THE UNIVERSITY OF MANITOBA

A STUDY OF FEEDING THYROPROTEIN TO BROILER BREEDER HENS: EFFECTS ON THE PRODUCTION PERFORMANCE AND PLASMA THYROID HORMONE LEVELS OF THE HENS AND ON THE PLASMA THYROID HORMONE LEVELS AND GROWTH RATE OF THE PROGENY.

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ABSTRACT

The effect of feeding broiler breeder hens, 0, 5.5, and 16.5 g. of thyroprotein (TP) per 100 kg. of ration for 196 consecutive days (22 to 50 weeks of age) was studied to ascertain if any response was elicited relative to egg production and weight, body weight gain, feed efficiency, fertility, hatchability, length of hatching period, viability of progeny, mortality and certain aspects of thyroid function. The Hycel Protein Bound Iodine (PBI) and a modified Tetralute method were used to determine plasma levels of thyroid hormone(s) in the hens and their day-old and two-week old progeny. As a segment of this study experiments were conducted with broiler replacement pullets from 8 to 22 weeks of age to determine if TP feeding could reduce their mature adult body weight. In conjunction with this, comparisons were made with similar birds on a lysine deficient ration and a TP free control.

At all levels used, broiler breeder hens fed TP produced significantly more eggs, and utilized their feed with greater efficiency than did the controls. At both levels of TP feeding body weight gain and egg weight was lower than for those on the control diet. Livability responded positively to TP feeding at all levels. However, it had no influence on yolk weight, shell

thickness or hatchability. On the other hand, only the high level of TP feeding (16.5 g/100 kg.) increased fertility. Eggs from birds fed the 16.5 g. level required from 6 to 12 hours longer to hatch than did eggs on the other treatments. The body weight of day-old chicks from TP treated hens was not significantly different from chicks on the control ration. However, after being fed a commercial starter diet for 2 weeks immediately post hatching, chicks from the dams that had been fed the 16.5 g. TP level weighed significantly less than did chicks from the hens that were fed the 0, or 5.5 g. levels of TP.

The TP dietary levels imposed between 8 and 22 weeks of age did not influence total feed intake, mortality, body weight gains of the birds. The birds on the lysine deficient ration consumed more feed than did the controls or the TP fed groups. In the lysine deficient group during the same period the birds consumed more feed than the controls or the TP fed group but their body weight gain and mortality levels showed no significant differences.

Throughout the laying period, the plasma PBI level of birds on the control ration tended to parallel their rate of production. Birds that received either level of TP were found to exhibit plasma PBI levels higher than and not in phase with the rate of lay. On the other hand the plasma thyroxine-iodine

(T_4-I) levels for birds fed both levels of TP did not follow the egg production pattern but they were also constantly above the T_4-I plasma levels present in birds on the TP free diet.

Plasma PBI and T_4-I values for day-old chicks from TP treated hens were consistently higher than the controls but when these levels were measured at two weeks of age they closely approximated the control values.

Thyroprotein fed to broiler breeder hens at the rate of 5.5 g/100 kg. diet enhanced egg production, lowered mortality, reduced adult body weight and produced no detrimental effects on fertility, hatchability and viability of offspring (to 14 days of age). It should be noted that hens fed TP at 16.5 g. per 100 kg. of diet showed evidence of delayed hatching time and the progeny at 2 weeks of age weighed less than the controls. In other respects they responded similarly to the hens on the lower level of TP feeding.

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INTRODUCTION

Dietary thyroprotein has had limited experimental use as a means of stimulating egg production with commercial strains of Leghorn hybrids. The results have not been consistent. It has been hypothesized that total egg production may be related to the level of thyroid activity and thyroxine secreted by the hen.

Broiler breeder females have long been noted for their obesity, short production cycle, high incidence of broodiness and low egg yield per bird. Virtually no experiments have been conducted with broiler breeder females that measured their response to dietary thyroprotein.

The study herein reported was conducted with heavy breed birds in an attempt to evaluate the effect of dietary thyroprotein on egg weight, fertility, hatchability and hatching time, feed consumption, feed efficiency, body weight gain, viability of the progeny from thyroprotein fed hens, mortality and certain aspects of thyroid gland function.

As an adjunct to but immediately preceeding the trials with broiler breeder layers, a study was conducted to determine whether dietary thyroprotein could be usefully employed to control the mature weight of broiler breeder pullets prior to and

following the onset of production. In addition to dietary thyroprotein a low-lysine diet was also fed to 8 to 22 weeks old pullets as a means of limiting body weight and providing comparative data.

LITERATURE REVIEW

The Role of the Thyroid Glands in Chickens

Several researches have studied the role of the thyroid gland in growth and egg production in the domestic fowl (Winchester, 1939; Taylor and Burmester, 1940; Winchester, Comar, and Davis, 1949; Winchester, and Davis, 1952; Winchester and Scarborough, 1953). Procedures used by these workers involved thyroidectomy (either having the gland removed anatomically or destroyed radioactively) and replacement therapy through the injection of thyroxine. The results were conclusive. Levels of circulating thyroxine play a definite role in the growth of the chicken, although the absence of it was found to exert no effect on body weight changes of fully grown hens. Thyroidectomy caused a decline in egg production and replacement therapy as described by Winchester and his associates was effective in restoring egg production to the control level.

In the early nineteen twenties and thirties, desiccated thyroid tissues of mammals were often utilized in thyroid function studies in chickens (Crew and Huxley, 1923; Crew, 1925; Cole and Hutt, 1927; Asmundson, 1931; Asmundson and Pinsky, 1935). Crew and Huxley (1923) failed to demonstrate

any appreciable increase in egg production in hens which were administered small quantities of desiccated thyroid for a period of 4 weeks. Later, Crew (1925) described a remarkable rejuvenescent effects on several five to eight years old hens and cocks. However, in the studies of Cole and Hutt (1927), no comparable response was observed. According to Asmundson and coworkers, when considerable amounts of desiccated thyroid were fed to laying hens, a depression in egg production and body weight gain were noticed.

The Development of a Thyroactive Material

The introduction of thyroportein (TP), also known as iodinated casein (trade name, Protamone) in 1942 (Reineke and Turner), enabled a more precise and meaningful comparison in thyroid function studies from different researchers. This thyroactive material was developed by the iodination of casein and the subsequent oxidative coupling of diiodotyrosine molecules in the protein. The thyroxine content present in this material was first estimated to be about 3% (Reineke et al. 1945). This was based on the application of the n-butanol-soluble iodine as an index. Reineke (1954) made use of radioactive isotope dilution technique in a group of 7 preparations and found 1.04% of true thyroxine which was present in an estimated 3.24% of thyroxine in the butanol-soluble fraction. This preparation was then standardized

by the manufacturer to contain 1% l-thyroxine. Hydrolysed TP may contain at least ten iodinated compounds including mono and diiodotyrosine, diiodo and triiodothyronine as well as thyroxine and certain inorganic iodides (Friedberg and Reineke, 1952).

The Use of Thyroprotein in Poultry Feeding Tests

Numerous tests carried out involving TP yielded contradictory results. More definitive results from Missouri seemed to warrant the use of TP in poultry feeding to enhance egg production in Leghorns (Turner et al., 1945a,b; Turner et al., 1946; Turner and Kempster, 1947; Turner, 1948c; Turner and Kempster, 1948, 1949). Their results were drawn from a 7 year study on the same group of hens. Oloufa (1954) reported a significant increase in egg production from Fayoumis, especially in the first two months of production. Recently, Proverbs (1971) reported that the response of broiler breeder hens to TP administration was manifested by greater egg production.

Gutteridge and co-workers reported a negative effect of TP on egg production in short term feeding trials (Gutteridge and Pratt, 1946; Gutteridge and Novikoff, 1947). In addition, according to Hutt and Gowe, 1948; Wheeler and Hoffmann, 1948b; Godfrey, 1949; Lillie et al., 1952; Oloufa, 1953, TP had no effect on egg production. Using TP in poultry feeds at levels

higher than 22 g/100 kg. of feed, was discouraged by some researchers (Hoffmann and Wheeler, 1948; Berg and Bearse, 1951; Savage et al., 1952).

Conflicting conclusions concerning the effect of TP on egg weight have been reported by different investigators. Turner et al. (1945a, b; 1946); Hutt and Gowe (1948); Hoffmann and Wheeler (1948); Turner (1948a); Berg and Bearse (1951); Savage et al. (1952) showed that TP had no effect on egg weight. However, Proverbs (1971) reported that egg weight was reduced when TP was fed at levels greater than 11 g/100 kg. feed during the later part of the laying cycle. Gutteridge and Pratt (1946), Gutteridge and Novikoff (1947) observed an increase in specific gravity of eggs and stronger egg shell from hens fed TP during the summer months. Improved shell quality was also obtained by Hoffmann and Wheeler (1948), when Rhode Island Red pullets were fed 22 g. TP/100 kg. feed from June to October. Wilson (1949) reported a decrease in egg size but an increase in egg shell thickness. Berg and Bearse (1951), Lillie et al. (1952), Oloufa (1953), obtained a similar response. However, Savage et al. (1952) reported a slight increase in shell quality.

Thyroprotein feeding may or may not elicit any effect on body weight and mortality. Turner and co-workers (1945a, b; 1946, 1948) found no appreciable effect on body weight and

mortality of hens when TP was administered at levels between 11 and 22 g. per 100 kg. of feed. This was in agreement with the results reported by other workers (Hutt and Gowe, 1948; Godfrey, 1949; Oloufa, 1953). However, Hoffmann and Wheeler (1948) reported a significant loss of body weight in hens fed TP. Lillie et al. (1952) observed contradictory effects of TP on body weight in their feeding trials. Savage et al. (1952) were able to demonstrate that TP fed hens weighed less throughout the year than hens fed the control diet but found no significant differences in mortality. A marked decrease in body weight gain was found only when 22 g. TP/100 kg. feed was fed during the latter part of the laying cycle (Proverbs, 1971). No differences in mortality were found between the control group and those receiving various levels of TP, as reported by the same investigator.

Literature reviewed did not contain any reports of the effect of feeding thyroprotein on the efficiency of converting feed to eggs though Wheeler et al. (1948), Berg and Bearse (1951) claimed a higher feed efficiency based on body weight gain in TP fed groups. Godfrey (1949) using the same criterion was not able to uncover any similar effect. It has been suggested that the inconsistency of the results of various experiments may be due to the fact that adipose tissue deposition is different in different strains of bird. (Proverbs, 1971).

Thyroprotein administration at a level of 22 g/100 kg. feed to Barred Rock roosters was found to have no effect on the volume or concentration of semen although fertility tests carried out by Shaffner (1948) showed a reduction in semen quality. On the other hand, work done by Huston and Wheeler (1949), in which the same level of TP was fed to Rhode Island Red cocks, did not reveal a decrease in semen quality. A retardation in the onset of egg production from the 20th to 25th week was reported by Wheeler and Hoffmann (1948) who also noticed significantly higher testes weights in treated males. Continuous feeding of TP (44 g/100 kg. diet) to pullets from hatching was found to have no effect on fertility of eggs laid. However, hatchability was markedly increased provided sufficient hatching time was allowed (Wheeler and Hoffmann, 1948b). In contrast, Godfrey (1949) found no effect on hatchability when TP was administered at 22 g. per 100 kg. of feed.

McCartney and Shaffner (1949, 1950) discovered that the eggs of TP fed hens required 12 hours more incubation time than those of the controls. This phenomenom was also suggested in Proverbs recent work (1971). He further reported that fertility in broiler breeder hens was depressed when TP was fed at a level of 22 g/100 kg. of feed, however, a more severe effect was not obtained when a higher level was

used. Hatchability was not in any case found to be affected by dietary level of TP except that on the initiation of the feeding, a depression in hatchability was observed by the above researcher but this was described as a temporary effect.

Thyroid Metabolism in the Chicken

The function of the thyroid gland is governed by the level of the circulating thyroid hormones and the effects of the pituitary release of thyrotrophic hormone (TSH) (Smelser, 1938; Adams and Beeman, 1942; Dvoskin, 1947; and Shellabargar, 1954). The ability of TSH to increase the formation and release of thyroid hormones and to influence iodine uptake has been observed by many investigators (Rawson and Salter, 1940; Keating et al., 1945; Larson et al., 1945; Shellabargar and Godwin, 1954; and Frey and Flock, 1958) although the mode of action of TSH on thyroid gland at the cellular level is as yet unknown.

Ma (1963) demonstrated that the hypothalamus which has been shown to play a role in the secretion of TSH in mammals might not be necessary for the feedback mechanism to function in chickens. This investigator, when studying the effect of exogenous thyroid hormone on the uptake of I^{131} by the thyroid glands of hypophysectomized cockerels with adenohypophyseal autotransplants, found that both thyroid weight and I^{131} uptake

by the thyroid gland were decreased by the exogenous thyroxine and that the decreased I^{131} uptake by the thyroid gland was roughly proportional to the level of thyroxine injected.

Information on thyroid hormone synthesis in avian species is lacking although the synthesis is assumed to follow the same route as demonstrated in most mammals. This involves, 1) the concentrating of iodide within the thyroid (iodide trap) against a concentration gradient over that of blood, 2) the conversion of the iodide to I_2 and 3) the conversion of iodine to I^+ in order that iodination of tyrosine can be accomplished. Chromatographic analysis confirmed the presence of both monoiodotyrosine and diiodotyrosine in chicks (Taurog et al., 1950; Frey and Flock, 1958) and in adult birds (Vlijm, 1958). The conversion of tyrosine to thyroxine and triiodothyronine is stimulated by TSH (Frey and Flock, 1958) and these two thyroid hormones have also been isolated radiochromatographically from thyroid extracts.

That only $1\text{-}T_4$ and $1\text{-}T_3$ are present in the blood stream of chicken have been shown by Shellabargar and Pitt-Rivers (1958) and Wentworth and Mellen (1961). The latter investigators also demonstrated that both T_3 and T_4 found in the blood of chickens, turkeys and ducks is present at the ratio of 60% T_4 to 40% T_3. In most mammals, an alpha-globulin plays a major role as a carrier of T_4 in the blood circulation

(Robbins,et al., 1961), whereas, in chickens and ducks, serum albumin and to a lesser extent T_4-binding prealbumin are considered to be the major carriers. No T_4-binding globulins have ever been discovered in chickens, turkeys, and pigeons (Farer, Robbins, Blumberg, and Rall, 1962).

