THE INFLUENCE OF A TRANQUILIZER FED IN COMBINATION WITH AN ESTROGENIC COMPOUND ON PHYSIOLOGICAL ACTIVITIES, GROWTH, AND MARKET QUALITIES OF CHICKENS

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By

Kenneth George Rood

AN ABSTRACT

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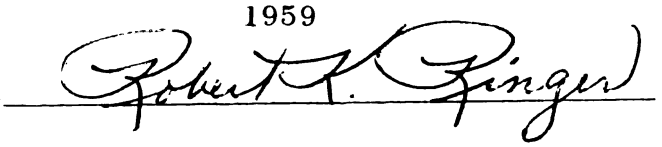
Approved 

The consequences of an estrogen and a tranquilizer, fed singly and in combination, to a known strain of White Plymouth Rocks was investigated. The tranquilizer (a perphenazine derivative) was incorporated in the feed at 4 grams per ton and a synthetic estrogenic compound (dienestrol diacetate) was incorporated in the feed at 21.14 grams per ton at two levels of protein intake.

The positions of the eight (8) dietary treatments were randomly selected and replicated among sixteen (16) identical pens of thirty-two (32) males each and sixteen (16) identical pens of thirty-two (32) females each. Body weight, feed efficiency, feathering, fleshing, pigmentation and serum xanthophyll was scored on the live birds. Carcass grade, abdominal fat weight, comb, gonadal and oviducal weights were determined after slaughter. At nine weeks of age, one-half of the birds in each pen were weighed, scored and slaughtered. The remaining birds in each pen continued on to thirteen weeks of age, at which time they were weighed, scored and slaughtered.

The lower protein recipients were from 5 to 8 percent lighter in body weight than those in corresponding groups on the higher protein ration. The oral administration of dienestrol diacetate brought body weight of the lower protein recipients up to the body weight of the higher protein control fed birds. The addition of the perphenazine derivative to a low protein ration suggested a protein sparing effect.

The addition of an estrogen or a tranquilizer to either a high or low protein ration did not increase fat deposition in the abdominal area of either sex.

The males fed the lower protein ration exhibited a higher serum xanthophyll content than males fed a higher protein level and slightly higher than that found in the blood serum of the females.

Dienestrol diacetate physiologically suppresses hypophyseal gonadotrophin release resulting in decreased testicular weight and ultimately comb size in the male and decreased ovarian weight with increased oviducal weight in the female compared to that exhibited by the birds fed the control ration. Similar results were observed when the perphenazine derivative was administered singly, suggesting an estrogen-like activity by this compound.

When the estrogenic compound and the tranquilizer were fed in combination ovarian weight was very similar to that exhibited by birds fed the control ration, indicating that the suppression of hypophyseal secretions, when either compound was administered singly, was relieved. Oviducal weights indicated that when fed in combination, the two compounds counteracted each other eliminating their estrogenic activity and giving ovarian weights similar to the controls. In contrast testicular weight was increased significantly, when the combination was fed, compared to testicular weight of birds fed the control ration.

These data concerning an estrogen and a tranquilizer, fed singly and in combination, would offed excellent tools in the study of reproduction in the fowl. Since it is possible to interrupt the reproductive cycle, apparently through endocrine interference, a more thorough investigation of endocrine inter-relationship can be made.

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INTRODUCTION

The search for products, either natural or synthetic, that will stimulate growth in chickens and improve appearance of the finished product is important to the grower and processor. Estrogens are examples of synthetic products that will improve carcass quality by promoting fat distribution and will render the edible portion more tender. Broilers, roasters and adult chickens fed an estrogenic compound are slightly heavier in body weight than birds not receiving the estrogen. This phenomena is caused primarily by increased fat deposition and is not considered by many investigators to be true growth.

