A STUDY OF THE EFFECT OF FEEDING THYROPROTEIN TO LAYING BIRDS OF ONE STRAIN OF RHODE ISLAND REDS

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Maurice S. Armstrong

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THESIS

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By

Maurice S. Armstrong

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Introduction

The process of reproduction of the fowl is highly complicated and involves considerable glandular activity in the body. The complete physiological processes have not as yet been worked out, however a great deal of information pertaining to them has been obtained. Among the more important of the glands associated with egg production is the thyroid. In the chicken the thyroid is a small, oval red body located on the ventral side of the common carotid artery at the point where it touches the jugular vein. The thyroid gland, which is protein in nature, secretes the hormone, thyroxine, which is an iodine containing derivative of the amino acid, tyrosine. Thyroxine is thought to be catalytic in that it raises the excitative capacity of cells, yet withstands digestion, Kendall (1929). The more general influences of thyroxine are that it is responsible for the maintenance of normal metabolism and alterations in the metabolic rate to counteract changes in the environment. In addition thyroxine plays an important role in the promotion of growth, this affect apparently resulting from its action on metabolism in general, Lerman (1942). These general effects are brought about by the following more specific influences: oxidation of carbohydrates, and more primarily oxidation of proteins and fats; heat production; and tissue consumption of oxygen.

Since environmental conditions affect body metabolism, it follows that they similarly influence the thyroid. Thyroid gland activity and

body metabolism are decreased by high temperatures and increased by low temperatures, Kendall (1929). Furthermore it is known that thyroid activity tends to decrease with advancing age.

Thyroid secretion has a two-fold influence on egg production. Since egg production is to some extent associated with body metabolism, it comes under the influence of thyroxine in this respect. In addition to the above effect, an excess of thyroxine in the tissues results in a depression of the thyrotropic activity and an increase of the gonadotropic activity of the anterior pituitary; the gonadotropic hormone in turn influences ovarian activity, Marine (1940). A deficiency of thyroxine in the tissues results in the opposite of this, Cameron (1940). In view of these facts it would appear that an optimum level of thyroxine in the body would be the most conducive to high egg production.

Literature Review

Effect of Feeding Raw or Desiccated Thyroid on the Egg Production of Fowls

Various experiments have been performed with the feeding of raw or desiccated thyroid to fowls and their influence on egg production. Crew and Huxley (1923) fed raw thyroid to six birds, using six other birds as controls, but were unable to obtain any significant results pertaining to egg production. Following this Crew (1925) administered desiccated thyroid to seven senile hens. All birds promptly molted, the new plumage being characteristic of younger fowls. In addition an enlargement and reddening of head furnishings took place. The low egg production of 6.67 eggs per hen for six months preceding the experiment increased to thirty-four eggs during the treatment, and twenty-four during the six months following the treatment. Desiccated thyroid (one mg. of thyroid iodine per 1750 gm. of body weight) was administered to laying hens by Asmundson and Pinsky (1935) with the following results: slight increase in the amount of shell, a reduction of yolk weight and rate of ovum growth, and a decrease in body weight and egg production.

Influence of Thyroidectomy on the Egg Production of Fowls

Winchester (1939) decreased the egg production of White Leghorn hens eighty-five percent by thyroidectomy. Weekly injections of thyroxine resulted in an increase of the egg production of these same fowls to forty and fifty to sixty percent of that of normal fowls. Complete thyroidectomy by Taylor and Burmeister (1940) brought about a decrease in egg production of one-third to one-fourth that of normal fowls, while incompletely thyroidectomized birds showed a decline in egg production of about two-thirds that of normal.

Relation of Thyroid Function to Molt and Feather Growth

Cole and Reid (1924) and Zawadorsky (1925) found evidence to the effect that thyroid-fed birds showed more rapid and uniform feather growth, and that the thyroid plays a role in the regulation of the growth and molting of feathers. In an experiment conducted by Cole and Hutt (1928) thyroid-fed birds exhibited a rapid molt, dropping all feathers simultaneously and growing new ones uniformly.

Association of Egg Production, Senescence, and Seasonal Cycles with Thyroid Function

Various investigations involving the association of environment, egg production, and thyroid function have been undertaken. Brody, Henderson, and Kempster (1923) and Clark (1940) obtained evidence that the average annual decline in egg production is twelve to twenty percent of that of the preceding year. Indications that egg production is definitely influenced by light and length of day were obtained by Bissonnette (1933) and Winchester (1940). Yearling Rhode Island Red hens were subjected to a fourteen hour day-light period by Byerly and Gardner (1943). These birds laid at a rate of fifty-eight percent from December through March, while the controls laid at the rate of twenty-four percent for the same period, being subjected to the normal daylight periods. The molt was not affected by the extended light period.

Dempsey and Atwood (1943) found evidence which indicated that the thyroid secretes its active principle more rapidly in cold than in warm environments, and that the basal metabolic rate is elevated by exposure to cold.

Development and Synthesis of Thyroprotein

Considerable work has been done relating to the development and synthesis of thyroprotein. Ludwig and Mutzenbecher (1936 and 1939), and Reineke and Turner (1943) were able to isolate thyroxine from artificially produced iodinated proteins including iodinated casein (thyroprotein). Reineke and Turner (1942) developed the currently used method of producing iodinated casein by treating casein, in the presence of manganese as a catalyst, with iodine and sodium bicarbonate in a warm water bath followed by an eighteen to twenty hour incubation period, and the iodinated casein was precipitated out by the isoelectric method, following dialysis. The thyroidal activity of an iodinated potency attains a maximum when two atoms of iodine have been added for each mole of tyrosine, and that additional iodination results in a definite decline in thyroidal activity. Thyroactive proteins have also been obtained with egg albumin, soybean proteins, silk fibroin, and serum globulin, but casein has been most widely used due to availability and ease of manipulation.

The iodinated casein (protamone--supplied by Cerophyl Laboratories, Inc., 2438 Broadway, Kansas City, Missouri) prepared above tested 2.73 thyroxine by biological assay and 3.03 percent by chemical assay. Biological assays have been accomplished in various ways. Reineke and

Turner (1942) employed the metamorphosis of tadpoles, and the oxygen consumption of guinea pigs due to thyroid stimulation as a measure of the thyroxine level of iodinated proteins. Another method of biological assay, developed by Mixner, Reineke, and Turner (1944), involved treating day old chicks with thiouracil (anti-thyroid hormone), and observing the rate at which thyroactive proteins caused a decrease in the size of the enlarged thyroids. Reineke, et. al. (1945) worked out a chemical assay of iodinated proteins, which consisted of the hydrolysis of iodocasein with forty percent barium hydroxide solution, the extraction of the thyroxine with n-butanol, and determination of the iodine content of the purified extract.

Effect of Thyroprotein on the Rate of Growth and Feathering of Baby Chicks

Although this work is concerned primarily with the egg production of laying birds, a few experiments are herein reviewed relating to the rate of growth and feathering of baby chicks, since molt and subsequent growth of feathers is a normal occurence during the summer and fall months of the laying year. Irwin, Reineke, and Turner (1943) fed thyroprotein at the rate of thirty-six grams per one hundred pounds of feed to White Plymouth Rock chicks, and at the end of twelve weeks the experimental chicks were slightly heavier and showed improved feather growth as compared with the controls. Rhode Island Red chicks raised to twelve weeks on rations containing .025 to .08 percent iodinated proteins by Parker (1943) showed slightly greater gains in body weight than did the controls. Turner, Irwin, and Reineke (1944) fed thyroprotein at the rate of forty-five grams per one hundred pounds of feed to Barred

Plymouth Rock cockerels with the result that the treated birds grew slightly slower than the controls, indicating that this amount was the uppper limit of tolerance without a marked depression of the growth rate.

Effect of Feeding Thyroprotein on the Egg Production of Laying Fowls

The first experiment involving the feeding of thyroprotein to laying fowls was conducted by Turner, Irwin, and Reineke (1945). Forty-eight two year old White Leghorn hens, selected on the basis of physical characteristics, were divided into four groups with one group as controls; the other groups receiving the following levels of thyroprotein (2.73 percent thyroxine by biological assay); five, ten, and twenty grams per one hundred pounds of feed. The average percentage egg production records obtained for one year were: controls, 22.6; five gram level, 38.1; ten gram level, 40.6; and twenty gram level, 30.7. The five and ten gram level groups attained a higher level of egg production and maintained the level longer during the summer, however the thyroprotein-fed birds dropped precipitously in egg production in late August and went into a molt. No marked differences were obtained in regard to egg weights, body weight, or mortality.

