, sperm concentration and consequently, in the total number of sperm. The results from young roosters are more consistent than in the older ones.

Higher levels of thyroprotein seemed to have a depressing effect on spermatogenesis, as can be seen in the data obtained from group C of the young roosters. We observed a decrease in semen volume, sperm concentration, and consequently, in the total number of sperm in this group.

CONCLUSIONS

1. Thyroprotein when given as 0.04 per cent of the ration produced an increase in spermatogenesis of young roosters. As compared to control birds both semen volume and sperm concentration increased to some extent with the result that the total sperm per ejaculate increased rather markedly. In older roosters similar but less pronounced trends were observed.
2. Thyroprotein when given at the higher levels of 0.08 and 0.16 per cent of the ration, depressed semen production and the total number of sperm decreased markedly.

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