Tranquilizers allay or diminish anxiety and motivation when administered to some mammals. In these mammals, increased weight gains and improved feed conversions were observed when a tranquilizer was fed singly or in combination with other compounds. A reduction in motor activity with a subsequent improvement in feed conversion without deleterious side effects would be an economic advantage to the poultry industry.

The consequences of a combination of an estrogen and a tranquilizer have not been adequately investigated; therefore, the main objective of this study was to determine the influence of a tranquilizer, administered singly and in combination with dienestrol diacetate at two levels of protein intake, on growing chickens.

REVIEW OF LITERATURE

Body weight gains and feed conversion:

When diets containing 15 percent and 18 percent protein were fed to 12-week old cockerels, Bird (1948) observed that the birds fed the high-protein diet gained more weight and showed an improved feed conversion when compared to those fed a lower protein ration, but were "lean, blue and lacking finish". Experimenting with various high protein broiler mashes, Gassner and Wilgus (1948) demonstrated improved rate of gain, improved feed efficiency and improved grade of the dressed carcasses over that obtained on the standard or low protein mash. The average grades obtained on these high protein rations were as good as those observed in birds treated with estrogens. Lorenz (1954) used rations containing varying amounts of fiber in a study of the effect of protein intake on the response of broilers (killed at 12 weeks of age) to different estrogen treatments. The low-fiber diet permitted the greatest response to the various treatments, both in additional gains and amount of fattening. Feed efficiency was decreased, as compared with control birds, by all treatments on the high-fiber ration, but dienestrol diacetate actually improved feed conversion on the low-fiber diet.

Lorenz and Bachman (1947) reported slight stimulation of total growth in 8 to 12-week old cockerels fed dienestrol

or abdominal fat weight, indicating that no advantage was derived from the tranquilizer in these respects. Wolterink et al. (1958) found that birds fed a combination of dienestrol diacetate and Reserpine in a 12 percent protein ration fed to White Rock cockerels from the first to the tenth week of age improved weight gain and resulted in greater fat deposition when compared to similar birds not receiving the additives.

Pigmentation:

In some geographical areas, yellow skinned, highly pigmented poultry meats are more desirable than the paler or white skinned varieties. This is especially true in the United States, where the yellow skinned breeds predominate, contrasted to England, where the consumer prefers poultry having white skin, white shanks, and white fat. Since Palmer (1915) first demonstrated that xanthophyll is the yellow pigment of chicken body fat, numerous investigators have confirmed this finding and demonstrated a relationship between xanthophyll content of feeds and the intensity of yellow pigmentation in shanks and body fat. Hammond and Harshaw (1941) demonstrated that yellow pigment was absent in the shanks and skin of Light Sussex chicks, regardless of the diet fed. White Wyandotte, White Leghorn and Rhode Island Red chicks were found to have approximately the same shank and skin color on any given diet. Collins et al. (1955) found the shank color of New Hampshires to be significantly darker than that of White Plymouth Rocks. A significant

diacetate at 0.01 percent of the ration. Quisenberry and Kruger (1948) also obtained an increased rate of gain, as well as greater feed consumption and increased net efficiency of feed conversion, by feeding dienestrol diacetate.

The administration of estrogens to chickens tends to decrease metabolism and activity and the fattening effects may be attributed to these factors (Bird, 1946). The current impetus for tranquilizers in animal feeding is directed toward these same effects. The quiting effect of tranquilizers on animal metabolism is believed to be obtained by the concentration of a greater share of the nutrients toward weight gains and production and less toward motor activity. In cattle feeding, tranquilizers in combination with an antibiotic and stilbestrol resulted in greater feed efficiency than either of these ingredients fed alone or in single combination with the tranquilizer (Anonymous, 1958).