In a later experiment Turner, Kempster, Hall, and Reineke (1945) used the same White Leghorns with some addition. A ten gram level of thyroprotein (2.73 percent thyroxine by biological assay) was fed to twenty-four of the birds, and another group of twenty-four birds was used as the control group. In addition ten and twenty gram levels of thyroprotein were fed in the ration to two groups of twelve Rhode Island Red pullets each, and a third group received the basal ration with

physical characteristics serving as the basis of selection. All the birds were subjected to a ten and one-half hour daylight period. The three year old thyroprotein group had an average egg production record of 38.8 percent during October to May, 36.3 percent during May to October, with an overall average of 37.7 percent; while the controls had an average of 42.0 percent during the first six months, 23.4 percent during the last half of the year, and 33.9 percent for the whole year. The egg production of the controls decreased 43.8 percent in the second half of the year, while that of the experimental group decreased only 6.4 percent. The three year egg production of those three year old birds surviving this and the previous experiment was as follows: controls, 81.3 percent and 89.5 percent of their previous years' production; thyroprotein-fed group, 72.5 percent and 102.3 percent of the previous years' production. Similar results were obtained with the pullets. During the first half of the experiment the controls laid at the rate of 70.8 percent, the ten gram level at 71.6 percent, and the twenty gram level at 61.7 percent. In the second half the average egg production was as follows: controls, 52.7 percent, ten gram level, 69.7 percent; and twenty gram level, 51.7 percent. The overall percentages were: for the controls, 62.8 percent; for the ten gram level, 67.2 percent; and for the twenty gram level, 52.0 percent. Decreases of 25.6 percent, 6.1 percent, and 14.8 percent during the final half of the experiment were recorded by the controls, ten gram level, and the twenty gram level, respectively.

Reasons for Undertaking Experiment

In view of the foregoing information further work involving the feeding of thyroprotein to laying birds to determine its effect on egg production is necessary, before its value in this respect can be definitely established. The significant results obtained by Turner, Irwin, and Reineke (1945), and Turner, et. al. (1945) indicate that thyroprotein has possibilities as a method of increasing egg production. Furthermore, there is an ever continuing need to increase egg production by delaying the seasonal decline, either by delaying molt, or speeding it up; this applies both to pullets and old hens. Therefore, it seemed that an experiment, involving the feeding of thyroprotein to laying birds, to determine its effect on egg production, would be of considerable interest. It would appear that this procedure would provide the birds with a uniform level of thyroxine in the body, particularly during the time, when the hot weather of summer results in a decrease of the body thyroidal activity. As a result it was believed that a test of this nature would be more apt to be significant if conducted during the spring, summer, and early fall.

Materials and Experimental Procedure

A. Basis for Selection of Pullets

In order to have the birds employed in this experiment as closely matched in relation to egg production ability as possible, they were paired up on the following basis: age at first egg, rate (intensity), total lay for the first four months of production, and where possible, the same dam. Out of ninety-six Rhode Island Red pullets, which were pedigreed from the Michigan State College flock, twenty-seven pairs were selected with ten days being the greatest variation in age at first egg, and eight eggs the extreme as to comparison in reference to total lay, while three of the pairs had the same dam. Following examination of the birds, three pairs of the twenty-seven were discarded, as one of the birds in each instance was unusually light, and noticeably dull and inactive. These birds had been reared on range and housed in laying cages during September and October, 1946.

B. Experimental Procedure for Pullets

The experiment was started on March 3, 1947, although the experimental birds received ten grams of thyroprotein per one hundred pounds of feed in their diet for a period of two weeks prior to the official starting date. Forty-eight pullets were divided into two groups of twenty-four birds each, and housed in laying cages. Most of the birds were in production at the start of the experiment, and none were molting. The birds were divided into groups I and II with the former being the control. These birds had been subjected to a thirteen and one-half hour daylight period prior to the beginning of the experiment, and this light period was extended to fourteen hours for the duration of the experiment. The controls were fed the regular college battery laying mash (15.0 percent crude protein), which was made up, as follows:

Feedstuff	Amount (pounds)
Corn meal	690
Ground oats	400
Wheat bran	300
Wheat middlings	200
Alfalfa meal (17% dehydrated)	60
Meat scraps	60
Dry milk	40
Fish meal	50
Soybean meal	50
Oyster shell flour	100
Steamed bone meal	30
Salt	12
Fish oil (400 A, 2000 D)	8
Total	2000

Group II received the same ration plus ten grams of iodinated casein (3.04 percent thyroxine by chemical assay) per one hundred pounds of feed. The ten gram level of thyroxine was selected, because it was found to be the most optimum in the experiments performed by Turner, et. al. (1945).

Inasmuch as the amount of thyroprotein added to the ration was very small, it had to be meticulously mixed to insure even distribution. The ten grams was first thoroughly mixed with one-half pound of feed. This one-half pound of feed was then mixed with one pound of feed. This

process was progressively continued, until ten pounds had been mixed. The ten pounds mixed in the above manner were then added to the remaining ninety pounds, and the final mixing was done with a McClellan Batch Mixer.

The experiment was divided into four week intervals to facilitate record keeping. The egg production, and feed consumption records were tabulated at the end of each four week period. At this same time the birds were individually weighed and their respective weights recorded. These birds were checked for molt on October 5 and 26, and November 9, 1947, on the basis of the number of new primaries present. The work herein reported was concluded on November 9, 1947.

C. Experimental Procedure for Two and Three Year Old Birds

Since thyroid activity as well as egg production decreases with age, a study of the influence of feeding thyroprotein on the egg production of two and three year old hens was also included in this experiment. This portion of the experiment was started on May 15, 1947. These birds were pedigreed Rhode Island Reds from the Michigan State College flock and were paired up on the basis of egg production for the first two and three years, and as near as possible, age at first egg. The greatest variation in two year old birds for first year production was three, for second year production, fifteen, while the differences in age at first egg varied from five to seventy-five days. For the three year old birds the largest difference for the first year egg production was sixteen, for the second year, twenty-seven, and for the third year, thirteen. Differences in age at first egg for these birds ranged from two to forty-nine days.

These birds were divided into groups of eight as follows: group III, two year old controls; group IV, two year old thyroprotein-fed birds; group V, three year old controls; group VI, three year old thyroprotein-fed birds. As with the pullets the two and three year old birds were housed in laying cages and exposed to a fourteen hour daylight period. Egg production and feed consumption records were tabulated at the end of four week intervals. The controls were placed on the regular college battery laying mash, while the experimental birds were given the same ration as the experimental pullets.

D. Experimental Procedure for Testing for the Effect of Thyropretein on Egg Shell Quality.

1. Experiments Relating to the Effect of Thyroid Material on Egg Shell Quality.

Involved in the overall effect of thyroxine on body metabolism is a further influence on calcium metabolism, and consequently a possible effect on egg shell quality. Asmundson and Pinsky (1935) were able to bring about a slight increase in the amount of shell by feeding desiccated thyroid to laying hens. Gutteridge and Pratt (1946) conducted an experiment involving the feeding of vitamins D_2 and D_3, and iodinated casein at the rate of fifteen grams per one hundred pounds of feed, to White Leghorn pullets. On the basis of the specific gravity test, the group receiving the iodocasein showed a significant increase in egg shell quality compared with those receiving vitamins D_2 and D_3, and the controls. No significant differences in egg production were recorded for the duration of the experiment, which extended from January through July. In a later experiment of similar nature Gutteridge and Novikoff (1947) obtained results which verified the previous work.

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In this case the thyroprotein was fed at the rate of ten grams per one hundred pounds of feed, and as before egg production for the various groups was about the same.

2. Experiments Involving the Various Tests for Egg Shell Quality

Various methods have been devised for testing for egg shell quality. Morgan (1932) tested for percent of shell, breaking strength, and thickness of shell by breaking the shell by applying increased weight to the egg at right angles to the long axis. A test for breaking strength of the shell by measuring the striking force of a mass of known weight falling as a free body through a given distance onto the egg was devised by Swenson and James (1932). Lund, Heiman, and Wilhelm (1938) employed a complicated device involving a pulley, fulcrum, carriage, weight, and graduated scale, as a means of testing the egg shell for breaking strength and shell thickness. An experiment using the moisture loss as a criterion of egg shell quality was conducted by Quinn, etc. al. (1945). The egg weight in grams (nearest tenth) was recorded the day after the eggs were laid, and the eggs were then subjected to a fourteen day incubation period in a forced-draft incubator (99.5° F. and sixty percent relative humidity). Following the incubation period the eggs were re-weighed to ascertain the moisture loss.