Tata and Shellabargar (1959) reported that the biological half-lives of T_3 and T_4 are identical in birds and this is apparently different from that in mammals. Triiodothyronine has been reported to be 3 to 7 times more potent that T_4 in preventing goiter in rats (Robbins et al., 1961) but T_3 was found to be no more potent than T_4 in chicks (Shellabargar 1955). As an antigoitrogenic substance in the chicken and as counteracting agent in reducing thyroid-stimulating hormone TSH secretion, Newcomer (1957), Mellen and Wentworth (1959) reported that T_4 is more potent than T_3 in this respect. However, the former investigator (Newcomer, 1957) was able to show that 1-T_4 and 1-T_3 are equally active in influencing oxygen consumption, suffocation time, heart rate, feather length in thiouracil-treated chicks. These phenomena which differ from the responses noted for mammalian species were partly explained on the basis that in mammals the T_4-binding globulin has a 4 fold affinity for T_4 as compared to T_3 whereas in avian species an equal protein-binding affinity of T_3 and T_4 for albumin and T_4-binding prealbumin exists (Tata and

Shellabargar, 1959).

Data in the literature on the PBI present in the circulation of birds were limited. Bumgardner and Shaffner (1957) reported a mean value of 1.12 ug. % for PBI, with no line differences when studying 4-week old New Hampshire chicks of 2 lines differing in response to thiouracil. Mellen and Hardy (1957) disclosed a mean value of about 1.13 - 1.22 ug. % in 8- and 20-month old chickens but were unable to alter PBI levels by either cold stress or thiouracil treatment. Apparently, these values are far below those for mammalians. This was explained on the same basis that has been discussed earlier that the absence of a specific thyroxine binding globulin in avian blood may contribute to the result. Thus, investigators (Mellen and Hardy, 1957; Bumgardner and Shaffner, 1957) questioned the sensitivity of this method to evaluate the level of the thyroid circulating hormones. It seems that a further study is needed.

Restricted Feeding of Growing Pullets

In the past 40 years, many investigators have studied the feeding and management of pullets during the rearing period with the object of reducing feeding costs and improving laying performance. Lee et al. (1971) reviewed this type of research and classified the treatments into the following basic 4 management procedures that retard body weight and delay

sexual maturity:

1. Limiting the time of birds' access to feed (Vondell,1943; Couch et al., 1957; Arbor Acres Review, 1965; Harms et al. 1968).
2. Quantitative feed restriction (Milby and Sherwood, 1953; Sherwood and Milby 1954; Singsen et al., 1954; Howes and Cottier, 1964).
3. The use of low energy diets (Singsen et al., 1954; Schneider et al., 1955; Couch et al., 1957; Isack et al., 1960; Howes and Cottier, 1964; Waldroup et al., 1966).
4. Dietary protein restriction (including the use of a diet low in certain amino acids e.g. lysine; Harms and Waldroup, 1962; Singsen et al., 1965; Waldroup et al., 1966; Couch, 1966; Couch, 1967, 1968; Sherwood et al., 1969; Couch and Trammell, 1970; Lee et al., 1971).

Of these 4 major methods, the use of a diet low in lysine under title 4, will be reviewed in greater detail.

Singsen et al. (1964, 1965) reported no effect on body weight at 21 weeks of age when birds were fed a normal ration for 4 weeks or longer and later changed to the lysine-deficient diet (0.65%), but when the lysine-deficient diets were given from one day of age up to 21 weeks of age, there was

a marked retardation of body weight gain at 21 weeks. These investigators, however, indicated that the growing treatment did not appear to have any influence on the cumulative total egg production, adult mortality, body weight, egg size and hatchability of fertile eggs.

Couch (1966) fed a simplified pullet developer (0.6% lysine) to birds from day-old to 26 weeks of age and reported that the average weights of the pullets of the low lysine fed group at 16 and 24 weeks of age were lower than those of the controls. In a second study (1967), the lysine levels used were varied from 0.32 to 0.64% and the feeding of these diets commenced at 7 and 9 weeks of age. It was found that an advantageous effect on retarding body weight gain and delaying sexual maturity could be achieved more effectively if the lysine-deficient diet was administered earlier (7 weeks of age).

Couch and Trammel (1971) reported a similar response indicating that restricted pullets consumed less feed during the growing period than ad libitum fed pullets and thus a smaller pullet was produced. It is not clear from the literature reports available if a diet deficient in lysine is to be preferred. However, the limited evidence as reported by these investigators suggests that the introduction of deficient diets at one day of age would possibly be of more value for the reduction of live-weight gain with resulting delays in sexual maturity.

From the foregoing review, it would appear that feeding TP at a level higher than 22 g/100 kg. of feed to either egg or meat-type birds is definitely undesirable. Dietary supplementation by TP below this level could effect a method whereby more satisfactory economical returns may accrue to producers either through increased egg production and/or more efficient feed utilization. Similarly the hope would be that TP will not exert an adverse effect on fertility and hatchability. In addition, the viability of the offspring produced by the TP fed hens must not be impaired. All of these considerations constitute the foundations of success for flock owners in general and in particular for the broiler breeder industry. Hence, a study was designed to evaluate the use of TP at low levels in the ration of broiler breeder hens.

EXPERIMENTAL

Housing and Feeding of Birds

A. Phase I (0 - 8 weeks of age)

Seven hundred and fifteen day-old Peels' broiler type chicks, comprising 93 males and 622 females, were allocated into 3 floor pens provided with electric heating brooder units. All birds were fed a commercial chick starter (21% protein) and water ad libitum to 8 weeks of age.

B. Phase II (8 - 22 weeks of age)

At 8 weeks of age all female birds were randomized into 3 treatment groups. Each group was assigned a different diet with one group receiving a control diet, one a low-lysine diet and the remaining group a thyroprotein (TP) supplemented diet. The low-lysine diet (Table 1) was prepared to contain 0.45% (calculated) lysine which represents approximately 50% of the lysine requirement for growing chicks (NRC, 1971). Supplementation of the low-lysine diet with 0.45% of crystalline lysine-HCl constituted the control diet. The TP diet was prepared by adding TP (16.5 g/100 kg. diet) to the control ration. At 12 weeks of age pullet chicks from the 3 treatment groups were divided randomly within each treatment into 8 subgroups of approximately 26 pullets in each. They were distributed

Table 1. Composition of Grower Diet[1], [2]used in Phase II of the experiment.

Ingredients	Amount (%)
Barley (9.7% Protein)	45.25
Wheat (14% Protein)	26.75
Corn distillers with dried solubles (27% Protein)	20.00
Alfalfa meal (17% Protein)	5.00
Defluorinated rock phosphate	1.50
Salt premix[3]	0.50
Vitamin premix[4]	1.00

[1]Calculated analysis of diet: Crude protein, 14.55%; Metabolizable energy (Kcal/kg), 2726.51; Crude fiber, 4.71%; Lysine, 0.45%; Methionine, 0.30%; Calcium, 0.7%; Phosphorous, 0.58%.

[2]Control and TP-supplemented diets were prepared by incorporating 0.45% of Lysine-HCl, with the exception that only the latter contained 0.0165% of TP at the expense of wheat midlings.

[3]Salt premix supplied the following per kilogram of diet: Manganese, 80.85 mg; Zinc, 11.45 mg; Sodium chloride, 4.72 gm.

[4]Vitamin premix supplied the following per kilogram of diet: Vitamin A, 8,250 I.U.; Vitamin D_3, 818.45 I.C.U.; Vitamin E, 25.3 mg; Vitamin B_{12}, 11 mcg; Menadione, 1.1 mg; Riboflavin, 2.8 mg; Choline, 137.5 mg; Niacin, 8.3 mg; Pantothenic acid, 5.5 mg; Santoquin, 250 mg.

evenly according to weight within their respective groupings and housed in floor pens in the laying house. The pullets were fed the diets specified at the beginning of Phase II of the experiment (Table 1). The average weight of the pullets at 12 weeks of age was determined by weighing 80 birds selected at random from each treatment group. Thereafter, 10 birds from each pen were randomly weighed when the pullets reached 16, 20, and 22 weeks of age. Throughout this entire 14-week period the males were housed separately and fed the low-lysine diet.

C. Phase III (22-50 weeks of age)

At 22 weeks of age all birds were weighed and a routine culling procedure carried out. Three breeder diets (A, control; B, control supplemented with 5.5 g. TP/100 kg. diet; and C, control supplemented with 16.5 g. TP/100 kg. diet) were prepared as indicated in Table 2. The birds in each of the 3 phase II treatment groups were each in turn randomly divided into 3 subgroups. These subgroups were further divided into 24 pens, so that 9 pens of birds received diets A and C respectively, while 6 pens of birds received diet B. There were 25 females in each pen and, in addition, 2 males were placed in each of the 24 pens.

The birds were housed in a windowless insulated and fan ventillated building having thermostatically controlled

Table 2. Composition of Broiler Breeder Control Diet A[1,2], used in Phase III of the experiment.

Ingredients	Amount (%)
Barley (9.7% Protein)	40.00
Wheat (14% Protein)	30.00
Soybean meal (44% Protein)	14.00
Fish meal (70% Protein)	2.00
Alfalfa meal (17% Protein)	2.00
Animal tallow	3.00
Limestone	5.00
Defluorinated rock phosphate	2.50
Mineral premix[3]	0.50
Vitamin premix[4]	1.00

[1]Calculated analyses: Crude protein, 15.68%; Crude fiber, 4.55%, Metabolizable energy (Kcal/kg), 2,701.78; Lysine, 0.8%; Methionine, 0.32%; Calcium, 3.0%; Phosphorous, 0.88%.

[2]Diets B, and C were prepared by incorporating 0.0055 and 0.0165% of TP respectively at the expense of wheat midlings.

[3]Mineral premix supplied the following per kilogram of diet: Manganese, 80.85 mg; Zinc, 44.19 mg; Sodium chloride, 4.8 g.

[4]Vitamin premix supplied the following per kilogram of diet: Vitamin A, 7,150 I.U.; Vitamin D_3, 818.45 I.C.U.; Vitamin E, 24.95 mg; Vitamin B_{12}, 11 mcg; Riboflavin, 7.7 mg; Pantothenic acid, 2.2 mg; Niacin, 3.3 mg; Choline, 5.5 mg; Santoquin, 250 mg.

temperature maintained within the range of 68 to 70°F. At the beginning of Phase III the daily light interval was increased by one half hour per week until a total light exposure time of 13 hours per day was attained. It was maintained at this level until one month past peak production. This was followed by an increase of light interval at the rate of 15 min. per week to 16 hours daily. This level of lighting was maintained until the completion of the experiment.

All birds were weighed at the beginning of Phase III. Thereafter, 10 hens were selected at random from each of the 24 pens and weighed at 28-day intervals. Throughout the laying period, egg production and mortality were recorded daily and all dead birds autopsied by the Provincial Veterinary Laboratory. The recording of feed consumption data and efficiency of feed utilization was calculated for each 28-day interval.

Egg weights were recorded during the third, fifth, and seventh 28-day periods. In the third period, 10 eggs were taken at random from each pen to ascertain whole egg and yolk weight, as well as shell thickness. Thickness of shell was measured with an Ames micrometer after the shells were water-soaked overnight, washed and shell membranes removed. In the fifth and seventh periods only egg weight was measured. This was accomplished by weighing daily, for 3

consecutive days, on a pen basis all of the normal eggs produced. Abnormal eggs (double yolked etc.) were rejected.

To study the influence of treatment on fertility and hatchability, eggs were collected at various stages throughout the production cycle. The first lot was incubated when the hens were in about 50% production, and this was followed by 3 weekly settings made at peak production, 3 more weekly settings made just subsequent to peak production and finally 2 settings were made toward the termination of the production cycle (declining egg production). Eggs for incubation were collected for 3 consecutive days just prior to each setting. Eggs slightly soiled were hand-washed and wiped dry. The eggs collected daily were stored at a temperature of 50°F. In selection of eggs, each was individually candled and those of low quality (cracks, misplaced air cell etc.) rejected. Ultimately 10 eggs from each pen were selected.

Eggs were placed in incubator trays and allowed to warm up to room temperature before being placed in the incubator. On the twentieth day, incubated eggs were candled and the infertile and dead embryos removed. The live embryos were then transferred to the hatching unit until the twenty-second day, at which time all unhatched eggs were broken out and examined for mal-formation or cause of death. The number of chicks hatched was obtained for each group set and per cent

fertility and hatchability were calculated. The hatchability and fertility data were calculated according to total number of eggs set.

To determine if chicks hatched from eggs laid by hens treated with the various levels of TP exhibited any carry-over effects of such treatments, the chicks were reared for a 2-week period. Out of the total 9 settings of eggs hatched throughout the laying period only 7 were used in this viability study (first and the last settings were not included). These chicks, individually weighed and wing-banded at hatching, were placed in a brooder house equiped with electric brooding units and were fed a commercial chick starter ad libitum. The general appearance of the chicks during the 2-week rearing period was observed and body weight recorded at the termination of the period.

A check on hatching time was made in 3 out of the 9 hatching studies. The time of hatch for all eggs was followed at 3 hours intervals commencing 9:00 a.m. on the twenty-first day of incubation and continued until the late morning of the twenty-second day. Number of chicks hatched during a specified time interval for each treatment group was expressed as a percentage of the total chicks hatched in that treatment group.

Blood Sampling Technique

A. Laying hens: Blood samples (5 ml.) were obtained from each of 5 randomly selected hens of each treatment group by puncture of Vena cutanea ulnaris. The samples were taken prior to the onset of egg production, at about 10% production, at about 50% production, at peak production and during the decline of egg production. The blood samples, drawn into a syringe were immediately transferred to centrifuge tubes containing ethylenediaminetetraacetic acid (EDTA). These tubes were prepared by evaporating 0.5 ml. of a 10% EDTA solution. Plasma was obtained by centrifugation (3,000 rpm for 20 min.) and the samples were stored (-22°F) for later analyses.

B. Progeny: Blood samples (approx. 4 ml.) were collected from day-old chicks by severing the left jugular vein with a scalpel. Each blood sample was allowed to drip through a funnel into a vial (5 ml. capacity) containing EDTA. These vials were prepared as described above. Since it was found to be difficult to get a large enough volume of blood from each day-old chick, pooled blood samples from 2 chicks were used. At least 4 pooled blood samples were secured from each treatment group. Blood samples from 2-week old birds were collected (at least 5 from each treatment group) by the same procedure as described above for day-old chicks except that

individual rather than pooled samples were obtained. Plasma was obtained for all samples by centrifugation and stored as indicated above for subsequent analyses.

Thyroid Hormone Analyses

A. Hycel Cuvette Protein Bound Iodine (PBI) Determination:

The PBI in plasma samples was determined using 0.1 ml. of plasma. A dilution technique was used for samples where the PBI value was found to be particularly high. Iodine of inorganic origin was removed through the action of an anionic-exchange resin. Plasma treated with resin was set for digestion in a cuvette at 230 $\pm$ 5°C for 6 min. with the addition of 2 ml. of a stable perchloric reagent (0.025% vanadic acid in 72% perchloric acid). The digestion was performed in a fume-hood by inserting the cuvettes into wells (1 x ¾") in a heating block especially designed for this procedure. The digested sample was then removed and allowed to cool to room temperature. Following digestion 2 ml. of ceric reagent (0.6% ceric ammonium sulfate in 27% sulfuric acid) was added to the tubes followed by the addition of 2 ml. of arsenious reagent (0.9% arsenious trioxide in 8.2% sulfuric acid). The cuvettes were then placed in a water bath for incubation at 37°C for 20 min. after which the effect of catalysis was determined photometrically (420 - 450 mu.) with a Coleman Spectronic "20" spectrophotometer. Protein bound iodine values were

determined by comparison with a standard curve.