Very little research has been conducted in the use of tranquilizers as an additive to poultry feeds. The results of Hewitt and Reynolds (1957) have shown Reserpine to be successful in reducing cannibalism in pheasants but the rate of body weight was also reduced. Meprobemate and promazine, when fed to White Leghorns, did not lessen characteristic nervousness but did retard body weight gains (Babcock and Taylor, 1957). Rood et al. (1958), feeding Reserpine to female broiler chicks from the fourth to the eleventh week of age with phenobarbital as a comparative compound, found no significant differences in weight gain, feed conversion,

sex difference in shank color was observed and some of the strains within each breed differed significantly. A further study of the same five strains of New Hampshires on each of three consecutive trials showed that environment markedly influenced shank color.

Culton and Bird (1941) presented evidence indicating that certain common poultry feedstuffs such as meat scraps, fish meal and soybean oil meal contain a factor, or factors, which inhibit the deposition of yellow pigment in the shanks of growing chicks. It would appear that some lots of these common protein supplements must be free of inhibiting effects, otherwise a high degree of pigmentation could not be produced under normal practical conditions.

Certain brands of cod liver oil were found to be potent sources of a pigment-suppressing factor (Hammond and Harshaw, 1941). Although the reason for the inhibition of pigmentation when these oils were fed was not determined, a large portion of the inhibiting factor could be eliminated by heating the cod liver oil to 230°C. in a vacuum for three hours. It was theorized that the pigment might be destroyed by oxidation induced by the unsaturated compounds in the cod liver oil. To test this theory, various oils and other so-called contaminants were fed. These supplements had no appreciable effect on pigmentation, resulting in the conclusion that the color of the shanks and skin in the yellow skin breeds was dependent on the interaction of the quantity of the pigment-inducing ingredient and the amount of yellow pigment-suppressing

factor present in the diet.

Day and Williams (1958) significantly increased the pigmentation exhibited in broilers by increasing the calorie: protein ratio the last two weeks of the feeding period.

Palmer's pioneer work proved that, in hens, dietary xanthophylls (but not carotene) occur in the blood plasma and in the fat and skin, especially in the shanks. Xanthophyll (lutein) is esterified and stored in the liver, skin, fat, face and beak of the hen. Hollander and Owen (1939) noted xanthophyll in the iridial carotenoids and demonstrated their lability by showing that alteration of the diet can alter the eye color of hens.

The contribution which carotenoids make to the pigmentation of the plumage of birds is considerable, and feather carotenoids can be considered analogous to the carotenoids stored in the external structures of sea animals. In both cases the carotenoids are xanthophyllic, produced by the animals from the alimentary carotenoids (Goodwin, 1952).

Xanthophylls are laid down in the skin and shanks as esters in a non-laying chicken. During the laying period these are mobilized in the free state and deposited into the eggs. In pullets, carotenoids are mobilized into the blood by heavy doses of estrogens and this is probably how the pigments are transferred to the eggs when the pullet comes into lay (Common and Bolton, 1946). The basic findings that striking increases in blood lipid levels occur during periods of ovarian activity have been confirmed. Further, Lorenz

et al. (1938) demonstrated that liver lipids are also increased in hens during periods of ovarian activity.

Contributions to the understanding of the lipogenic mechanism were made by Entenman et al. (1938). who demonstrated lipemia in intact immature female birds following administration of crude gonadotrophin. and by Lorenz et al. (1938), who produced lipemia in immature birds of both sexes by the administration of crude estrin.

The blood lipids of the laying female are considerably higher than in the non-laying female or in the male. and most of the surplus fat is deposited in the egg in the form of yolk. Since the blood lipids of males receiving estrogen are also increased. the surplus blood fat in males must be stored in the tissue. thus making the bird fatter and more desirable as a meat bird.

Feathering:

In birds with six-dimorphic feathers. the administration of an estrogen can alter the shape of the feather and the nature and distribution of feather pigment from the sexually-indifferent pattern typical of the male into the direction of female plumage normal for the species and breed. Of additional practical importance to broiler growers is the effect of estrogens on rate of feather growth. By experimentally plucking small areas, Lorenz (1954) observed that estrogen treatment tends to inhibit the formation of new pinfeathers but does stimulate the growth of those already erupted. The number of pinfeathers present when the birds

are marketed is thus reduced.