3. Reasons for Employing Moisture Loss Test

The moisture loss test has the advantage of being a simple total score test for egg shell quality. It does not involve testing specifically for such factors as, shell strength, porosity, thickness, percent of shell, and the shell membrane characteristics. Individual tests for these numerous factors tend to bring in such variables, as the manner of breaking or crushing the shell and the fact that different parts of the egg shell respond differently. Further the size of the air cell has long been used as one of the standards of quality in eggs, and the less of moisture, which is accompanied by the loss of weight, results in increased size of the air cell. Though this type of test does not test individually for the various shell quality factors, it does serve as an overall measure of these factors.

4. Egg Shell Quality Tests and Records

A record including the results of the incubation of the eggs of each group for the week of May 19 to 23, 1947, was made. The average moisture loss rates of the two groups of pullets were compared to determine the effect, if any, of thyroprotein on egg shell quality. In order to further investigate the possible influence of iodocasein on egg shell quality, a second test was conducted, which included the incubation of those eggs laid from October 13 to 20, 1947. The latter records were summarized individually first, then compared with those of the first test to determine whether or not, the feeding of thyroprotein might cause a seasonal variation in egg shell quality.

E. Experimental Procedure to Test for the Effect of Iodinated Casein on Egg Weights

In order to obtain an idea of the possible effect of iodocasein on egg weights, the eggs of the two groups of pullets were weighed once each week for the first two and one-half months of the experiment, then twice in June and once in July, and the weighings of the eggs of October 13 to 20 were also included. These weighings were then divided into five different intervals. The egg weight which was nearest the mean for that particular period for each bird that laid during that period was recorded. The average egg weight of each period was then computed. Finally an overall average was computed. The average egg weights of both groups of the pullets for the five periods were then compared and summarized.

Experimental Results

Pullets-Egg Production

The Rhode Island Red pullets employed in this experiment laid at their greatest rate during the first four week interval, which began March 3, 1947 (Tables 1 and 2, and Figure no. 1). This peak performance by both groups was followed in the second four week period by a decrease of 0.7 percent in production by the controls, and a decrease of 15.2 percent by the experimental birds. The two groups continued to decrease throughout the third interval of April 28 to May 26. The controls declined more rapidly through this third period, but their production remained above that of the thyroprotein-fed birds. The period from May 26 to June 23 found both groups showing an increase in production at about the same rate.

The experimental birds apparently began their seasonal decline in late June, while the control birds registered a slight increase in their production during the period of June 22 to July 21, at which time they began their seasonal decline. A rapid reduction in production was recorded by the controls from July 21 to September 15. The thyroprotein-fed group also exhibited a similar decrease during this period, but at a slightly more retarded rate. Both groups fell off precipitously in production from September 15 to October 13, as a result of their going into a melt. During the last four week interval the experimental birds increased their production 8.7 percent, while the controls increased only 0.5 percent.

The two groups were nearly equal in egg production on the basis of their laying performances during the first five months of the laying year, the controls laying at the rate of 38.3 percent during this period, with the experimental birds laying at the rate of 37.9 percent. Except for the first and last intervals the control birds consistently laid at a greater rate, this rate varying from 4.6 to 20.8 percent, or at an average of 11.7 percent. The controls had an average egg production of 47.8 percent for the first four intervals, while the thyroprotein-fed birds averaged 41.9 percent for the same period.

The seasonal decline of the controls extended over three four-week intervals, and their production average for this period was 28.3 percent with the average decrease being 12.8 percent. In contrast to this the seasonal decline of the experimental group covered a duration of four four-week intervals, while the average production for this period was 19.8 percent and the average decrease for this period was 8.5 percent. During the second interval of their seasonal decline, that is, from July 21 to August 17, the experimental birds showed a tendency to resist the decline, by dropping only 2.0 percent in their average production, while the controls decreased 7.4 percent during this time. As indicated by Table 9, this is the period of the highest mean temperature. In referring to this table it will be noted that these were outside atmospheric temperatures. During March, April, and May, artificial heat was supplied to maintain a constant temperature of sixty degrees Farenheit in the battery room. Therefore those temperatures listed in the table after May are the only ones that apply for this experiment.

Statistical analysis, employing standard deviations and standard error, showed that the difference in the overall average egg production of the two groups of pullets was not large enough to be significant. The standard deviation of the overall average egg production of the

TABLE 1 -- Egg Production

Let I - Controls	Egg production by 4 week intervals									
Legband Number	Mar 3 to Mar 31	Mar 31 to Apr 28	Apr 28 to May 26	May 26 to Jun 23	Jun 23 to Jul 21	Jul 21 to Aug 18	Aug 18 to Sept 15	Sept 15 to Oct 13	Oct 13 to Nov 10	Total
8705	7	7	13	13	12	6	1	0	2	61
8711	21	18	11	16	16	15	12	2	3	114
8712	9	17	20	19	15	16	12	2	5	115
8714	19	14	16	14	17	18	11	5	7	121
8717	9	2	7	9	9	6	3	4	4	53
8731	12	3	0	1	0	0	1	1	0	18
8739	15	19	17	11	17	10	6	1	0	96
8740	14	13	10	8	14	10	3	7	4	83
8749	9	15	18	15	13	5	3	0	1	79
8752	15	13	6	5	4	5	3	2	2	55
8754	18	11	2	9	16	15	13	13	11	108
8762	15	18	4	15	18	19	11	0	3	103
8772	21	20	17	19	18	17	13	0	9	134
8784	7	11	11	10	11	9	10	2	0	71
8789	16	8	3	20	19	13	4	0	0	83
8793	19	20	18	13	13	16	13	2	2	116
8794	1	18	20	19	19	17	16	9	2	121
8795	25	21	0	25	22	17	19	6	5	140
8800	22	22	20	20	16	13	9	6	5	133
Total	274	270	213	261	269	227	163	62	65	1804
% E.P.	51.5	50.8	40.0	49.1	50.1	42.7	30.6	11.7	12.2	37.7

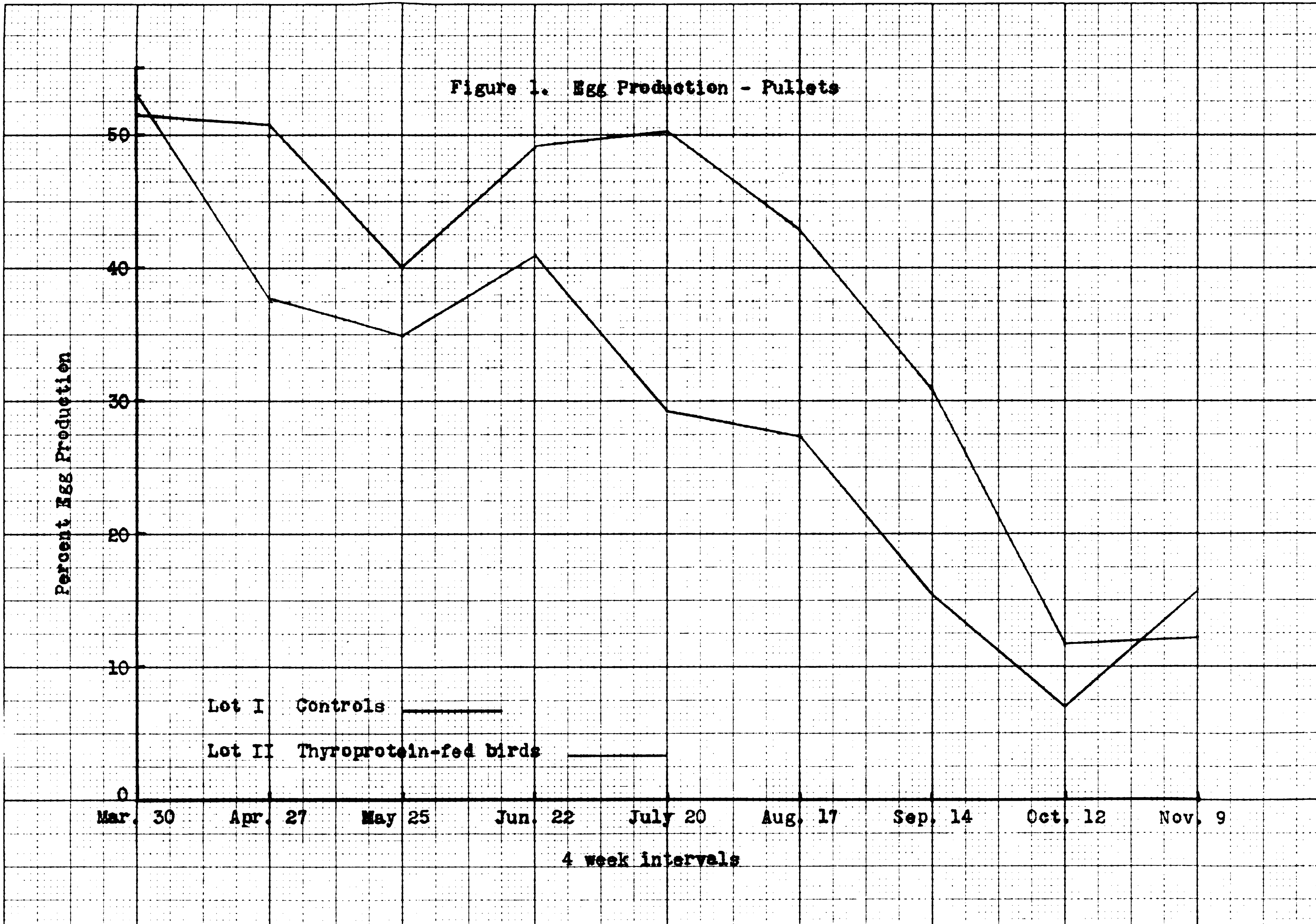
Figure 1. Egg Production - Pullets
Percent Egg Production
50
40
30
20
10
0
Mar. 30
Apr. 27
May 25
Jun. 22
July 20
Aug. 17
Sep. 14
Oct. 12
Nov. 9
4 week intervals
Lot I Controls
Lot II Thyroprotein-fed birds