B. Tetralute Method (^{125}I Column T_4 Test):

This analytical method involves the use of a miniature Sephadex G-25 column which serves as a secondary binding agent. The column is prepared in a small plastic syringe barrel with a porous disc resting on the bottom. The Sephadex G-25 (450 mg.) is suspended in 0.1 N NaOH solution. The syringe barrel holding the Sephadex column is capped both at the top and at the bottom with removable seals (Fig. 1). For the determination of thyroxine-iodine (T_4-I) the top cap was removed from all columns and 0.1 N NaOH was poured off into a plastic tray. With the aid of an Oxford pipetter (0.5 ml.), 0.5 ml of ^{125}I-T_4 (which would give an initial count of 34,000 cpm) in 0.1 N NaOH solution was added to each column. A standard or test-plasma (the latter should not exceed 0.4 ml.) was then added to the column and shaken gently. The bottom cap of each column was then removed and the column allowed to drain. Approximately 4 ml. of barbital buffer, pH 8.6 was then delivered to each column and the columns again allowed to drain. These columns were blotted dry at the tips and recapped before recording the initial radioactivity in a well-type gamma detector (Nuclear Chicago DS202 2¼" x 2¼" crystal). After determination of the initial radioactive count the bottom caps were again removed prior to the addition of 1 ml. of

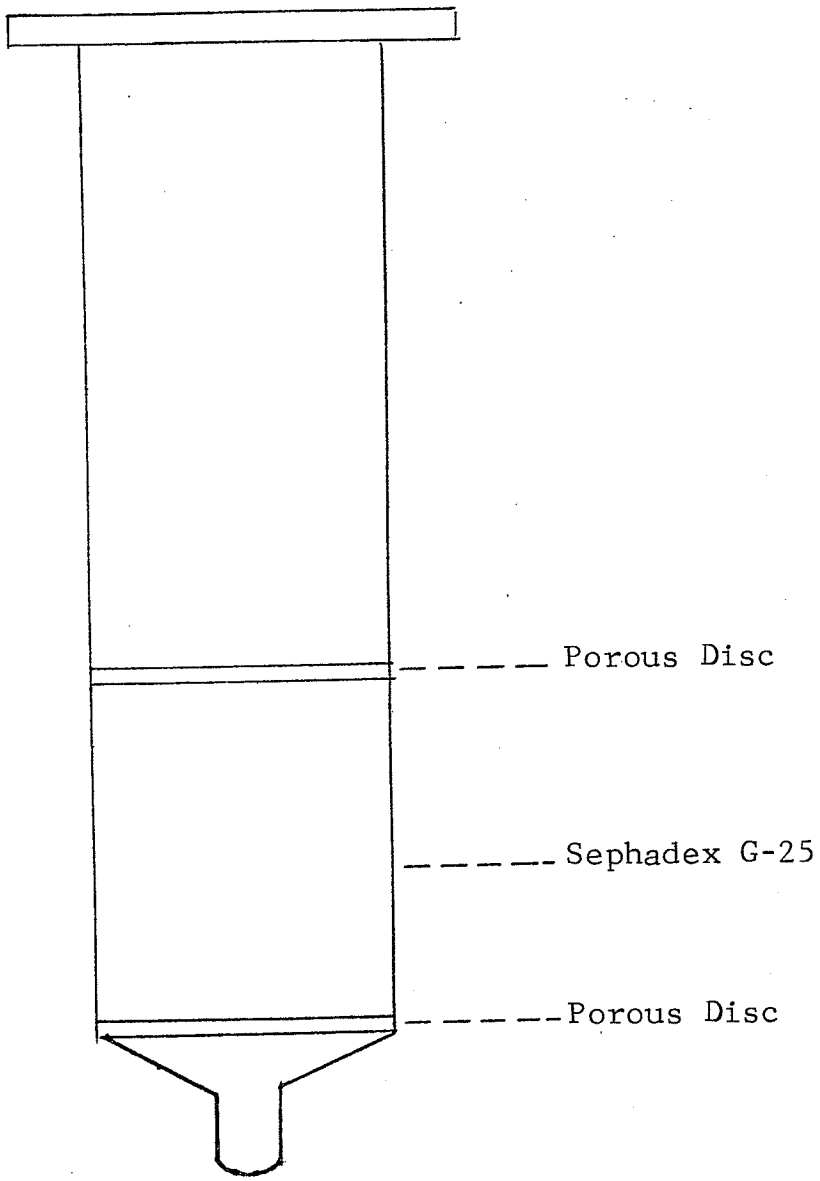

Fig. 1. Structure of Column.

eluting reagent. All columns were allowed to drain for a critical period of 5 min. Then, barbital buffer (4 ml.) was delivered to each of the columns which were allowed to drain, blotted dry at the tip, recapped and set in the gamma well counter for final counting. The percentage of retention of ^{125}I-T_4 was calculated by the formula:

$$\frac{\text{Final cpm}}{\text{Initial cpm}} \times 100$$

A standard curve was prepared by plotting concentration of standards in microgram per cent versus the respective per cent retention. Test sample determinations were compared with the standard curve.

It had been shown that copper-free water should be used for all reagents preparation. In the determinations reported herein, a triple-distilled (millipore) water was used. Due to the low plasma T_4 levels of chickens as is also true for the fish, the eluting reagent source was made from a 1:30 dilution of human serum with barbital buffer. This is a modification of the procedure used for human thyroid hormone determination (Bauer et al, 1970). Another modification involved the time interval employed after drainage of the eluting reagent (TBG) before eluting with buffer. This change of timing is critical at least for T_4-I determination in fish plasma as reported by Higgs and Eales (unpublished data). The time limit was extended from 2 min. to 5 mins.

The regeneration technique for the used columns as adopted by Higgs and Eales (unpublished data) was followed. They reported that if columns were washed with NaOH (pH11) within 24 hours of use, virtually all radioactivity could be removed. It has also been found that no limit as yet has been reached for the number of times columns may be regenerated. In regeneration of used columns, approximately 50 ml. of 0.1 N NaOH was diluted to a total volumen of 2,200 ml. with triple-distilled water. The columns were washed and the radioactivity determined until the residual ratioactivity in the column was about 300 cpm or less. Then each column was re-equilibrated to pH13 by the addition of approximately 40 ml. of 0.1 N NaOH.

Statistical Analyses of Data:

Data were tested by analysis of variance for unequal observations as described by Snedecor (1956) and multiple range comparisons were performed according to Duncan (1955).

RESULTS

Phase II of the Experiment

Body weight gain, feed conversion, and mortality rate of birds during the growing period were not significantly ($P>0.05$) influenced by grower treatment (Table 3). The TP fed group consumed a similar quantity of feed as did the low-lysine group which was shown to consume significantly ($P<0.05$) more feed than the control group (Table 3).

Phase III of the Experiment

A. Egg Production

The grower treatments employed seemed to have little influence on subsequent per cent hen-day egg production of the hens except in period 1 where hens that had been fed 16.5 g. TP per 100 kg. of grower diet showed a slightly lower egg production as compared with the control (Table 4). However, this effect did not persist throughout the laying cycle. Although no influence of grower treatment on onset of egg production was found statistically, pullets fed the TP diet came into production 2 days later than did pullets fed from the other treatment groups. The effect of TP feeding during the laying phase on egg production for all treatment groups over the seven 28-day periods is presented in Table 5 and Fig. 2.

Table 3. Influence of grower diet on feed consumption, body weight gain, feed conversion and mortality of broiler breeding pullets.

Treatment*	Total feed[1] Consumption (kg/bird)	Body weight gain (g/bird)	Average feed conversion (g feed/g gain)	Mortality (%)
Control	13.6[a]	1845	4.2	2.9
Control + thyroprotein[3]	13.9[b]	1796	4.4	3.8
Low Lysine[4]	14.0[b]	1793	4.4	1.9

[1]Means with same superscript are not significantly different ($P>0.05$). See Appendix Table 1 for Statistical analyses.

[2]Control diet was prepared by adding 0.45% of supplemental lysine-HCl to the low lysine diet described in footnote 4.

[3]Control diet supplemented with 0.0165% of thyroprotein.

[4]Low lysine diet was prepared to contain 0.45% (Calculated) of lysine which is approximately 50% of the recommended requirement (National Academy of Sciences, #1, Nutrient Requirements of Poultry, 6th revised edition, 1971).

*Actual analysis revealed 0.72%, 0.80% and 0.44% of lysine for the control, control + thyroprotein, and low lysine diets, respectively.

Table 4. Effect of grower diet on hen-day egg production (%) for each 28-day period throughout the laying cycle.

Grower Treatment	Period						
	1	2	3	4	5	6	7
Control	19.7	63.1	67.8	65.9	59.7	51.5	43.4
Control + Thyroprotein[1]	15.5	63.1	66.1	65.0	60.5	52.0	46.6
Low Lysine[2]	19.1	63.3	67.2	64.7	60.1	52.7	46.3

[1]Thyroprotein, 16.5 g/100 kg. of feed.

[2]Lysine, 0.45% (calculated). Sample analysed contained 0.44% of lysine.

Table 5. Effect of level of thyroprotein feeding on hen-day production (%) of broiler breeder hens illustrated for each 28-day period throughout the laying cycle.

Treatment	Level of TP in Diet (g/100 kg)	Period 1	2	3	4	5	6	7	Overall Production
A	0	16.1	62.1	64.5	62.2	57.2	50.4	45.8	51.2[a1]
B	5.5	18.9	64.2	69.7	68.0	61.9	50.5	44.6	53.9[b]
C	16.5	19.5	63.6	67.8	66.3	61.9	57.8	45.7	54.2[b]

Means with same superscript are not significantly different ($P > 0.05$). See Appendix Table 2 for statistical analyses.

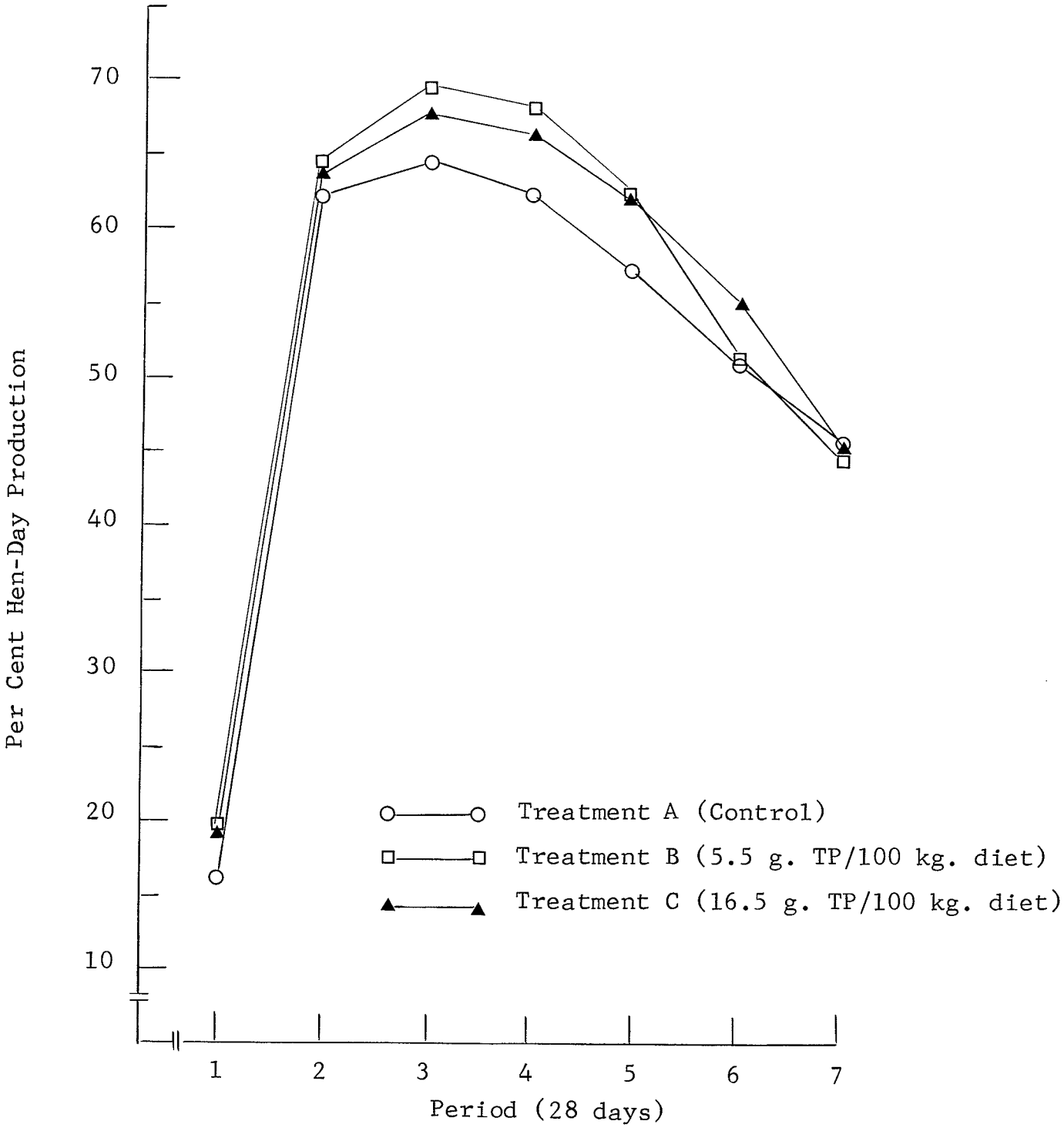

Fig. 2. Egg production (% Hen-day) of hens fed various levels of thyroprotein.

A significant increase ($P < 0.05$) in egg production was observed when supplemental TP was included in the diet (Treatment B and C). The production rates for treatment B (5.5 g. TP/100 kg. of diet) and C (16.5 g. TP/100 kg. diet) were 53.9 and 54.2% respectively while that for the control was 51.2%. The advantage in laying performance of the hens in treatment B and C over that of the control group appeared to be consistent (except period 7). No difference was observed between treatment groups B and C.

B. Plasma Thyroid Hormone Levels of Broiler Breeder Hens:

A homogeneity of variance test was performed according to the method of Bartlett (1937) for the PBI and T_4-I data. These data were found not to be homogeneous (Appendix Table 3) and hence an analysis of variance to detect treatment differences was not performed. However, anlaysis of variance was performed with the PBI and T_4-I data from the control to determine the influence of stage of egg production on these two plasma parameters.