Carcass quality:

The degree of external fatness designates finish, while the degree of internal fat deposited in the muscle tissue determines quality and tenderness of the flesh. An even distribution of fat in all parts of the body is essential to optimum quality in the carcass. Maw (1935) found that the different cereals cause the deposition of fats in entirely different manners; for instance, cornmeal causes a high percent of the total body fat to be deposited in the flesh and much less fat in the abdominal cavity and in the skin of the bird, whereas the cereals, oats, barley, and wheat, show the reverse in varying degrees. Wheat gave an excellent external appearance, a uniform layer of fat deposited over the entire carcass, but very little deposition in the actual flesh. Several investigators have shown that fat deposited in body tissues replaces moisture, thus increasing the edible portion of the carcass.

Harms et al. (1958) demonstrated that as the amount of yellow corn was increased in the diet, finish of birds receiving the diet was improved.

The first suggestion that birds might be fattened for market by estrogen administration was made by Lorenz (1943) who sued subcutaneous implantation of diethylstilbestrol (DES) pellets on cockerels. A single 12 to 15 mg. pellet produced a noticeable effect on growing birds within a week. DES has a relatively low oral estrogenic potency on chickens

and large doses are required to produce fattening. Jaap (1944), Munro and Kosin (1946). Thayer et al. (1945) observed that a slight improvement in finish was obtained when 8-week old Barred Plymouth Rock cockerels consumed 75 mg. of the estrogen during a three week feeding period and 100 mg. in a four week period. Birds that consumed 50 mg. of the estrogenic compound in a two week period did not show an improvement in grade compared to the group receiving no estrogen. Feeding DES to 6- and 11-week old White Leghorn cockerels for 4 weeks produced only questionable increases in abdominal fat deposition when over 200 mg. of estrogen were consumed, and none when the total dose was lower (7 to 144 mg.)(Lorenz, 1945). Glazener and Jull (1946) obtained excellent fattening in 6-week old Barred Plymouth Rocks x New Hampshire cross-bred cockerels fed DES for 3 weeks; but they used massive doses (630 mg. per bird). Some improvement in grade has been reported on relatively low doses by Sykes et al. (1945), who fed DES at the rate of 1 mg. per day. Rhode Island Red and Barred Plymouth Rock cockerels 5- to 12-weeks of age were used, and various treatment periods permitted total doses of 30 to 130 mg. of the estrogenic compound per bird. Black (1946) also reported improvement in 17-week old Light Sussex cockerels fed 68 mg. of DES over a period of 7 weeks. The reasons for these discrepancies are not obvious but may be related to different criteria of carcass quality or the effect of breed on responsiveness to estrogens.

Dianisylhexene, fed to chickens, is much more potent as an estrogen by the oral route than is DES and has produced better fattening responses at lower doses. Lorenz (1945) obtained good fat deposition by feeding 144 and 236 mg. of the compound over a period of 4 weeks to 6-week old White Leghorn cockerels. Thayer et al. (1945) fed levels varying from 44 to 220 mg. of dianisylhexene per kilo of feed and reported improvement in grade over their entire range of experimental conditions. Birds receiving the higher levels exhibited symptoms of overdosage, with loss of appetite, leg weakness, and occasional death.

Due to its relatively high estrogenic potency when fed to cockerels, dienestrol was the most promising of the compounds tested. In preliminary trials it appeared to be an excellent fattening agent. Its solubility characteristics made the possibility of practical application seem remote. The diacetate of dienestrol, however, is more easily handled and the lability of the ester linkages would have the same physiological action as the free phenol. Dienestrol is effective when fed at the level of 0.01 percent or less of the feed for 3 to 6 weeks. Lorenz and Bachman (1947) found that this estrogen gave satisfactory results when fed at levels of 0.0033 percent, but more time was required to produce results than that at higher levels. At the o.01 percent level or above, lipemia was produced, but not at the 0.0033 percent level.