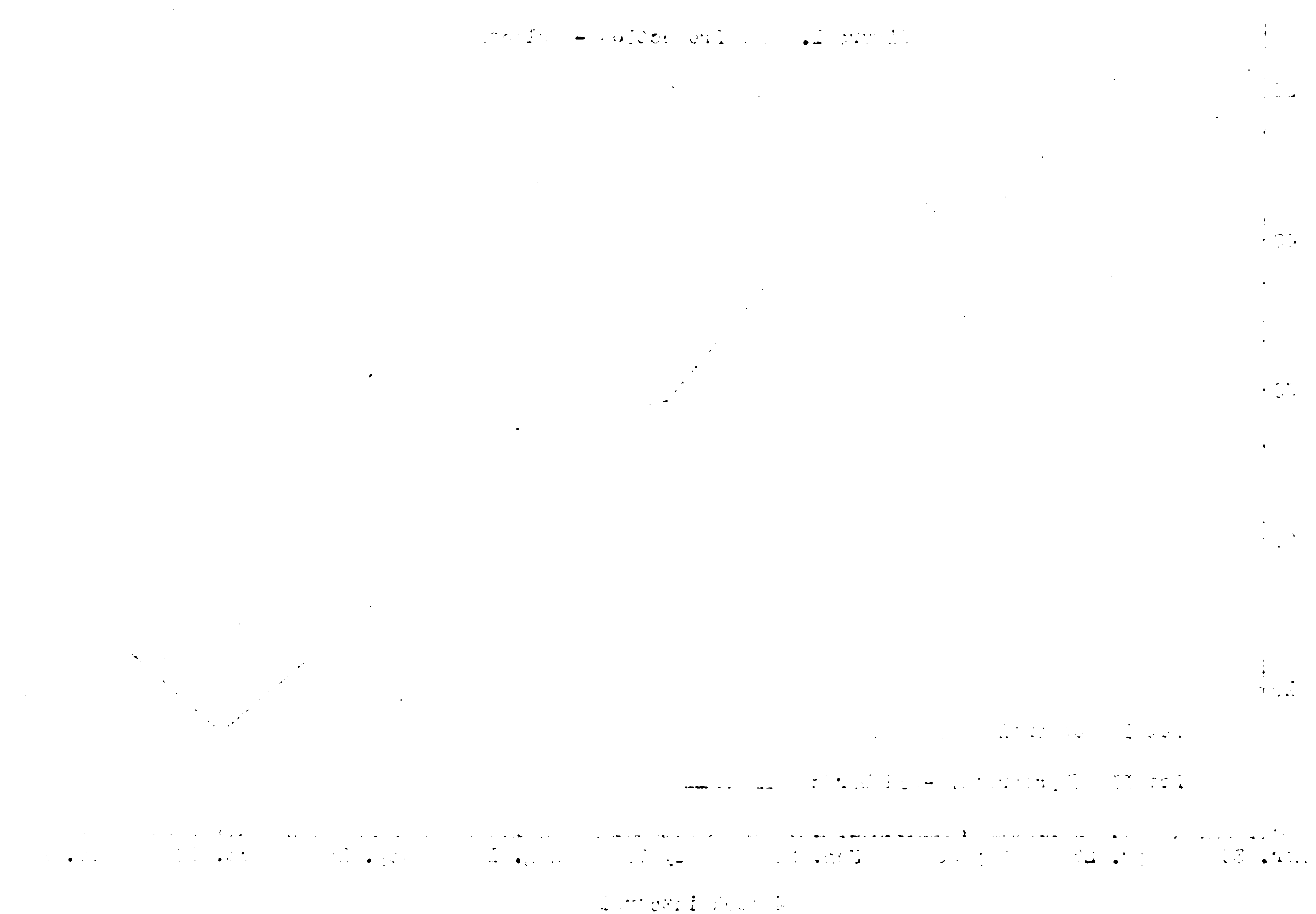

TABLE 2 -- Egg Production

Lot II - Feed contained 10 grams thyroprotein per 100 pounds. Egg production by 4 week intervals										
Legband Number	Mar 3 to Mar 31	Mar 31 to Apr 28	Apr 28 to May 26	May 26 to June 23	Jun 23 to Jul 21	Jul 21 to Aug 18	Aug 18 to Sept 15	Sept 15 to Oct 13	Oct 13 to Nov 10	Total
8708	18	1	0	2	15	1	0	0	0	37
8727	15	14	10	7	4	3	4	2	10	70
8728	7	2	2	5	6	0	11	4	8	45
8738	9	8	11	14	12	15	5	4	11	89
8745	20	13	13	10	12	13	0	7	10	98
8748	16	15	9	13	13	9	6	2	16	99
8750	14	11	2	17	13	8	2	3	0	70
8758	21	20	20	19	16	14	11	7	1	129
8759	0	1	1	12	0	16	3	0	0	33
8766	11	14	9	14	2	5	0	0	0	55
8768	12	15	10	4	9	7	0	2	0	59
8770	14	5	7	16	7	3	15	7	6	80
8773	19	1	2	11	0	11	0	0	0	44
8777	18	3	15	18	4	10	7	0	0	65
8785	15	13	17	12	6	0	0	0	7	70
8788	16	14	15	11	6	7	6	0	15	90
8790	17	16	16	13	9	12	3	0	0	86
8791	23	20	17	17	16	3	1	0	0	97
8799	17	15	10	13	6	8	8	0	0	77
Total	282	201	186	218	156	145	82	38	84	1392
% E.P.	53.0	37.8	35.0	41.0	29.3	27.3	15.4	7.1	15.8	29.1

control birds was 31.9, while for the thyroprotein-fed birds it was 24.0. The standard error, using these standard deviations, for the two groups was 9.16. The difference between the means of the two groups was 21.6, which was considerably below three times the standard error, or 27.5. The fact that this difference of 21.6 was more than twice the standard error, or 18.3, would indicate that it approached significance. However, it would appear that other factors, such as, variation in the amount of thyroxine available, or environmental conditions, are involved here, and further work would be necessary to determine whether or not this difference was definitely significant.

Pullets-Body Weight and Feed Consumption

The body weights of the two groups of pullets followed a rather similar pattern for the duration of the experiment (Tables 3 and 4). The controls outweighed the experimental group by about 0.45 of a pound on March 31. In general, this difference continued throughout the experiment, except for a difference of 0.3 of a pound in favor of the controls on August 18, and 0.6 of a pound, again in favor of the controls, on October 13. Both groups showed a slight decline for the next two intervals following the March 31 weighing. A slight increase occurred from May 26 to June 23, and this was followed by a negligible drop during the next interval. From July 21 to September 15 the two groups both increased about 0.25 of a pound. Although the thyroprotein-fed birds showed a small decrease in body weight from September 15 to October 13, while the controls remained about the same for this period, both groups increased a little during the last interval. On the basis of the overall average, the controls weighed 5.38 pounds, and the experimental birds weighed 4.93 pounds.

The feed consumption of the two lots of pullets included those birds that died and their partners. Because of this the number of birds in the two groups differed beginning with the interval of July 21 to August 18. The fact, that four of the controls died, while only one of the experimental birds died, would infer that the latter group would have the greater feed consumption. Actually the controls consumed the most feed, although their consumption was not noticeably larger, the difference ranging from 0.2 to 0.7 of a pound per bird (Tables 3 and 4).