Protein bound iodine in the plasma of the laying hens receiving the control diet rose significantly from the onset of egg production, peaked at the highest level of egg production, and declined thereafter (Table 6 and Fig. 3). However, such a pattern was not evident in the same group of control hens for plasma T_4-I (Table 7 and Fig. 4). Both PBI and T_4-I

Table 6. Effect of thyroprotein feeding on the plasma PBI level (ug% ± S.E.) of broiler breeder hens illustrated with respect to approximate level of egg production.

Treatment	Level of TP in Diet (g/100 kg)	Approximate Level of Egg Production (%)				
		0	10	50	70	50
A	0	0.73[a]	1.75[bd]	2.08[bc]	2.62[c]	1.22[ad]
		±0.08	±0.11	±0.20	±0.17	±0.29
B	5.5	3.97	6.78	4.80	5.93	8.87
		±0.12	±1.40	±0.90	±1.59	±0.86
C	16.5	7.63	13.81	11.62	14.71	13.77
		±1.38	±2.01	±0.40	±1.00	±1.32

[1]Means with same superscript are not significantly different ($P > 0.05$). See Appendix Tables 3 and 4 for statistical analyses of data from control group.

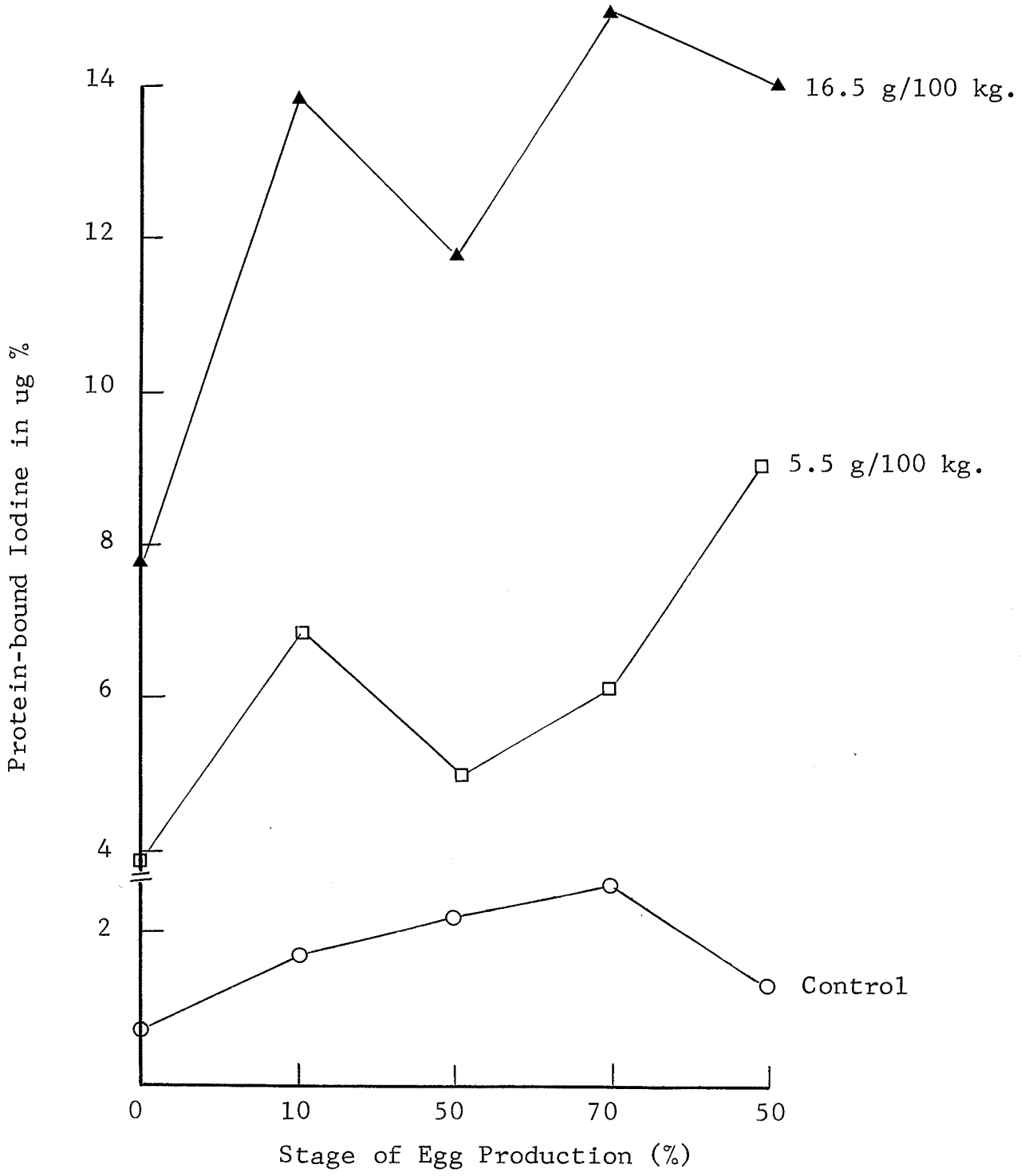

Fig. 3. Plasma PBI level of broiler breeder hens fed various levels of thyroprotein.

Table 7. Effect of thyroprotein feeding on the T_4-I level (ug % ± S.E.) of broiler breeder hens illustrated with respect to the approximate level of egg production.

Treatment	Level of TP in Diet (g/100 kg)	Approximate Level of Egg Production (%)				
		0	10	50	70	50
A[1]	0	0.46	0.44	0.47	0.45	0.51
		±0.08	±0.04	±0.08	±0.09	±0.08
B	5.5	1.10	1.91	1.39	1.96	1.19
		±0.38	±0.19	±0.14	±0.50	±0.24
C	16.5	4.53	3.72	3.54	4.35	3.36
		±1.44	±0.72	±0.23	±0.49	±0.84

[1]See Appendix Tables 3 and 4 for statistical analyses of data from the control group.

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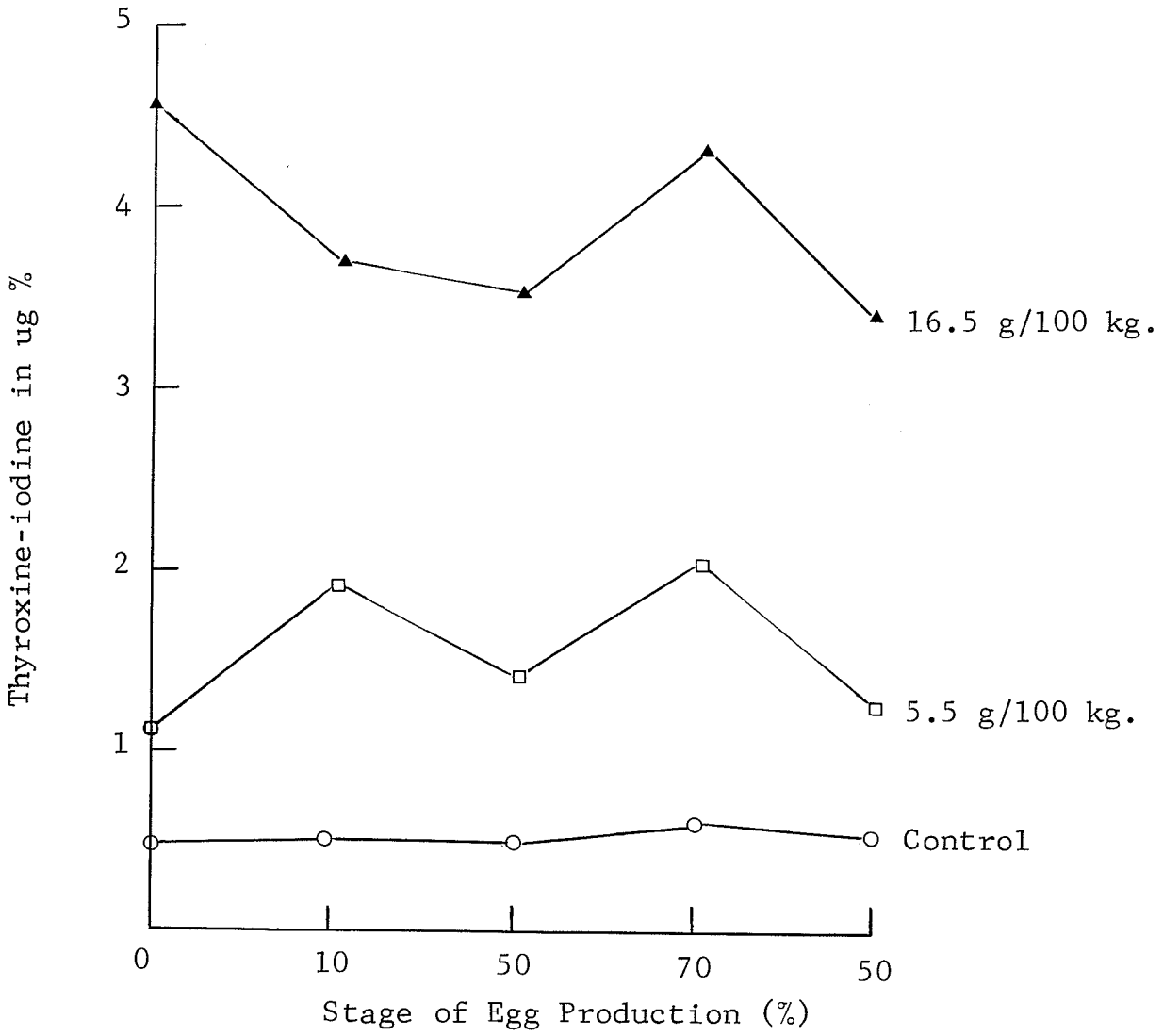

Fig. 4. Plasma T_4-I levels of broiler breeder hens fed various levels of thyroprotein.

plasma levels of the treated hens (treatments B and C) were markedly higher than those of the control group throughout the laying cycle. In addition, plasma levels of thyroid hormones (PBI or T_4-I) were higher in hens fed 16.5 g. as compared with those fed 5.5 g. of TP per 100 kg. diet. However, the PBI and T_4-I plasma levels of the treated hens revealed no consistent pattern with respect to stage of the laying cycle.

C. Feed Consumption, Feed Efficiency, and Body Weight Gain:

Thyroprotein feeding throughout the laying cycle of the broiler breeder hens did not seem to have a marked influence on feed consumption, although there was a trend toward a higher feed consumption by the two TP treated groups relative to the control (Table 8). The average total feed intake per bird during the seven 28-day periods was 30.5, 30.8 and 31.3 kg. for treatment groups A, B, and C respectively.

The efficiency of feed utilization (kg. feed required to produce one dozen eggs) throughout the laying cycle, as shown in Table 8, appeared to have been improved in the 2 TP treated groups (4.30 and 4.21 kg/doz., for treatments B and C, respectively) when compared with the control (4.60 kg/doz.). This would indicate an increase of approximately 11 and 6% (treatments B and C respectively) in feed utilization over the control. However, this improvement in feed utilization was not

Table 8. Effect of level of thyroprotein feeding throughout seven 28-day laying periods on total feed intake, feed efficiency, body weight gain, and mortality of broiler breeder hens.

Treatment	Level of TP in Diet (g/100 kg)	Feed Intake (kg/hen)	Feed Efficiency (kg/doz. eggs)	Body Weight Gain (g)	Mortality (%)
A	0	30.5	4.60	1,024[a1]	7.9[a]
B	5.5	30.8	4.30	820[b]	3.5[b]
C	16.5	31.3	4.21	805[b]	4.2[b]

[1]Means within the same column not having the same superscript are significantly different ($P<0.05$). See Appendix Tables 2 and 5 for statistical analyses.

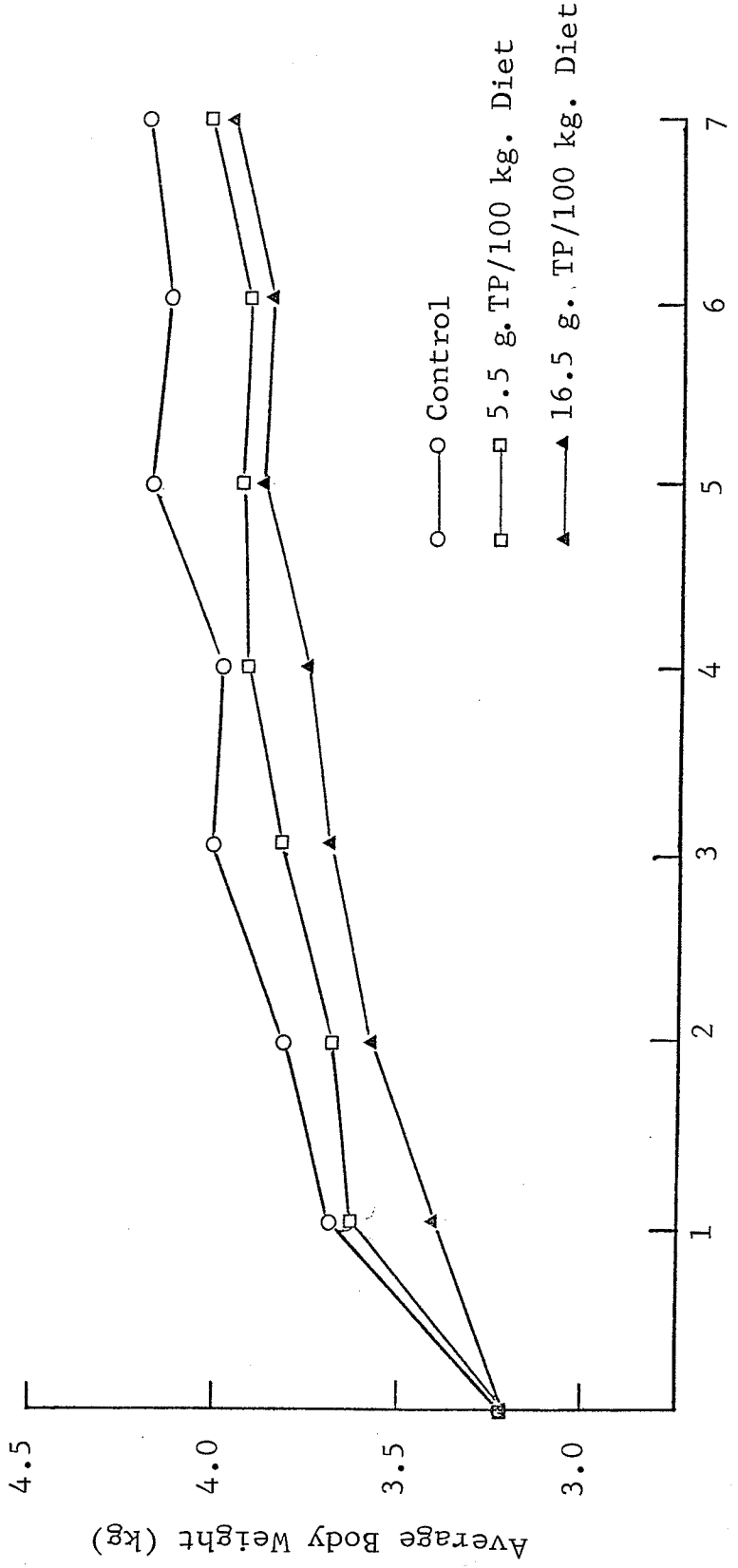

Fig. 5. The average body weight gain throughout the laying cycle of hens fed various levels of thyroprotein.

statistically significant ($P>0.05$).