The primary objective of estrogen treatment of poultry is improvement of carcass quality. Estrogen treatment has at least some influence on each component of carcass quality, and for the most part the influence is favorable. Fatness is the best-known advantage gained; extra fat is deposited in all parts of the bird. Muscle fat, important for "juiciness" of the meat, is consistently increased, and, in the leg tissue especially, may be more than doubled (Lorenz, 1943; Sykes et al. 1945). Increases in subcutaneous fat improve the appearance of the dressed carcass. Visceral fat may be increased many fold (Lorenz, 1943 and Bird, 1946).

Warden et al. (1958) demonstrated that the administration of dienestrol diacetate at 1/3 pound per ton, significantly reduced the deposition of abdominal fat in the males but not as markedly in the females compared to that exhibited by similar birds receiving an implant of DES. They concluded that this effect may be due to a decreased calorie: protein ratio of the fattener rather than to the influence of dienestrol diacetate.

Finish is improved and a smooth "silky" texture is imparted to the dressed carcass by the increased subcutaneous fat and by the effects of estrogen on skin structure and feather growth. Improved skin texture is a definite consequence of estrogen treatment, presumably due to hypertrophy of the epidermal strata (Bird, 1948). Dienestrol diacetate was more effective in producing this hypertrophy than dianisylhexene when both were administered orally and in the same

dosage.

Endocrine system and secondary sex characteristics:

The experiments of Berthold (1949) on transplantation of fowl testes are regarded as the first demonstration of endocrine secretion. The relation of the gonads and their endocrine products to the development of secondary sexual characteristics, to the functions of primary and accessory sex organs, and to overt sexual behavior have been the center of interest to numerous researchers. Surgical castration has been practiced for many years; however, since the isolation of the hormonally active material by Dodds et al.(1937) and the synthesis of DES and other potent estrogenic stilbene derivatives, the older method is rapidly being replaced by the administration of the synthetic compounds.

Behavioral responses to administered estrogens include diminished aggressiveness and cessation of fighting and crowing. Davis and Domm (1943) presented evidence to show that estrogen is a direct though weak stimulator of masculine sex behaviour in the male, but not in the female. Estrogens most striking direct effect is stimulation of sexual receptiveness. Chickens treated with adequate estrogen dosage readily assume the typical feminine receptive position, squatting with slightly spread wings when approached by a normal cock or by some other stimulus such as the human caretaker.

Retention of estrogen in edible tissue:

The significance to human health of tissue retention of estrogens was examined by Bird et al. (1947). Menopausal women were fed diets containing livers or rendered fat from treated birds daily for 6 days. Some vaginal cornification was obtained when tissues of dianisylhexene-treated birds were fed, but no demonstrable effect was produced by tissues of birds treated with dienestrol diacetate. Lowe (1949) made biological assays to determine the quantity of residual estrogen remaining in the flesh and skin, liver, gizzard, heart, and abdominal adipose tissues of cockerels which had been fed dienestrol diacetate. All the tissues assayed contained negligible amounts of estrogen and the conclusion drawn was that a person would have to consume at least 12 to 120 pounds of chicken skin and flesh every other day to obtain a clinical dose of estrogen of the magnitude that is used in practice (1 mg. to 10 mg.) (Selye, 1947; Robson, 1947).

The edible tissue of chickens fed dienestrol diacetate were examined by Umberger and Goss (1959) for estrogenic residues. Within limits of the assay method, no estrogenic residues could be detected in muscle, abdominal fat, skin fat, heart, gizzard or spleen. The results suggest that dienestrol diacetate is not stored in the edible tissues but that a period of 24 hours off the estrogen feed before slaughter is not sufficiently long to insure complete removal of the estrogen from the usual pathways of excretion.