Pullets-Molt

As indicated by Table 5, the molt of both groups of the pullets was well underway when the first check was made. At the time of the first observation on October 5, 1947, the thyroprotein-fed birds had molted 32.2 percent, while the controls had molted 19.7 percent. During the five weeks following October 5, the birds were checked twice, first on October 26, and again on November 9. Both of these observations showed the two groups to be molting at about the same rate. The controls increased 13.7 percent in the progress of their molt during the five weeks, while the experimental birds showed an increase of 14.5 percent.

Pullets-Egg Shell Quality

The first test for differences in egg shell quality was made with the eggs of May 19 to 23, 1947 (Table 6). The average moisture loss in grams for the two groups of pullets varied daily from 0.2 to 0.6 of a gram. The overall average for the controls was 2.85 grams, or 4.8 percent; for the experimental birds it was 3.14 grams, or 5.5 percent.

The next test involving egg shell quality included those eggs of October 13 to 20, 1947 (Table 7). The daily variations in moisture loss

TABLE 3 -- Body Weight and Feed Consumption

Lot I - Controls Body weight and feed consumption per bird by 4 week intervals										
	Mar 3 to Mar 31	Mar 31 to Apr 28	Apr 28 to May 26	May 26 to Jun 23	Jun 23 to Jul 21	Jul 21 to Aug 18	Aug 18 to Sept 15	Sept 15 to Oct 13	Oct 13 to Nov 10	Overall Average
Body wgt.	5.37	5.31	5.25	5.34	5.25	5.28	5.47	5.51	5.63	5.38
Feed Con.	7.2	7.1	6.7	7.1	6.9	6.7	6.7	6.7	6.1	

TABLE 4 -- Body Weight and Feed Consumption

Lot II - Feed contained 10 grams thyroprotein per 100 pounds Body weight and feed consumption per bird by 4 week intervals										
	Mar 3 to Mar 31	Mar 31 to Apr 28	Apr 28 to May 26	May 26 to Jun 23	Jun 23 to Jul 21	Jul 21 to Aug 18	Aug 18 to Sept 15	Sept 15 to Oct 13	Oct 13 to Nov 10	Overall Average
Body wgt.	4.95	4.83	4.82	4.89	4.82	4.99	5.12	4.92	5.02	4.93
Feed Con.	7.0	6.4	6.3	6.6	6.2	6.2	6.0	5.6	5.9	

TABLE 5 -- Molt

Lot I - Control Pullets, Progress of molt for 5 weeks				Lot II - Thyroprotein-fed Pullets, Progress of molt for 5 weeks			
Legband Number	New Primaries			Legband Number	New Primaries		
	October 5	October 26	November 9		October 5	October 26	November 9
8705	3-6	4-0	4-2	8708	0-0	0-0	0-0
8711	0-3	0-6	1-3	8727	1-0	1-4	1-6
8712	2-5	3-6	5-0	8728	0-0	0-0	0-0
8714	2-2	3-3	4-0	8738	1-3	2-4	2-6
8717	0-0	0-0	0-0	8745	5-3	6-2	6-4
8731	8-5	10-0	10-0	8748	0-6	1-4	1-6
8739	0-5	2-0	3-5	8750	6-0	6-3	6-5
8740	0-0	0-0	0-0	8758	0-5	2-3	3-6
8749	5-2	5-5	6-1	8759	5-2	5-1	6-1
8752	0-0	0-3	0-5	8766	5-0	5-4	6-0
8754	0-1	0-4	1-0	8768	2-0	2-5	3-2
8762	3-1	4-4	4-6	8770	4-0	4-1	4-3
8772	2-1	4-2	5-2	8773	5-4	7-6	8-4
8784	0-4	1-1	1-5	8777	4-1	5-2	6-1
8789	4-2	6-2	7-0	8785	7-5	8-0	8-0
8793	0-0	0-0	0-6	8788	2-1	2-6	3-0
8794	0-0	0-0	1-3	8790	4-0	5-3	6-2
8795	1-3	3-1	3-5	8791	4-3	6-0	6-1
8800	2-0	2-0	2-2	8799	1-3	2-4	3-1
Total	37-5	52-2	63-3	Total	61-1	76-3	84-6
Average	2.0	2.8	3.3	Average	3.2	4.0	4.5
% Molt	19.8	27.5	33.4	% Molt	32.2	40.2	44.7

EXPLANATION: Partially grown new primaries scored as follows, old primary just dropped, 1; one week grown, 2; two weeks grown, 3; three weeks grown, 4; four weeks grown, 5; five weeks grown, 6; Total of partially grown new primaries divided by seven (full grown new primary), and added to total number of full grown new primaries. For example, no. 8705 had 3 full grown new primaries, and one partially grown new primary - 5 weeks.old.

TABLE 6 -- Egg Shell Quality

Moisture loss in grams for eggs of May 19 to 23							
Lot I - Controls	May 19 to June 2	May 20 to June 3	May 21 to June 4	May 22 to June 5	May 23 to June 6	Total	% Moisture Loss
Number of birds	5	7	9	7	7	35	
Total Moisture Loss	14.0	18.8	20.0	23.8	23.1	99.7	
Average Moisture Loss	2.8	2.7	2.9	3.4	3.3	2.85	4.8
Lot II - Thyroprotein fed birds							
Number of birds	9	4	8	3	8	32	
Total Moisture Loss	23.2	9.8	27.7	10.0	29.8	100.5	
Average Moisture Loss	2.6	2.4	3.5	3.3	3.7	3.14	5.5

TABLE 7 -- Egg Shell Quality

Moisture loss in grams for eggs of October 13 to 20										
Lot I - Controls	Oct 13 to Oct 27	Oct 14 to Oct 28	Oct 15 to Oct 28	Oct 16 to Oct 29	Oct 17 to Oct 30	Oct 18 to Oct 31	Oct 19 to Nov 1	Oct 20 to Nov 2	Total	% M.L.
No. of birds	3	4	5	5	2	4	3	2	29	
Tot. of M. L.	7.4	18.4	15.6	21.1	5.1	13.1	10.5	8.3	99.5	
Ave. Moist. Loss	2.4	4.6	3.1	4.2	2.6	3.3	3.5	4.2	3.42	6.1
Lot II - Thyro-protein-fed birds										
No. of birds	2	3	3	3	2	5	3	4	25	
Tot. Moist. Loss	5.3	13.7	12.6	9.9	5.7	16.1	9.0	13.5	83.8	
Ave. Moist. Loss	2.6	4.6	4.2	3.3	2.8	3.2	3.0	3.4	3.43	6.2

in grams for the two groups for these eggs ranged from 0.2 to 1.2 grams. The controls had an overall average moisture loss of 3.42 grams or 6.1 percent, and the experimental birds had an overall average of 3.43 grams or 6.2 percent.

Pullets-Egg Weights

On the basis of two weighings from March 11 to March 18, the controls had an average egg weight of 58.0 grams, while the experimental birds had an average egg weight of 56.9 grams (Table 8). The average egg weight of the controls increased to 59.1 grams for the weighings of May 7 to May 23, and the final weighings of October 13 to 20 showed a decrease to 55.8 grams. The average egg weight of 58.6 grams recorded during the weighings of May 28 to July 21 was the peak for the experimental birds, and their lowest average, 55.8 grams occurred during the October 13 to 20 weighings. The controls had an overall average of 57.9 grams, and the experimental birds had an overall average of 57.7 grams.

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TABLE 8 -- Egg Weights

Egg weights for 5 different intervals						
Lot I - Controls	Mar 11 to Mar 18	Apr 2 to Apr 30	May 7 to May 23	May 28 to Jul 21	Oct 13 to Oct 20	Average
Number of weighings	2	5	7	4	8	
Number of birds	10	18	15	17	13	
Average egg weight	58.0	58.2	59.1	58.0	55.8	57.9
Lot II - Thyroprotein-fed birds						
Number of weighings	2	5	7	4	8	
Number of birds	13	14	15	17	8	
Average egg weight	56.9	57.4	57.2	58.6	55.3	57.7

TABLE 9 -- Outside Temperatures

Mean temperatures in degrees Farenheit								
	March	April	May	June	July	August	September	October
Monthly mean	29.0	43.8	52.1	63.6	68.2	75.2	62.4	57.8

Two and Three Year Old Birds - Egg Production

Both groups of the two year old birds showed a marked drop in egg production during the second interval which extended from May to June 22 (Tables 10 and 11, and Fibure no. 2). This was followed by an increase of 15.4 percent by the experimental birds, and 10.2 percent for the controls during the next period. The two groups both started their seasonal decline at the beginning of the fourth interval on July 21. From July 21 to August 17 the controls dropped 11.8 percent, while the thyroprotein-fed birds dropped only 0.5 percent for the same period. The controls decreased 20.9 percent to an average egg production of 5.1 percent from August 18 to October 13. For the same period the experimental birds declined 10.2 percent to an average egg production of 4.6 percent. During the last interval the controls increased their production to 9.2 percent, while the thyroprotein-fed group remained the same. Except for the interval of September 15 to October 13, the controls maintained a markedly higher average egg production than did the experimental birds. This superiority ranged from 4.6 to 22.5 percent. In addition three of the controls laid more than forty-five eggs for the entire period, while none of the experimental birds laid more than forty-five eggs during the experiment. One of the thyroprotein-fed birds died during the experiment, and the records of this bird together with the records of the corresponding paired bird in the control group, were eliminated from the final compilation.