The effect of TP feeding on the body weight gain for the seven 28-day periods is presented in Table 8 and is illustrated in Fig. 5. Hens fed 5.5 g. and 16.5 g. of TP per 100 kg. of feed were shown to gain significantly ($P<0.05$) less body weight than controls.

D. Mortality:

The effect of dietary TP during the laying cycle on the mortality rate is presented in Table 8. Livability, as shown by the present data, was improved significantly ($P< 0.05$) when either level (5.5 or 16.5 g/100 kg. of feed) of TP was fed to broiler breeder hens.

E. Egg Weight, Egg Yolk Weight and Thickness of Egg Shell:

Egg laid by hens fed either 5.5 or 16.5 g. TP per 100 kg. diet were significantly lower ($P<0.05$) in weight than those laid by control hens (Table 9). However, no difference was found between the 2 TP treated groups. Apparently, this reduction in egg weight appeared after the egg production rates started to decline as shown by the data in Table 10 although a statistically significant layer treatment x egg collection period interaction was not evident. Egg weight was also found to be affected by grower treatment (Appendix Table 6). The groups fed 16.5 g. of TP per 100 kg. of diet during the growing phase had a mean egg weight of 61.56 g. which was signi-

Table 9. Effect of level of thyroprotein fed to broiler breeder hens on egg weight, egg yolk weight, and egg shell thickness.

Treatment	Level of TP in Diet (g/100 kg)	Egg[1] Weight (g)	Egg Yolk Weight (g)	Egg Shell Thickness (mm)
A	0	62.77[a]	16.96	0.325
B	5.5	61.80[b]	17.01	0.333
C	16.5	61.93[b]	16.80	0.331

[1]Means with same superscript are not significantly different ($P > 0.05$). See Appendix Table 6 for statistical analyses.

Table 10. Effect of level of thyroprotein feeding on egg weight illustrated with respect to period of data collection.

Treatment	Level of TP in Diet (g/100 kg)	Period of Egg Collection[1]		
		3rd	5th	7th
A	0	58.87	63.58	65.88
B	5.5	59.24	62.73	63.94
C	16.5	58.47	62.90	64.41

[1]Eggs were collected during the 3rd, 5th, and 7th 28-day experimental periods.

ficantly ($P<0.05$) lower than the control and the low-lysine treated group which were 62.46 and 62.62 g. respectively. Since a significant ($P<0.05$) grower treatment x egg collection period was not evident (Appendix Table 6) this effect of grower diet persisted through the entire laying cycle.

Observations on egg yolk size during the third 28-day period (peak production) indicated no differences among treatments (Table 9). Similarly, no differences in shell thickness were observed among treatments although shell thickness of the eggs laid by both TP treated groups was slightly higher than that of the eggs laid by the control hens.

F. Fertility and Hatchability:

The data presented in Table 11 and 12 indicate that fertility was improved ($P<0.05$) by the high (16.5 g/100 kg.) level of TP feeding during the laying period although the grower treatments were shown to have no influence on the level of fertility. The layer treatment x grower treatment interaction (Appendix Table 7) is difficult to explain.

No differences were found among treatments when hatchability (% of total eggs set) was studied.

G. Livability of Goiterous Chicks Hatched from Eggs Laid by Broiler Breeder Hens Fed Various Levels of TP:

Table 11. Effect of thyroprotein feeding on fertility and hatchability of eggs from broiler breeder hens.

Treatment	Level of TP in Diet (g/100 kg)	Fertility (%)	Hatchability (%)
A	0	85.31[a][1]	79.51
B	5.5	87.41[a]	80.74
C	16.5	92.10[b]	81.60

[1]Means not having the same superscript are significantly different ($P<0.05$). See Appendix Table 7 for statistical analyses.

Table 12. Effect of grower treatment on fertility and hatchability of eggs from broiler breeder hens.

Treatment[1]	Level of TP in Diet (g/100 kg)	Fertility (%)	Hatchability (%)
Control	0	89.31	81.94
Control + TP	16.5	87.78	79.58
Low Lysine	0	88.06	80.26

[1]Chemical analyses of the diets showed that control, control + TP, and low-lysine rations contained 0.73, 0.80 and 0.44% of lysine respectively. See Appendix Table 7 for statistical analyses.

As shown in Table 13, the average body weight of the day-old chicks hatched from eggs laid by the broiler breeder hens fed various levels of TP did not differ significantly ($P>0.05$) from each other. However, at 2 weeks of age, after being fed commercial starter diet (21% protein), the average body weight of chicks from hens fed 16.5 g. TP/100 kg. diet weighed an average of 132.3 g. which was significantly lower than chicks from either control hens or hens fed 5.5 g. TP/100 kg. diet. The average body weights of these latter two groups of chicks were not significantly ($P>0.05$) different from each other. In addition, there was a significant ($P<0.05$) effect attributable to grower treatment in that the grower diet containing 16.5 g. TP per 100 kg. diet was shown to result in smaller chicks but only at 2 weeks of age (Appendix Table 8). Results also indicate a difference in mean body weight of day-old chicks among different hatches which was a normal response resulting from a gradual increase in mean egg weight within each treatment group through the laying cycle.

H. Plasma PBI and T_4-I levels in Day-old and Two-week old Chicks:

Data illustrated in Table 14 and Fig. 6 represent the influence of the diet fed to hens on the PBI levels in chicks hatched from eggs laid by the hens at different stages of

egg production; onset of peak production (hatch 2), peak production (hatch 4), and declining production (hatch 9). The results for all three hatches indicate that day-old chicks from treatment C had a mean PBI level that was significantly ($P<0.05$) higher than that of chicks from treatment B which, in turn, was significantly ($P<0.05$) higher than the value for control chicks (treatment A). The significant ($P<0.05$) hatch x treatment interaction would indicate that the magnitute of these differences was somewhat different for each of the hatches (Appendix Table 9). A similar result was obtained with regard to plasma T_4-I levels (Table 15 and Fig. 7) although the diferences were not statistically significant ($P>0.05$).

While the PBI levels of chicks hatched from eggs laid by hens receiving TP were found to decrease to the control level 2 weeks after placing the chicks on a commercial starter diet, the PBI levels of progeny of the control hens were found to increase slightly. This response was responsible for the highly significant ($P<0.01$) age effect and age x treatment interaction (Appendix Table 9). In addition the magnitude of the differences in PBI levels between the chicks of different ages was not the same for all hatches as indicated by the significant ($P<0.05$) age x hatch interaction. In all hatches, the PBI levels of the 2 weeks old chicks of all groups were virtually similar. The T_4-I data of all 3 hatches appear to

Table 13. Body weight of chicks hatched from eggs of broiler breeder hens fed various levels of thyroprotein.

Treatment	Level of TP in Diet (g/100 kg)	Body Weight (g) Day-old Chick	Body Weight (g) 2-week-old Chick
A	0	39.8	137.1[a][1]
B	5.5	40.0	139.2[a]
C	16.5	40.0	132.3[b]

[1]Means not having the same superscript are significantly different ($P < 0.05$). See Appendix Table 8 for statistical analyses.

Table 14. Plasma PBI levels (ug % ± S.E.) at day of age and two weeks of age in the progeny of broiler breeder hens fed various levels of thyroprotein.

Hatch[1]	Treatment[2]	Day-old Chick*	2-week old Chick*
Second	A	0.98 ± 0.16	2.01 ± 0.30
	B	4.76 ± 0.24	2.96 ± 0.61
	C	21.82 ± 3.82	3.32 ± 0.29
Fourth	A	2.46 ± 0.13	3.69 ± 0.44
	B	7.47 ± 0.53	4.46 ± 0.59
	C	15.09 ± 2.21	4.88 ± 0.41
Nineth	A	1.52 ± 0.16	2.39 ± 0.26
	B	5.43 ± 0.48	2.24 ± 0.19
	C	17.73 ± 3.82	3.00 ± 0.43

[1]Eggs for the second, fourth, and ninth hatches were collected approximately at the onset of peak production, peak production, and declining egg production respectively.

[2]A, denotes chicks hatched from eggs laid by control hens; B and C, denote chicks hatched from eggs laid by hens fed TP at 5.5 and 16.5 g. per 100 kg. diets respectively.

*For statistical analyses, see Appendix Table 9.

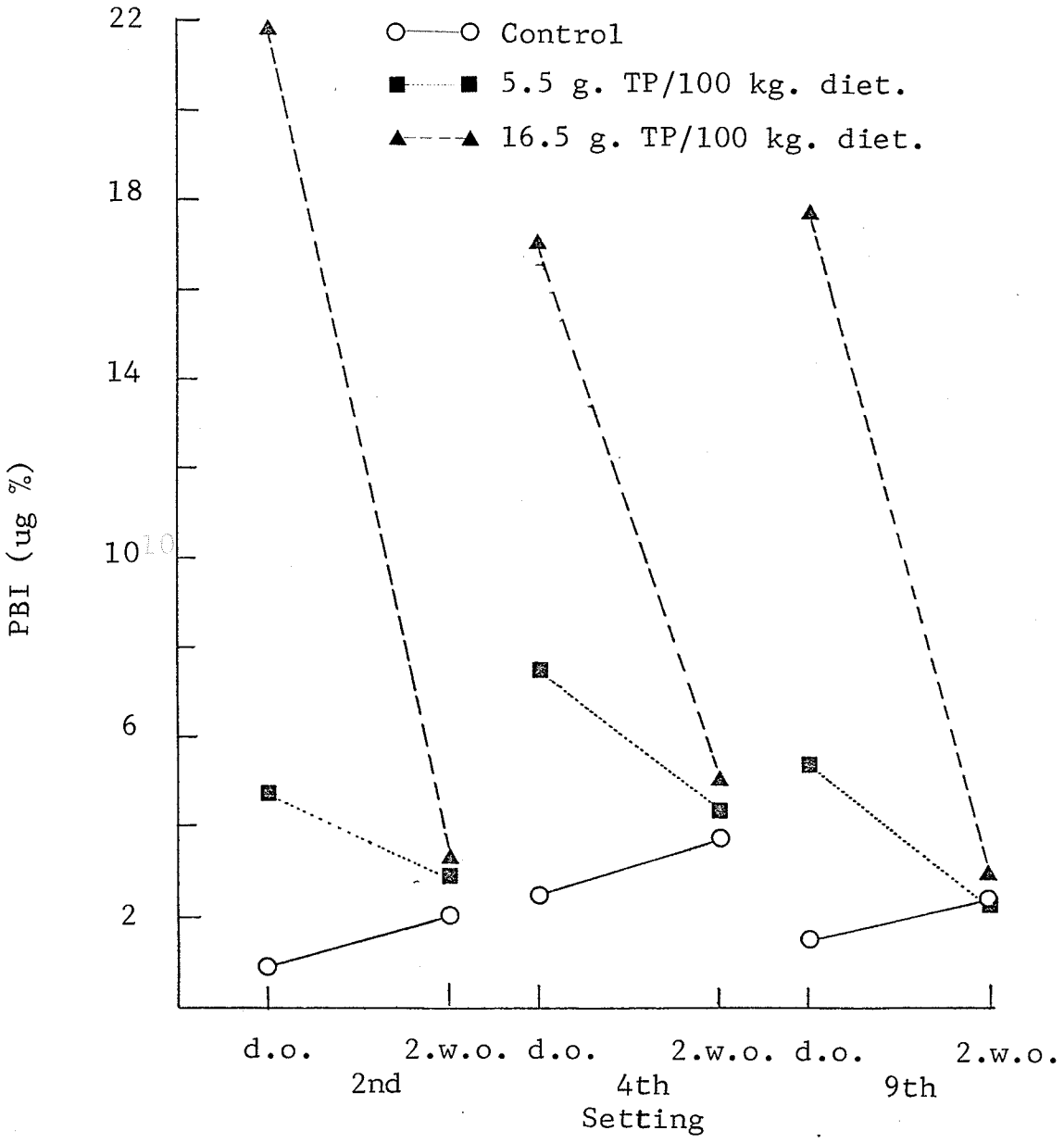

Fig. 6. Plasma PBI levels (ug %) at day of age and two weeks of age in the progeny of broiler breeder hens fed various levels of thyroprotein.

Table 15. Plasma T_4-I levels (ug % ± S.E.) at day of age and two weeks of age in the progeny of broiler breeder hens fed various levels of thyroprotein.

Hatch[1]	Treatment[2]	Day-old Chick*	2-week old Chick*
Second	A	0.54 ± 0.04	0.58 ± 0.04
	B	0.52 ± 0.03	0.72 ± 0.05
	C	0.99 ± 0.13	0.59 ± 0.06
Fourth	A	0.48 ± 0.04	0.56 ± 0.09
	B	0.53 ± 0.10	0.67 ± 0.14
	C	0.91 ± 0.16	0.53 ± 0.03
Nineth	A	0.48 ± 0.08	0.51 ± 0.05
	B	0.74 ± 0.10	0.50 ± 0.09
	C	1.39 ± 0.13	0.83 ± 0.06

[1]Eggs for the second, fourth, and ninth hatches were collected approximately at the onset of peak production, peak production, and declining egg production respectively.

[2]A, denotes chicks hatched from eggs laid by control hens; B and C, denote chicks hatched from eggs laid by hens fed TP at 5.5 and 16.5 g. per 100 kg. diets respectively.

*For statistical analyses, see Appendix Table 9.