Following an average egg production of 16.9 percent during the initial period, the three year old controls went out of production completely during the next interval, and laid only one egg during each of the next two intervals (Tables 12 and 13, and Figure no. 3). The experimental

TABLE 10 -- Egg Production

Lot III - Control Two Year Old Birds Egg production by 4 week intervals								
Legband Number	May 15 to May 26	May 26 to Jun 23	Jun 23 to Jul 21	Jul 21 to Aug 18	Aug 18 to Sept 15	Sept 15 to Oct 13	Oct 13 to Nov 10	Total
6214	2	4	17	14	0	3	6	46
6237	0	14	19	19	9	0	0	61
6276	3	0	10	0	12	2	10	37
6299	7	4	8	5	3	3	2	32
6304	9	21	17	13	7	2	0	69
6326	4	1	0	0	0	0	0	5
6331	2	0	3	0	0	0	0	5
Total	27	44	74	51	31	10	18	255
% E.P.	35.1	22.4	37.8	26.0	15.8	5.1	9.2	20.4

TABLE 11 -- Egg Production

Lot IV - Two Year Old Thyroprotein-fed Birds Egg production by 4 week intervals								
Legband Number	May 15 to May 26	May 26 to Jun 23	Jun 23 to Jul 21	Jul 21 to Aug 18	Aug 18 to Sept 15	Sept 15 to Oct 13	Oct 13 to Nov 10	Total
6209	2	1	2	1	3	0	2	11
6211	4	0	0	11	4	2	0	21
6275	0	0	0	0	0	0	0	0
6292	3	4	10	1	8	3	0	29
6308	3	1	9	8	3	4	7	35
6309	8	4	9	8	1	0	0	30
6330	2	0	0	0	0	0	0	2
Total	22	10	30	29	19	9	9	128
% E.P.	28.6	5.1	15.3	14.8	9.7	4.6	4.6	10.2

TABLE 10 -- Egg Production

Lot III - Control Two Year Old Birds

Egg production by 4 week intervals

Legband Number	May 15 to May 26	May 26 to Jun 23	Jun 23 to Jul 21	Jul 21 to Aug 18	Aug 18 to Sept 15	Sept 15 to Oct 13	Oct 13 to Nov 10	Total
6214	2	4	17	14	0	3	6	46
6237	0	14	19	19	9	0	0	61
6276	3	0	10	0	12	2	10	37
6299	7	4	8	5	3	3	2	32
6304	9	21	17	13	7	2	0	69
6326	4	1	0	0	0	0	0	5
6331	2	0	3	0	0	0	0	5
Total	27	44	74	51	31	10	18	255
% E.P.	35.1	22.4	37.8	26.0	15.8	5.1	9.2	20.4

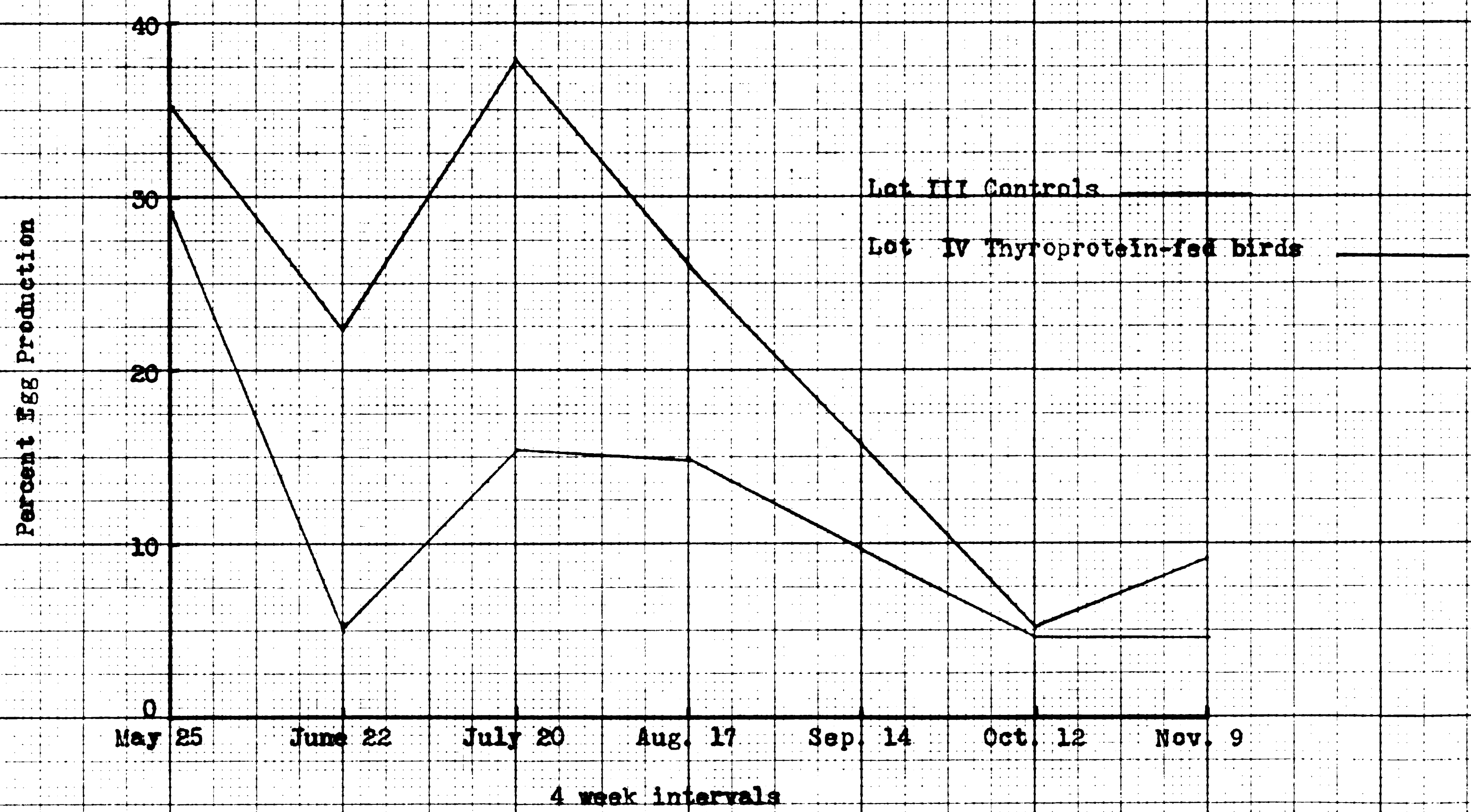
TABLE 11 -- Egg Production

Lot IV - Two Year Old Thyroprotein-fed Birds

Egg production by 4 week intervals

Legband Number	May 15 to May 26	May 26 to Jun 23	Jun 23 to Jul 21	Jul 21 to Aug 18	Aug 18 to Sept 15	Sept 15 to Oct 13	Oct 13 to Nov 10	Total
6209	2	1	2	1	3	0	2	11
6211	4	0	0	11	4	2	0	21
6275	0	0	0	0	0	0	0	0
6292	3	4	10	1	8	3	0	29
6308	3	1	9	8	3	4	7	35
6309	8	4	9	8	1	0	0	30
6330	2	0	0	0	0	0	0	2
Total	22	10	30	29	19	9	9	128
% E.P.	28.6	5.1	15.3	14.8	9.7	4.6	4.6	10.2