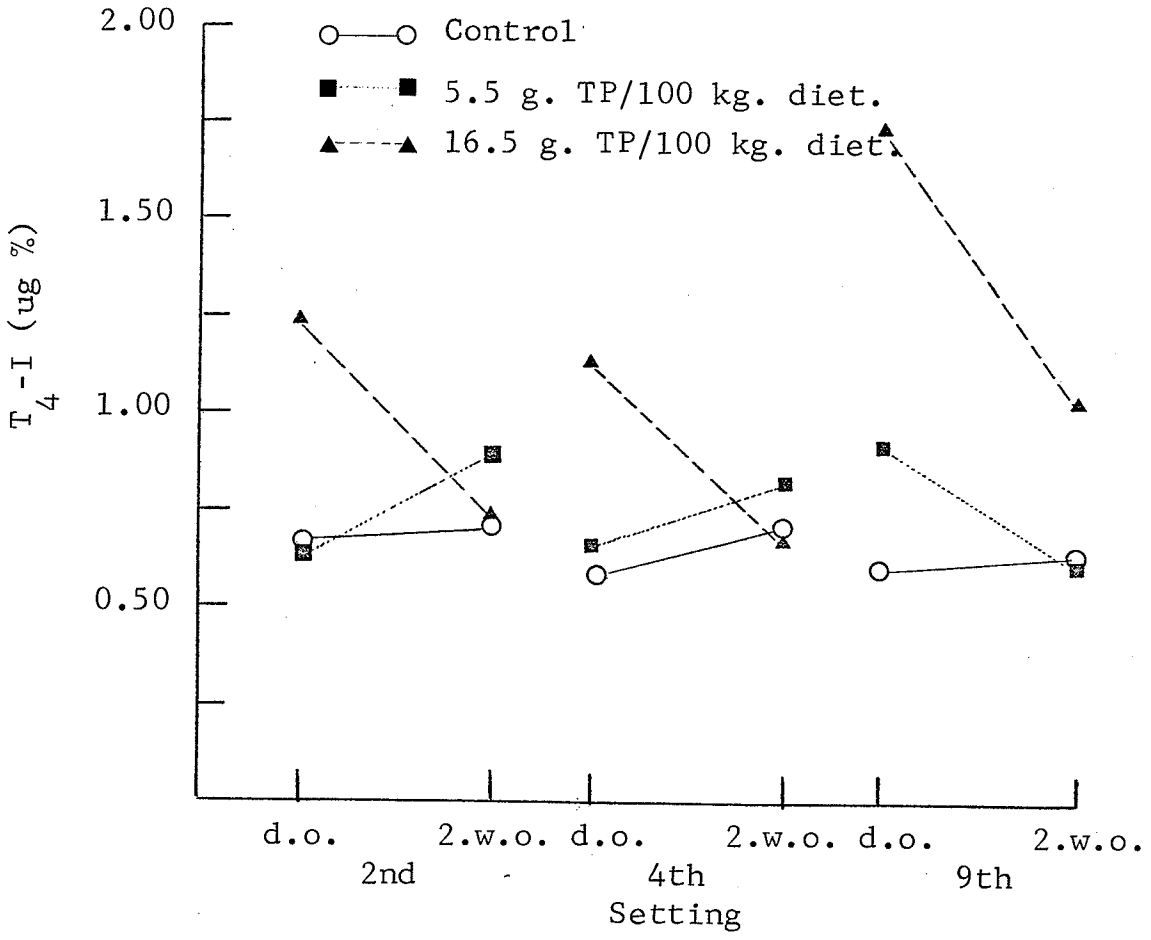

Fig. 7. Plasma T_4-I levels (ug %) at day of age and two weeks of age in the progeny of broiler breeder hens fed various levels of thyroprotein.

reveal a trend that T_4-I level from the progeny hatched from the eggs laid by the control hens increased slightly after placing them for 2 weeks on commercial chick starter diet. An opposite trend was observed for progeny from hens receiving 16.5 g. TP/100 kg. diet which showed a decrease in T_4-I level 2 weeks after. No consistent trend was observed in chicks from hens fed lower level of TP (5.5 g. TP/100 kg. diet).

I. Studies on the Effect of TP Feeding on the Hatching Time:

Observations on hatching time for eggs from each treatment group indicated that eggs laid by hens fed 16.5 g TP/100 kg. diet (treatment C) required 12 hours longer to hatch than control eggs (Fig. 8). However, the delay in hatching time could have been less than 12 hours as observed because some of the chicks from this late hatching group showed relatively dry down feathers when examined at 9:00 a.m. on the 22nd day of incubation. In order to make a more precise and accurate study in hatching time, one more observation at 3:00 a.m. in the morning of 22nd day of incubation was scheduled in the 8th setting of the fertility and hatchability study. When this was done, it was found that chicks from group C (from hens fed 16.5 g. TP per 100 kg. diet) required at least 6 hours more but definitely less than 12 hours in hatching time than chicks from the control group (Fig. 9). This finding was corroborated by the observation

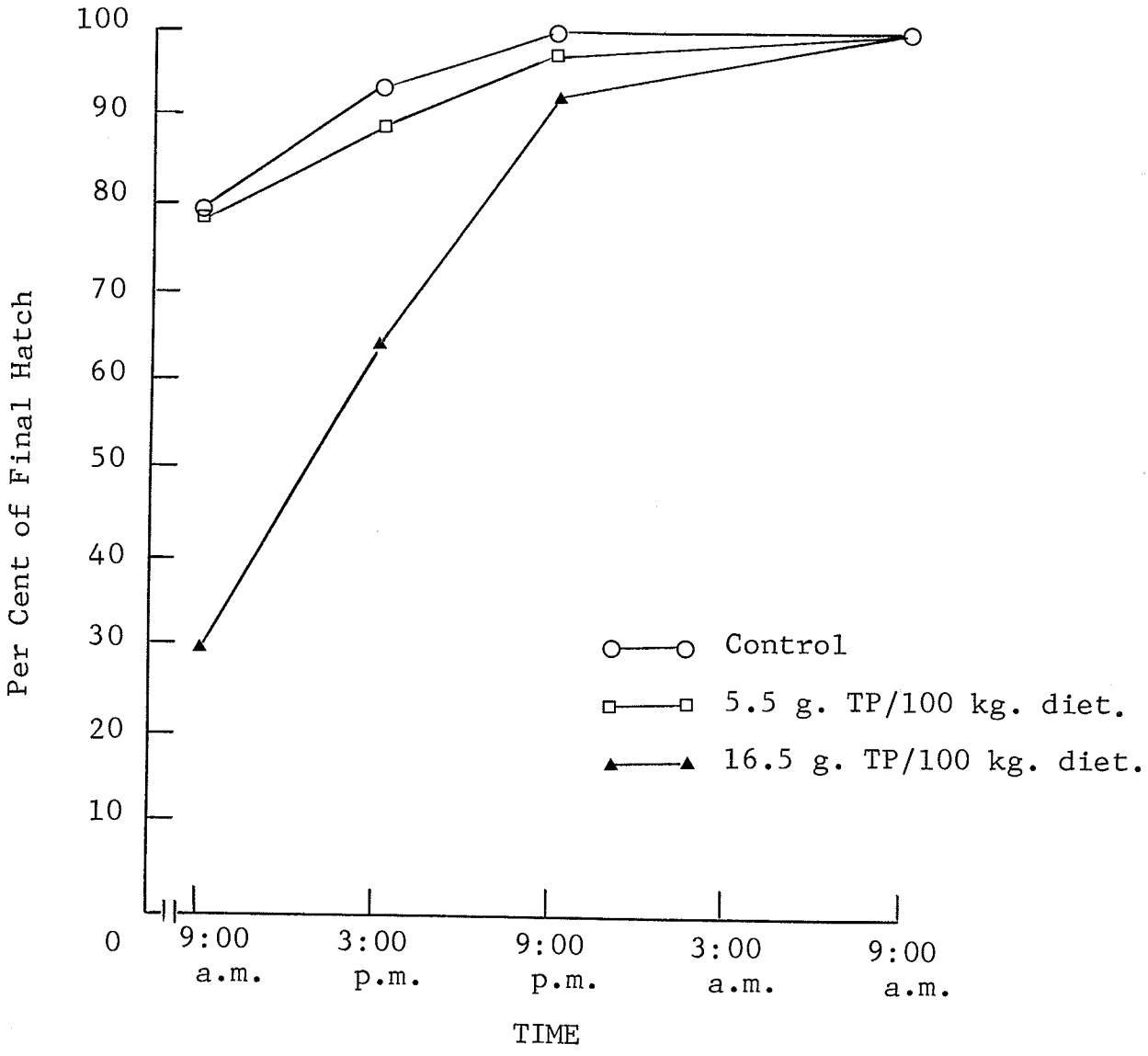

Fig. 8. Effect of thyroprotein feeding on hatching time (7th setting).

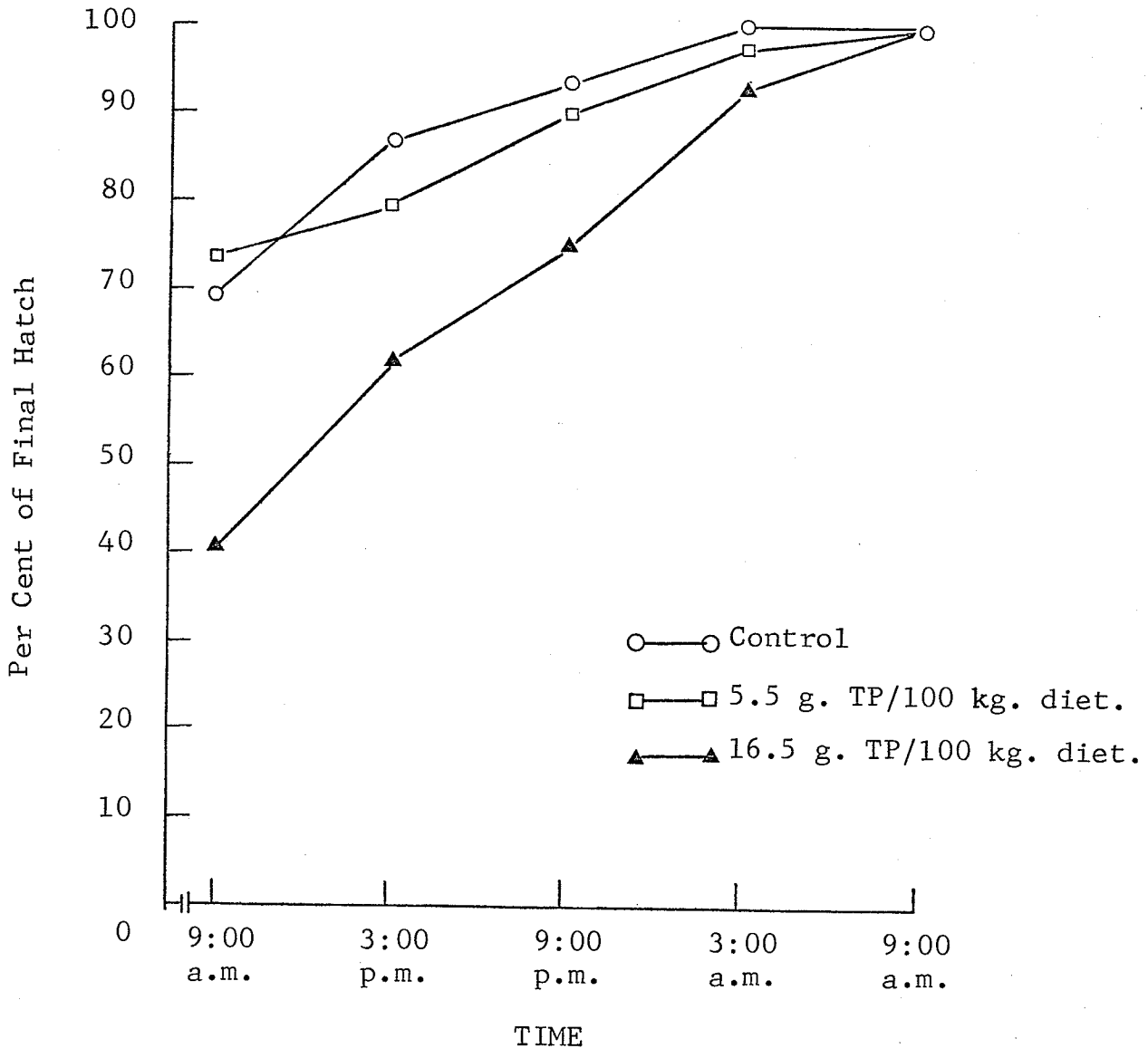

Fig. 9. Effect of thyroprotein feeding on hatching time (8th setting).

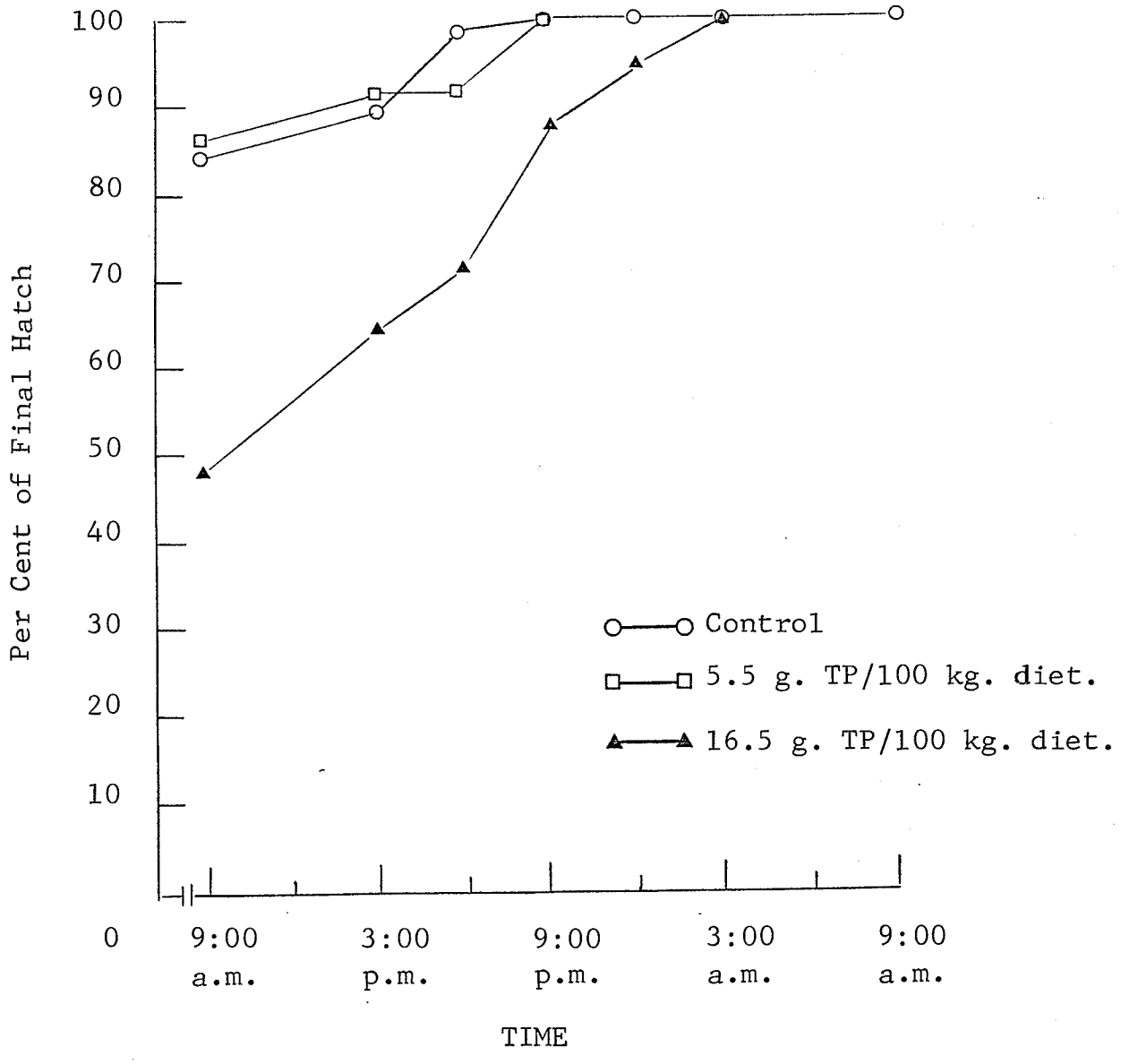

Fig.10. Effect of thyroprotein feeding on hatching time (9th setting).

made in the 9th setting of the fertility and hatchability study in which an additional observation time was scheduled at 12:00 mid-night between the 21st and 22nd day of incubation (Fig.10). In all cases studied, the incorporation of 5.5 g. of TP into 100 kg. of feed which fed to breeder hens had little or no influence on hatching time.

DISCUSSION

Phase II of the Experiment

Thyroprotein feeding at the rate of 16.5 g/100 kg. diet did not seem to influence body weight gain and mortality rate (Table 3) during the growing period of this group of broiler breeder pullets. However, their significantly higher ($P<0.05$) feed consumption contributed to less efficient feed conversion (Table 3) compared with the control. The rational for feeding TP during the growing phase was that an alteration in efficiency of feed utilization as a consequence of the exogenous thyroid hormone treatment might result in lighter and less obese birds at the onset of egg production. As this result was not obtained, it can be suggested that TP feeding is probably not a useful technique to use for reducing weight gain in growing broiler breeder pullets. It is, however, possible that higher levels of TP might have been more effective but due to the possible detrimental effects of high levels (22 g/100 kg. of feed or higher) this is probably not feasible.

No differences were obtained in body weight gain due to low-lysine treatments (Table 3). This finding is contrary to the results reported by Couch et al. (1971) but agrees with that of Singsen et al. (1964, 1965). The latter investi-

gators indicated that when chicks were fed for any period of time, on the normal diet and later changed to the deficient diet (0.65% of the diet) no effect on body weight at 21 weeks of age was found. Couch and co-workers were able to show that pullets fed (7-22 weeks) diets containing 0.42% lysine grew at a slower rate than did controls. The difference between results reported herein and those of Couch and associates may be attributed to the fact that in the present experiment birds on low-lysine diet consumed significantly ($P<0.05$) more feed than did the controls, which is contrary to the results obtained by Couch et al. The data seems to coincide with that reported by Lee et al. (1971) who indicated that daily feed intake would tend to increase if lysine intake falls below about 0.65 g. A similar effect was also well illustrated by other workers (Berg and Bearse, 1958; Berg, 1959; Waldroup and Harms, 1962; Lillie and Denton, 1966). It is likely that birds on lysine-deficient diet were able to overcome the limiting barrier presented by the low-lysine diet by consuming more feed.

Phase III of the Experiment

A. Egg Production:

The data indicating a 6% increase in egg production in response to TP feeding as observed in this study are in agreement with those reported by Turner and his associates

(Turner et al., 1945a, b; Turner et al., 1946; Turner and Kempster, 1947; Turner, 1948a; Turner and Kempster, 1948, 1949); Oloufa (1954) and more recently Proverbs (1971). However, the data are in disagreement with those of Gutteridge and Pratt (1946), Gutteridge and Novikoff (1947), Hutt and Gowe (1948), Lillie et al. (1952) and Oloufa (1953). This discrepancy of results as reported by these investigators may be explained on the possible basis that a particular strain of birds would tend to have a particular inherent degree of thyroid activity to cope with its genetic potential of productivity. This could be directly or indirectly related to the effect of higher or lower rate of general body metabolism or to the body fat deposition. This latter effect is important since it will pose a physiological and/or physical problems or barrier in terms of optimum egg production to the bird during its production cycle.

Since the feeding of TP at these two levels (5.5 and 16.5 g/100 kg. diet) to broiler breeder hens resulted in an approximately 5 to 6% increase in egg production over controls it may be suggested that the thyroid hormone(s) released into the blood stream in thyroid gland in the control hens might not have been adequate to cope with the maximum production potential inherent to the hens. The effect might have been through a direct influence on the reproductive system, interaction between the pituitary anterior and the ovary or through

an increase in metabolic activity per se.

B. Thyroid Hormones in the Blood Circulation of Broiler Breeder Hens Fed Various Levels of Thyroprotein:

Since a homogeneity test of variance (Bartlett, 1937) indicated that the PBI and T_4-I data for broiler breeder hens fed various levels of TP were not homogeneous (Appendix Table 3), no attempt was made to detect treatment differences statistically. However, in absolute terms, both PBI and T_4-I plasma values of the treated hens (treatment B and C) were markedly higher than those of the control group throughout the laying cycle. Also, the PBI and T_4-I were higher in hens fed 16.5 g. TP per 100 kg. diet. Endogenous production of thyroid hormones would seem unlikely in these two TP treated groups since the plasma levels of the hormones observed should have presumably been adequate to inhibit the relevant production of TSH in the anterior pituitary which in turn would have suppressed the endogenous output of thyroid hormones. This might also be held responsible indirectly, for the fluctuation observed for PBI and T_4-I levels (Fig. 3 and 4) with the two TP treated groups or the variation observed which might have been due solely to an occasional uneven distribution of the TP incorporated into the respective diets.

A one-way analysis of variance was performed on PBI and

T_4-I data for controls (Appendix Table 4). The data indicate that PBI in the plasma rose significantly ($P<0.05$) from the onset of egg production, peaked at the highest level of egg production, and declined thereafter (Table 6, 7, and Fig. 3 and 4). No consistent variation pattern was evident for T_4-I throughout the laying period. The data would seem to indicate that the changes in the concentration of T_3 instead of T_4 had developed during the laying cycle. This is based on the fact that Hycel PBI method would theoretically account for all protein-bound iodo-hormones in question. However, this supposition would be invalid unless a test has been performed to verify that all inorganic iodine is presumably trapped and removed in the anion-exchange process, that is, to assume that no inorganic iodine would compete with the iodohormones in binding to the specific proteins (albumin, prealbumin etc.) concerned. It is in doubt that T_3 was the only component that varied in concentration in the blood circulation of the hens in this study. It is suspected that inorganic iodine might indeed be able to compete for the specific proteins that bind the iodohormones and that not all of them were being removed during the anion-exchange process. Therefore, further work is required to elucidate the exact compounds responsible for the effect noticed for PBI levels in response to stage of egg production.

C. Feed Consumption, Feed Efficiency, Body Weight Gain and Mortality:

Feeding of TP to broiler breeder hens did not influence feed intake (Table 8). These data are in agreement with those of Berg and Bearse (1951) and Proverbs (1971) but at variance with those of Godfrey (1949). Since egg production was improved and feed consumption was not affected an improvement in feed utilization of the two TP treated groups relative to control was observed. This improvement of approximately 9.4 and 9.2% (B and C respectively) over the control would imply a greater economic return which is definitely highly desirable to broiler breeding industry. As has been mentioned elsewhere in the literature review, this criterion of expressing feed utilization has not been reported in the earlier work on TP feeding except by Proverbs (1971) and his findings corroborate that reported herein.

Data on body weight gain revealed that hens fed either level of TP were significantly lighter in weight than controls throughout the laying period. This disputes the data reported by Turner and his associates (1945, b; 1946, 1948), Hutt and Gowe (1948), Godfrey (1949), Oloufa (1953) but corroborates those of Hoffmann and Wheeler (1948), Lillie et al. (1952), and Savage et al. (1952). Proverbs (1971) showed that when 22 g. of TP was incorporated per 100 kg. of diet, and was

fed during the latter part of the laying cycle, a marked decrease in body weight gain was observed.

It is apparent that the loss of body weight (approximately 200 g. less than the controls) in the birds receiving the TP supplemented diets was not due to a reduced feed consumption. It is possible that the difference in body weight gain observed, may be explained on the basis fundamentally attributable to the effect of TP on the increase of general body metabolism. Adipose tissue deposition may have been altered and hence a relatively lighter bird produced. The possibility that feed energy absorbed was selectively utilized for more efficient egg production rather than general body weight gain in those birds receiving TP also might have contributed to the body weight reduction. This reduction of body weight is not necessarily detrimental since an ability to control body weight gain, as a measure to prevent birds from becoming too obese and subsequently affect the laying performance during the laying cycle, is desirable. This is particularly important in broiler breeding stock since in this case the birds in question have been intensively selected for high meat yield; they have a tendency toward greater fat deposition, and at the same time egg production potential has been sacrified.

A better livability was observed for TP fed groups in

this study (Table 8). Turner (1948), reported that the degree of hyperthyroidism exerted by feeding 22 g. TP/100 kg. of diet did not overstimulate any glands or organs to result in a detrimental effect. This assumption was further extended to include a higher level (44 g/100 kg.) of TP feeding in another study without detrimental effects (Proverbs, 1971). In addition, data reported by other investigators (Turner et al., 1945a, b, 1946, 1947; Turner, 1948; Hoffmann and Wheeler, 1948) also illustrate this similar supposition.

D. Egg and Yolk Weight and Thickness of Egg Shell:

The data (Table 9) showed that a reduced egg weight was observed when hens were fed either 5.5 or 16.5 g. TP/100 kg. of diet during the production cycle. This detrimental effect of TP on egg weight does not agree with the data reported by Hutt and Gowe (1948) and Proverbs (1971). However, it seems likely that the degree of reduction of egg weight might become more pronounced toward the decline of egg production. The periodical differences (values were in ascending order, i.e. egg weight increases as time lapses) in egg weight were a normal phenomenom (Appendix Table 6). In addition, the influence due to grower treatment would further substantiate the discouragement of the use of TP fueing the growing stage of birds.

The observation that yolk weight was not influenced by

TP feeding (Table 9) is at variance with the data reported by Asmundson and Pinsky (1935). These researchers were feeding high levels of desiccated thyroid which was not characterized chemically although it may be assumed that it contained a much higher level of thyroidal substances than those present in the diets here reported. Hence, the significant reduction in yolk weight which was claimed to be due to a reduction in the rate of secretion of yolk material as a consequence of the hyperthyroid condition produced by the desiccated thyroid treatment might have been expected under their set of conditions but not under the conditions of the experiment reported here. Due to the fact that no differences were found in yolk weight in the present study, the differences observed in egg weight were likely due to differences in egg albumin (presumably the water content) among treatment groups. This assumption was further substantiated by the fact that no differences were observed in egg shell thickness in this study (Table 9). This finding with regard to the influence of TP on egg shell thickness is in agreement with the data reported by Hutt and Gowe (1948) and Proverbs (1971). Other investigators (Gutteridge and Pratt, 1946; Gutterridge and Novikoff, 1947, Hoffmann and Wheeler, 1948, Wilson, 1949, Berg and Bearse, 1951, Lillie and others, 1952, Oloufa, 1953), however, have detected a significant increase in egg shell quality in response to TP feeding. Savage et al.

(1953) reported a slight increase in shell thickness. Proverbs (1971) explained this inconsistency of the results of different researchers on the basis that different strains of birds might differ in thyroid gland activity. In corroboration of this, a difference in thyroxine secretion rate of different strains of chickens has been reported (Biellier et al., 1957, Premachandra et al., 1957, 1958, Pipes et al., 1957, 1958).

E. Fertility and Hatchability:

Fertility data revealed that TP administered at the high level (16.5 g/100 kg.) tended to improve fertility (Table 11), thus, TP fed at this level might be beneficial in improving the fertility of the broiler breeder hens. This could have been due to the fact that TP treated males and/or females might have tended to be more active than the controls and also the excess abdominal fats in the latter (about 200 g. more) may have interferred with the normal mating process especially in the females. These results would tend to agree with those reported by Wheeler and Hoffmann (1948b) who were not able to demonstrate any ill-effect on fertility through TP feeding from the day of hatching (44 g. TP/100 kg. diet). However, these investigators did report a significant increase in fecundity for TP fed breeding birds as compared with that of control birds. Proberbs (1971) reported that no ill-effect was observed when a low (11 g. TP/100 kg. diet)

level of TP was used. The observation by Proverbs (1971) that either the age of hens at the time when TP was fed or the length of time for which it was fed might be associated with a decline in fertility was not in agreement with the results as described in this report. It should be pointed out that no similar test involving a two-way reciprocal cross by using White Leghorn cocks and hens as the other group, were run on the fertility capacity of semen from TP fed and control males in this study.

Hatchability results (Table 11) do not indicate that TP feeding was detrimental. This is not in agreement with the work reported by Proverbs (1971). There was no effect which could be traced to the grower treatments. These results corroborate those reported by Godfrey (1949) Huston and Wheeler (1949); McCartney and Shaffner (1950) and Savage et al. (1952), and dispute the finding as reported by Proverbs (1971) that TP feeding to hens at 26 weeks of age was detrimental. The so-called adjustment period of the birds to adapt physiologically to the TP described by Proverbs was not observed in this study.

F. Viability of Chicks Hatched from Eggs Laid by Broiler Breeder Hens fed various Levels of TP:

Wheeler and Hoffmann (1948b) found that chicks hatched from eggs laid by hens fed TP (22g/100 kg.) contained en-

larged thyroid glands. This phenomenom was also noted by McCartney and Shaffner (1949), and Godfrey (1949). Wheeler and Hoffmann (1948b) also reported that when the goiterous chicks were placed on a standard chick starter diet at the time of hatch, their thyroids became reduced in size to within the normal range in about 15 days. In the present study, no attempt was made to determine the size of the thyroid glands of the chicks. However, the data on chick plasma T_4-I and PBI levels at day one and 2 weeks of age (Table 14 and 15; Figs. 9 and 10) would suggest that a change at least in thyroid function if not thyroid size occured in the 14 days post-hatching. It is possible that the PBI and T_4-I level of chicks from eggs laid by hens receiving the high level (16.5 g/100 kg.) of TP would have gone down still further relative to the control if the growth period was extended. These results would explain the observation made by Hoffmann (1948b) that the thyroids of the chicks had become reduced in size to within the normal range in about 15 days.