Figure 2. Egg Production - Two Year Old Birds



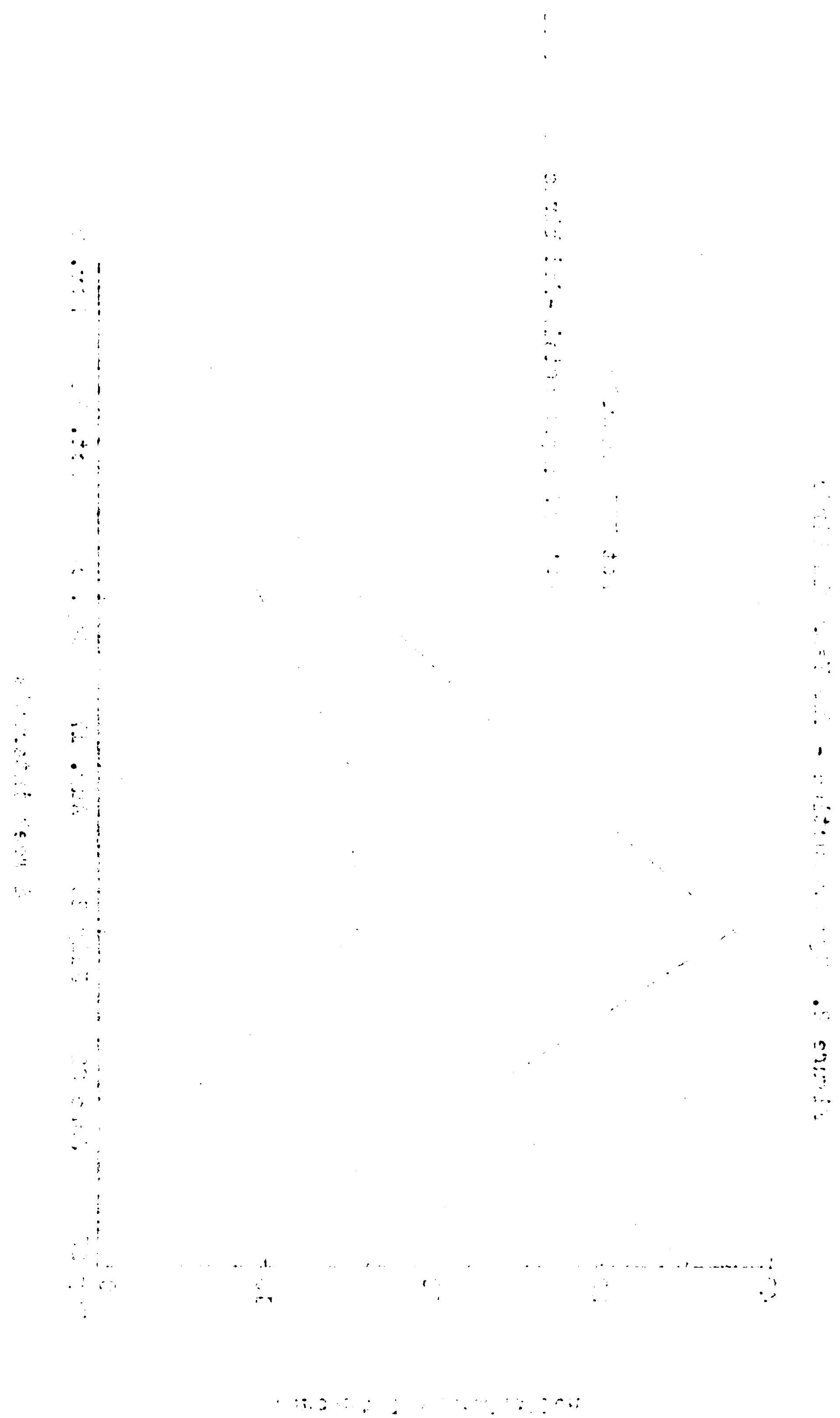


TABLE 12 -- Egg Production

Lot V - Control Three Year Old Birds

Egg production by 4 week intervals

Legband Number	May 15 to May 26	May 26 to Jun 23	Jun 23 to Jul 21	Jul 21 to Aug 18	Aug 18 to Sept 15	Sept 15 to Oct 13	Oct 13 to Nov 10	Total
161	4	0	0	1	0	0	0	5
3214	2	0	0	0	0	0	0	2
3242	1	0	1	0	0	0	0	2
3256	0	0	0	0	0	0	0	0
3287	0	0	0	0	1	0	0	1
3296	3	0	0	0	5	3	0	11
3316	3	0	0	0	0	2	8	13
Total	13	0	1	1	6	5	8	34
% E.P.	16.9	0.0	0.5	0.5	3.1	2.6	4.1	2.7

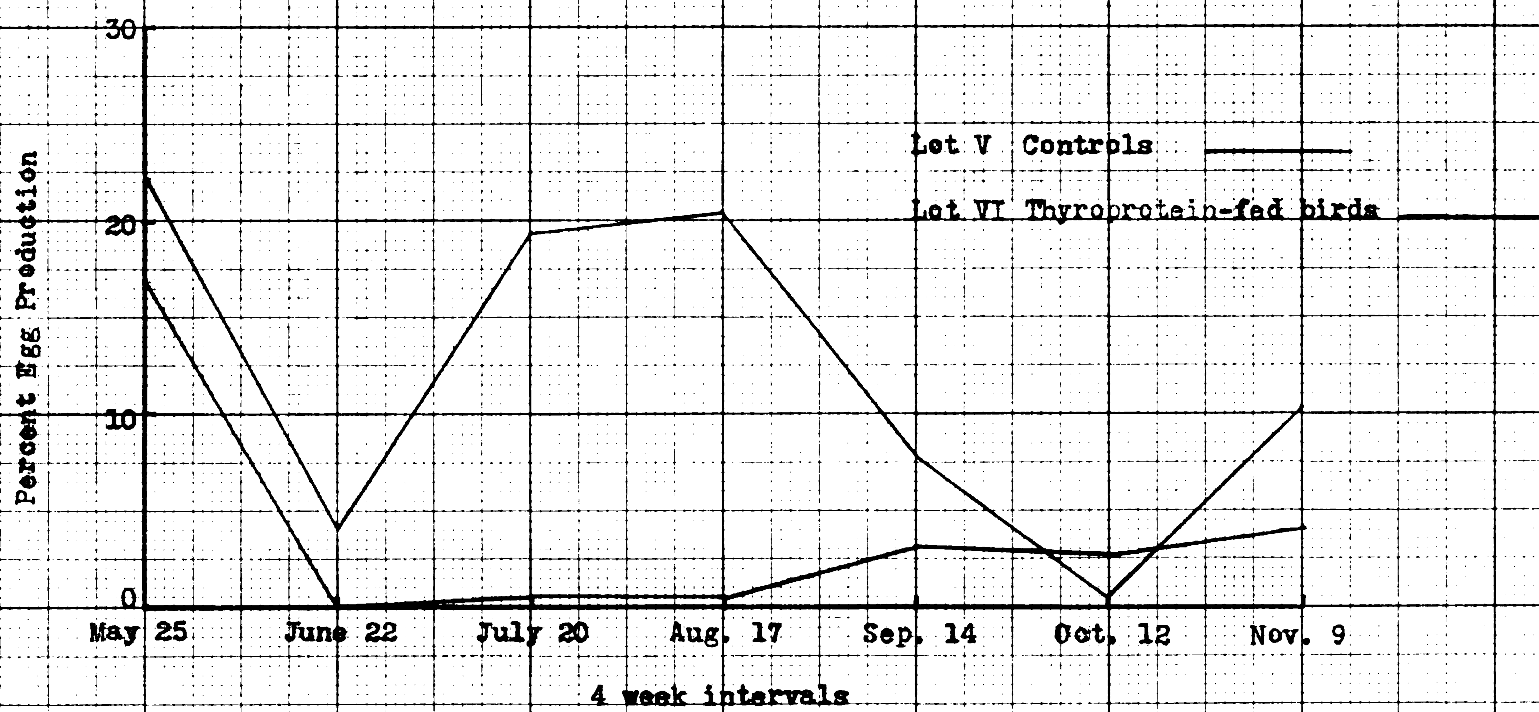
TABLE 13 -- Egg Production

Lot VI - Three Year Old Thyroprotein-fed Birds

Egg production by 4 week intervals

Legband Number	May 15 to May 26	May 26 to Jun 23	Jun 23 to Jul 21	Jul 21 to Aug 18	Aug 18 to Sept 15	Sept 15 to Oct 13	Oct 13 to Nov 10	Total
Y-3	3	6	10	2	5	0	5	31
3208	2	0	5	9	0	1	0	17
3212	2	0	0	4	0	0	10	16
3237	2	0	0	0	4	0	5	11
3276	4	0	7	8	6	0	0	25
3281	3	0	16	17	0	0	0	36
3303	1	2	0	0	0	0	0	3
Total	17	8	38	40	15	1	20	139
% E.P.	22.1	4.1	19.4	20.4	7.7	0.5	10.2	11.1

Figure 3. Egg Production - Three Year Old Birds



birds laid at a rate of 22.1 percent during the first period, then dropped to 4.1 percent from May 26 to June 23. From June 23 to August 18, while the controls were laying only two eggs, the experimental birds increased their average egg production to 19.4 percent on July 21, and 20.4 percent on August 18. The thyroprotein-fed birds showed a seasonal decline from August 18 to October 13, dropping from 20.4 percent to 0.5 percent during this time. Although the controls averaged 3.3 percent during the last three intervals, only three birds were laying during this time. The average egg production of the experimental birds climbed to 10.2 percent during the last period. The thyropretein-fed birds maintained a much higher egg production throughout the experiment except for the interval of September 15 to October 13, and their overall average was 11.1 percent, while that of the controls was 2.7 percent. As with the two year old birds, one of the experimental birds died during the experiment, and the records of this bird along with those of the corresponding paired bird in the control group were not included in the final tabulation.

Two and Three Year Old Birds - Feed Consumption

The feed consumption of those birds that died during the experiment, and the feed consumption of their partners was included in the final computation (Table 14). In addition the feed consumption of the two and three year old controls was combined, as was that of the experimental two and three year olds. The thyroprotein-fed birds consumed slightly more feed throughout the experiment than did the controls, the difference ranging from 1.1 to 0.2 pounds per bird.

TABLE 14 -- Feed Consumption

Feed consumption per bird by 4 week intervals						
	May 26 to Jun 23	Jun 23 to Jul 21	Jul 21 to Aug 18	Aug 18 to Sep 15	Sep 15 to Oct 13	Oct 13 to Nov 10
Lots III & V - Two and Three Year Old Controls	6.1	6.0	6.1	6.0	5.5	5.8
Lots IV & VI - Two and Three Year Old Experimental Birds	6.3	6.2	6.2	7.1	6.7	6.7

Discussion

Egg Production

The level of ten grams of thyroprotein per one hundred pounds of feed was found to be the most nearly optimum in the experiments conducted by Turner, etc. al. (1945), however it would appear that this level was not optimum in this particular experiment. This would indicate that the optimum level for different strains and breeds of birds would tend to vary. Since the previously mentioned experiments were performed in Missouri, the further possibility of the environment affecting the optimum level exists. In addition the thyroprotein used in this experiment tested 3.04 percent thyroxine by chemical assay, while that used at Missouri tested 2.73 percent thyroxine by biological assay, which would imply that there was a difference in the actual amount of thyroxine available.

Inasmuch as the results of this experiment indicate that the level of thyroprotein, or at least the amount of available thyroxine, was not optimum, the question arises, was the level too high or too low? The egg production of both the experimental pullets and two year old birds was consistently depressed in comparison with that of the control birds of these two groups (Tables 1, 2, 10, and 11). However, the egg production of the experimental three year old birds was decidedly stimulated in comparison to that of the three year old controls (Tables 12 and 13). Since thyroid activity is known to decrease with advancing age, Crew (1925), there is a possibility that the optimum level for the three year old birds was slightly lower than that of the two year olds or the pullets. Due to the limited number of birds available only eight of

both the two and three year old birds could be employed in this experiment, and this is a rather small number from which to draw any definite conclusions. Despite this the average egg production of the experimental three year olds was definitely greater, particularly during the months of highest mean temperature, than that of the corresponding controls.

The ten gram level of protamone (2.73 percent thyroxine by biological assay) supplied the chicken with an amount of thyroxine in an excess of that amount normally secreted by the body, Turner, Kempster, Hall, and Reineke (1945). In the experiments of Turner, et. al. (1945), a twenty gram level of thyroprotein was found to have a depressing effect on egg production and was therefore considered in excess of the optimum level. While the results of this experiment imply that there is a possibility that the ten gram level was slightly below the optimum in this particular case, the above information indicates, that the ten gram level need be increased only a few grams, before favorable results might be effected. Therefore, it follows, that this work might have produced more conclusive results, if levels of twelve and possibly fifteen grams of thyroprotein had been included; however, a limited number of birds prevented such procedure.

It is interesting to note that in each of the three different age levels of birds employed there was a definite tendency on the part of the experimental birds to resist the normal seasonal decline in egg production during the period of July 21 to August 18, which was the period of the highest monthly mean temperature. In addition the seasonal decline of the experimental pullets and the experimental two year old birds, was somewhat retarded in comparison with that of the control birds of these two age groups. Although these results can not be considered decisive, there does exist some evidence that the feeding of thyroprotein may yet be found to have value in increasing egg production by delaying the normal seasonal decline. Apparently the feeding of thyroprotein supplies the birds with a constant level of thyroxine throughout the laying year, whereas high temperatures probably cause a reduction in the normal rate of secretion of thyroxine by the thyroid gland, and consequently the egg productien undergoes a seasonal decline during the summer and early fall.

Body Weight and Feed Consumptien

The body weight of the control pullets was greater than that of the experimental pullets (Tables 3 and 4). The greater weight of the controls remained at a fairly constant rate throughout the experiment. This would indicate that the controls were heavier as a group than the thyroprotein-fed birds, and that the level of thyroprotein (available thyroxine) employed here had no noticeable effect on body weight.

The control pullets consumed more feed during the experiment than did the experimental pullets. In general, however, the feed consumption of the controls was not markedly greater than that of the thyroprotein-fed birds. Furthermore the controls maintained a higher average egg production and were a heavier group of birds, and for these reasons would naturally consume more feed. Therefore, it can be assumed that the ten gram level of protamone used here, had no marked effect on feed consumption.

The two and three year old thyroprotein-fed birds consumed slightly more feed than did the controls of these two age levels (Table 14). Since the average egg production of the two and three year old experimental birds was slightly more than that of the corresponding controls, it follows that their feed consumption would tend to be a little greater. As a result this slight difference cannot apparently be attributed to any influence of the iodinated casein.

Molt

The experimental pullets had progressed much further in their molt than had the controls, when the first check was made (Table 5). However, the results obtained from the next two observations, indicate that the two groups were molting at approximately the same rate. These results would further imply that the thyroprotein-fed birds started their molt at an earlier date than did the controls. The fact that the controls began their molt at a later date would account to some extent for the superior average egg production of the thyroprotein-fed birds during the last four week interval of the experiment. There is some evidence here to indicate that the feeding of thyroprotein may have had some effect on the molt, since the experimental birds began their molt at an earlier date than did the controls.

Egg Shell Quality and Egg Weights

The eggs laid by the control pullets had a slightly smaller rate of moisture loss in grams than those laid by the experimental birds during the time that the first egg shell quality test was conducted (Table 6). This difference was not great enough to be significant, however. The moisture loss rate of the eggs of the two groups used in the second egg shell quality test differed even less than that of the first test. Comparison of the moisture loss rate of the first and second tests for egg shell quality (Table 7), indicate no significant differences due to seasonal variation. The negligible variations in moisture loss rate obtained in this experiment indicate that the feeding of protamone at the level employed had no effect on egg shell quality.

The differences in the egg weights of the two groups of pullets followed a similar pattern throughout the experiment (Table 8). Here again, the results did not differ sufficiently to infer that the level of thyroprotein herein employed had any influence on the egg weights.

Summary

Three different age groups of Rhode Island Reds received a ten gram level of thyroprotein (3.04 percent thyroxine by chemical assay) per one hundred pounds of feed. The average production of the experimental pullets was 29.1 percent, as against 37.7 percent for the controls. The two year old controls outlaid the experimental birds 20.1 percent to 10.2 percent. Three year old experimental birds were apparently stimulated to a production level of 9.0 percent above that of the controls.

The rate of decrease of the seasonal decline of the experimental birds was somewhat retarded in comparison with that of the controls, particularly during the interval of the highest temperature, July 21 to August 18.

In two tests made for egg shell quality, no significant differences between the moisture loss rate of the two groups of pullets were obtained. In addition it was found that the level of thyroprotein, or at least the amount of available thyroxine used here, had no noticeable effect on body weight, mortality, egg weights, or feed consumption.

Although the two groups of pullets molted at about the same rate from October 5 to November 9, 1947, the experimental birds had molted about 12.4 percent more by October 5, than the controls had. This difference indicated that the feeding of thyroprotein apparently resulted in the experimental birds going into the molt sooner.

The lack of agreement of these results with those of the work of Turner, et. al. (1945) indicates that results obtained in one experiment may not hold for a similar experiment performed with different birds in a different environment, or with different amounts of available

thyroxine. Furthermore, more work is necessary to establish the optimum levels of thyroprotein (available thyroxine) necessary for different strains, varieties, and breeds, and for different environments.

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