The depression of body weight (Table 13) at 2 weeks of age of the chicks hatched from eggs laid by hens receiving the high (16.5 g/100 kg.) level of TP could be caused by the considerable amount of the thyroactive materials being transferred to the eggs. These compounds would be ingested

by the chicks and could result in an accelerated metabolism during the two-week post-hatching period. The fact that the mean body weight of chicks at 2 weeks of age, hatched from eggs laid by hens fed lower level of TP (5.5 g/100 kg.) was comparable to that of control chicks at this age, indicates that TP fed to hens at this level does not exert a detrimental carryover eggect on the chicks.

The significant (P 0.05) effect attributable to the grower treatment revealed at 2 weeks of age, which was manifested by smaller chicks, relative to the control in the TP fed group is difficult to explain. It is not likely a carryover effect because a period x grower treatment interaction was not evident and hence may be an artifact.

The fact that a difference among layer treatments in chick weight at day of age was not evident is also difficult to explain in light of the significant response at 2 weeks of age. It would seem logical to expect that the chicks hatched from the eggs suspected to contain the thyroactive substance would have been lighter in weight than the controls. The rationale being that any material that accelerates general metabolism should result in a reduction in hatch weight. One of the possible ansers to this might be that embryonic tissue of chicks during incubation might be less sensitive to the action of thyroactive substances. However, the possibility that inorganic iodine was deposited in considerable

amounts in the eggs should not be overlooked. The presence of inorganic iodine would tend to inhibit the TSH production in the anterior pituitary and elicit a negative feed-back reaction thus preventing the endogenous production of thyroid hormones. This would suggest that the T_4-I detected in the day-old progeny from TP fed hens was of exogenous origin.

Embryologically the thyroid of the chick has been shown to concentrate radioactive iodine at 7 days of incubation, although by this time, no follicles or colloid are discernible. On the 9th day of incubation, droplets of colloid are visible and the radioiodine accumulated was found to be protein-bound. Abrupt increases of radioiodine accumulation in the thyroid upon injection of I^{131} was also observed (Waterman, 1959). Secretion of thyroidal hormones starts after 10 to 11 days of incubation (Hansborough and Khan, 1951). An even greater radioiodine uptake was observed just before hatching (Rogler et al., 1959). These latter authors also reported that hens could concentrate radioiodide in the yolk in the ovary although only a small amount of this radioiodine was protein-bound (Okonski, 1961). The iodide was thought to be utilized by the developing embryo (Wollmann and Zwilling, 1953, Okonski et al., 1960). Watermann (1959) reported that both in vitro and in vivo studies of the thyroid reveal the same I^{131} concentration pattern and the thyroid gland was reported to be able to

develope morphologically and functionally in chorioallantoic grafts or in vitro. This would suggest that the thyroid gland of the embryonic chick possesses an intrinsic ability to develop autonomously. The result obtained in the present study that a significantly ($P<0.05$) higher PBI and T_4-I levels observed in day-old chicks from the TP fed hens might then be explained on this basis. In this case, the functional differentiation of the embryonic thyroid gland would not be related to the elaboration of thyroid stimulating hormone produced by the anterior pituitary. Therefore, the thyroid gland of embryonic chicks might have been at least relatively insensitive to the TSH but continued to accumulate and eventually produce thyroid hormones by utilizing the resources available in the embryonic eggs. Further studies are necessary in this area to clarify the various hypotheses.

G. Effects of TP Feeding on Hatching Time:

Approximately 12 hours of additional hatching time was reported by McCartney and Shaffner (1949, 1950) to be necessary for the progeny of the hens fed 22 g. TP/100 kg. diet. This phenomenom was also noticed by Proverbs (1971) though no detailed study was undertaken. It can be concluded from the present study (Figs.8, 9, and 10) that 6 to 12 hours of additional hatching time are required for the progeny of hens

fed 16.5 g. TP per 100 kg. diet as compared with controls. However, this did not apply to the lower level of TP feeding (5.5 g/100 kg. diet) where little or no additional hatching time was required.

It is without doubt that delay of hatching time was the result of TP feeding (at 16.5 g. level). However, which component(s) of the thyroactive substances being transferred to incubating eggs was (were) responsible for the effect is not clear at the present time. A delayed hatching time is consistent with a slow rate of metabolism rather than a rapid rate of metabolism and hence this result is at variance with the observed high PBI and T_4-I levels noticed in the chicks. However, the fact that the eggs laid by the hens fed the high (16.5 g/100 kg. diet) level of TP were lighter than those of the other two treatment groups but the weight of the chicks at hatching did not differ is consistent with a lower rate of metabolism. It is also possible that the differences in egg weight were due to differences in water content of the albumin (Kondra and Sell, unpublished data) and hence a difference in chick weight at hatching would not be expected. Further work is required to solve these discrepancies.

GENERAL DISCUSSION AND CONCLUSION

An experiment was conducted to study the effect of feeding thyroprotein at three different levels (0, 5.5, and 16.5 g/100 kg. of diet) to broiler breeder hens throughout the seven 28-day laying periods. One level of TP (16.5 g/100 kg. of diet) was also employed as a means in controlling mature body weight of one group of broiler breeder pullets during their growing stage (8 - 22 weeks of age). Concurrently, a low-lysine diet was fed to another similar group of pullets to act as a possible control in addition to another similar group fed a standard control diet. The Hycel PBI method and a modified Tetralute method were used to evaluate plasma levels of thyroid hormones in both the hens throughout various stages of egg production and their progeny at day-old and 2 weeks of age. The results obtained in this study are summarized as follows:

1. Grower treatment (8 - 22 weeks) did not influence the mature body weight, feed conversion, and mortality rate but pullets fed the low-lysine diet were shown to consume significantly ($P<0.05$) more feed than the control and TP fed groups. This increased feed consumption may have accounted for the inability of the low-lysine diet to restrict body weight gain.

2. Thyroprotein feeding had a favorable effect in enhancing egg production throughout the seven 28-day laying periods. An increase of approximately 5 to 6% in hen-day production over the control was obtained with both levels of TP feeding.

3. Feed intake was unaffected by either level of TP feeding.

4. Feed utilization data (kg. feed required to produce one dozen of eggs) indicated that TP treated hens tend to utilize feed more efficiently than do controls. Treatment B and C (5.5 and 16.5 g/100 kg. diet, respectively) represent an increase of 11 and 6% in feed utilization over the control.

5. Thyroprotein feeding resulted in a reduction of body weight gain of about 200 g. per hen over the seven 28-day periods.

6. Reduction of egg weight was observed in those hens fed either level of TP during the laying period but no influence on egg yolk weight or egg shell thickness was noted.

7. No detrimental effects on fertility or hatchability were noted with either level of TP feeding.

8. Additional hatching time of 6 to 12 hours was required for eggs laid by hens fed the high (16.5 g. TP/100 kg. of diet) level of TP. A normal hatching time was observed for the eggs from hens fed 5.5 g TP/100 kg. diet.

9. Thyroprotein feeding was shown to have no influence on the body weight of the day-old chicks but when these chicks were placed on a commercial starter diet, a lower body weight gain than the controls was observed for chicks hatched from eggs laid by hens fed 16.5 g. TP/100 kg. of diet. A similar effect was not noted for chicks hatched from eggs laid by hens fed 5.5 g. TP per 100 kg of ration.

10. The PBI level of the hens receiving the control diet rose significantly ($P<0.05$) from the onset of egg production, peaked at the highest level of egg production and declined thereafter.

11. Both PBI and T_4-I plasma levels of the TP fed hens were markedly higher (in absolute terms) than those of the control throughout the laying cycle.

12. The PBI and T_4-I levels in the day-old chicks from TP treated hens were markedly higher (except T_4-I in hatch #1 the level for the chicks from TP treated hens on 5.5 g. level was comparable to the controls) than the controls, with essentially no difference at 2 weeks of age.

13. After placing these birds for 2 weeks on a commercial starter ration, a marked decrease in PBI plasma level for chicks hatched from eggs laid by TP fed hens was observed. The same trend was observed in T_4-I level only from chicks hatched from hens fed 16.5 g. TP/100 kg. of ration. However,

plasma T_4-I level from chicks hatched from hens fed lower level of TP (5.5 g/100 kg.) did not reveal a definite trend in these three hatches observed.

14. A trend was observed in both plasma PBI and T_4-I level from the progeny hatched from the control hens that increased slightly 2 weeks later.

Thus, the results indicate that TP when incorporated into a standard broiler breeder diet at 5.5 g. per 100 kg. of diet can result in increased egg production without detrimental effects on fertility or hatchability. Thyroprotein fed at this level was also effective in controlling body weight gain throughout the laying cycle. The fact that no additional hatching time was required for these chicks to hatch is particularly desirable. Moreover, the mean body weight of the 2 weeks old chicks from this group of TP treated hens was virtually comparable or slightly heavier than the controls.

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APPENDIX Table 1. Analysis of variance of total feed consumption, body weight gain, feed conversion, and mortality obtained with broiler breeding pullets fed three different grower diets.

Source of Variance	Degrees of Freedom	Mean Square[1] Feed Consumption	Body Weight Gain	Feed Conversion	Mortality
Treatment	2	10.5*	1,403	0.055	0.23
Time	3	607.8*	696,033*	0.002	2.09*
Error	6	1.71	309	0.051	0.28

[1]Significantly different (*) at $P<0.05$.

APPENDIX Table 2. Analysis of variance of egg production, feed consumption and feed efficiency data obtained with broiler breeder hens fed various levels of thyroprotein throughout the laying cycle.

Source of Variance	Degrees of Freedom[1]			Mean Square [1,2]		
	EP	FC	FE	EP	FC	FE
Treatment Layer Phase (L)	2	2	2	171.92*	1.23	0.81
Treatment Grower Phase (G)	2	2	2	6.43	0.02	1.39
Periods (P)	6	-	-	7,102.52*	-	-
L x G	4	4	4	20.11	0.99	0.29
L x P	12	-	-	17.96	-	-
G x P	12	-	-	14.27	-	-
L x G x P	24	-	-	33.11	-	-
Error	105	15	15	19.48	1.58	0.49

[1](EP), egg production (% hen-day); (FC), feed consumption (kg);(FE), feed efficiency.

[2]Significantly different (*) at $P<0.05$.

APPENDIX Table 3. Chi-quare values obtained with PBI and T_4-I data.

Data	Degrees of Freedom	Chi-square
PBI	2	48.7**
T_4-I	2	72.9**

Statistically significant (**) at $P < 0.01$.

APPENDIX Table 4. Analysis of variance of plasma PBI and T_4-I data obtained with broiler breeder hens fed the control diet throughout the laying cycle.

Source of Variance	Degrees of Freedom		Mean Square[1]	
	PBI	T_4-I	PBI	T_4-I
Level of Egg Production	4	4	2.99*	0.003
Error	21	15	0.17	0.030

[1]Significantly different (*) at $P<0.05$.

APPENDIX Table 5. Analysis of variance of body weight gain and mortality data obtained with broiler breeder hens fed various levels of thyroprotein throughout the laying cycle.

Source of Variance	Degrees of Freedom[1]		Mean Square[1,2]	
	BWG	M	BWG	M
Treatment,				
Layer Phase (L)	2	2	127,934.06*	94.14*
Treatment,				
Grower Phase(G)	2	2	11,494.98	26.00
L x G	4	4	3,431.07	35.95
Error	15	15	16,485.97	24.98

[1](BWG), body weight gain and (M), mortality.

[2]Significantly different (*) at $P<0.05$.

APPENDIX Table 6. Analysis of variance of egg weight, egg yolk weight, and egg shell thickness data obtained with broiler breeding hens fed various levels of thyroprotein.

Source of Variance	Degrees of Freedom[1]			Mean Square[2]		
	EW	EYW	EST	EW	EYW	EST($\times 10^{-4}$)
Treatment in Layer Phase (L)	2	2	2	6.87*	0.10	1.45
Treatment in Grower Phase (G)	2	2	2	7.85*	0.18	0.46
Collections(C)[3]	2	-	-	417.76*	-	-
L x G	4	4	4	2.07	0.25	1.26
L x C	4	-	-	1.97	-	-
G x C	4	-	-	0.05	-	-
L x G x C	8	-	-	1.13	-	-
Error	45	15	15	1.37	0.20	0.97

[1](EW), egg weight; (EYW), egg yolk weight; (EST), egg shell thickness.

[2]Significantly different (*) at $P<0.05$.

[3]Eggs were collected at 3rd, 5th, and 7th periods throughout the seven 28-day laying periods.

APPENDIX Table 7. Analysis of variance of fertility and hatchability data obtained with eggs of broiler breeder hens fed various levels of thyroprotein.

Source of Variance	Degrees of Freedom[1]		Mean Square[1,2]	
	F	H	F	H
Treatment, Layer Phase (L)	2	2	967.67*	89.90
Treatment, Grower Phase (G)	2	2	47.69	106.02
Setting (S)[3]	8	8	89.01	320.95
L x G	4	4	282.02*	287.11
L x S	16	16	50.48	105.34
G x S	16	16	82.06	197.16
L x G x S	32	32	77.33	102.31
Error	135	135	81.23	192.59

[1](F), fertility and (H), hatchability.

[2]Significantly different (*) at $P<0.05$.

[3]One setting was made at about 50% hen-day production, 3 settings (weekly) at peak production, 3 more weekly settings just subsequent to peak production and 2 settings at decline of egg production.

APPENDIX Table 8. Analysis of variance of the average day-old and two-week old body weights obtained with chicks hatched from eggs of broiler breeding hens fed various levels of thyroprotein.

Source of Variance	Degrees of freedom		Mean Square[1]	
	Day-old	2-week old	Day-old	2-week old
Treatment in Layer Phase (L)	2	2	0.76	682.1*
Treatment in Grower Phase (G)	2	2	2.84	338.2*
Hatches[2] (H)	6	6	113.23*	616.7*
L x G	4	4	1.64	34.6
L x H	12	12	1.37	71.8
G x H	12	12	1.68	54.8
L x G x H	24	24	0.82	73.1
Error	105	105	1.53	72.1

[1]Significantly different (*) at $P<0.05$.

[2]Out of a total of nine different settings of eggs throughout the laying period, only seven were used in the viability study (the first and last setting were not included.

APPENDIX Table 9. Analyses of variance of plasma PBI and T_4-I level data obtained with day-old and 2-week old progeny of broiler breeder hens fed various levels of thyroprotein throughout the seven 28-day periods of egg production.

Source of Variance	Degrees of Freedom		Mean Square[1]	
	PBI	T_4-I	PBI	T_4-I
Hatch (H)	2	2	2.27	0.12
Age (A)	1	1	712.27**	0.47
Treatment (T)	2	2	477.14**	1.09
H x A	2	2	28.71**	0.23
H x T	4	4	14.93*	0.15
A x T	2	2	613.22**	0.51
H x A x T	4	4	1.47	0.01
Error	87	70	5.31	0.52

[1]Significantly different (*) at $P< 0.05$ and highly significantly different (**) at $P<0.01$.