OBJECTIVES

The poultry industry is an extremely competitive business, continually searching for new products and methods to improve the efficiency of production and the acceptance of the finished product. This study was designed to furnish information for the eventual practical application of a tranquilizer as an additive to poultry feeds. The tranquilizer, administered singly and in combination with an estrogenic compound at various levels of protein intake, was studied to test the following objectives:

a. To measure the physiological activity of the tranquilizer administered alone and in combination with an estrogenic compound.

b. To determine the influence of the dietary treatments on weight gain and feed efficiency.

c. To establish the effect of protein intake and the addition of a tranquilizer and an estrogenic compound on pigmentation, deposition of abdominal fat, gonadal and oviducal weight.

d. To determine the influence of the dietary treatments on live market quality and dressed carcass grade.

EXPERIMENTAL PROCEDURE

The chicks used in this study were a known broiler strain of White Plymouth Rocks purchased from a local hatchery. Thirty-seven (37) wing-banded chicks of one sex were placed in each individual pen. Sixteen (16) identical pens of males were housed on one side of a brooder house with sixteen identical pens of females on the opposite side (see appendix for experimental design). The sexes were reared separately to eliminate sexual differences in behavior and feed conversion. During the first five weeks of the experimental period, a high energy pigment starter (Table 1) was fed to all pens (rations formulated by Poultry Nutrition Department, Michigan State University). This was a control period to facilitate selection of birds that exhibited relatively uniform growth rate before initiation of the dietary treatments.

Randomization of experimental chickens:

Just prior to the thirty-fifth day of age, the birds were weighed and segregated by weight into 30 gram increment lots. All birds in the middle or average weight division were uniformly distributed into the pens. The same procedure was followed in the weight increment above the average and then the group below the average, then the group that weighed two increments (60 grams) above the average, then two increments below and so on until 32 birds were placed in each of

the experimental pens. The same procedure was followed for both males and females assuring a uniform distribution of weight throughout the experimental pens. This selection aided in the elimination of any significant differences in weight at the initiation of dietary treatment.

Table 1 - High energy pigment starter ration (basal from 1 to 5 weeks)

Ingredient	Lbs. or gms./ 100 lbs.
Corn, grd. No. 2 yellow	45.00
Alfalfa leaf meal, 20 percent protein	2.50
Soybean oil meal, 50 percent protein	36.00
Menhaden fish meal, 60 percent protein	2.00
Meat and bone scraps, 50 percent protein	2.50
Ground limestone	0.75
Dicalcium phosphate	1.25
Salt, iodized	0.50
Whey, delactose (50 percent lactose)	0.50
Brewers dried yeast	0.50
No. 2 yellow grease	8.00
Vitamin supplement No. 1	34 grams
Vitamin D_3 (3,000 ICU/gm.)	10 grams
Choline chloride (25 percent supplement)	30 grams
Vitamin B_{12} supplement (6 mg./lb.)	0.10
Methionine	0.10
Delamix	0.10
Niacin	0.10
	1 gram
	100 lbs.

Calculated analysis

Crude protein percent	25
Crude fat percent	10.5
Crude fiber percent	2.5
Productive energy (Cal./lbs.)	1025

Dietary treatments:

At the end of the fifth week and after the experimental pens were formed as previously described, the following dietary treatments were initiated.

Code	Treatment Description
HPC	High protein basal control
HPL	High protein basal plus Lipamone[a]
HPLQ	High protein basal plus Lipamone plus Quietrol[b]
HPQ	High protein basal plus Quietrol
LPC	Low protein basal control
LPL	Low protein basal plus Lipamone
LPLQ	Low protein basal plus Lipamone plus Quietrol
LPQ	Low protein basal plus Quietrol

The positions of the various treatments were randomly selected to eliminate positional variables. The estrogenic compound (Lipamone) was administered orally at one-third pound per ton (21.14 gms./ton). The tranquilizer (Quietrol) was administered orally at 2 mg. per pound of feed (4 grams per ton). During the growing period, 5 to 9 weeks, the two levels of protein intake were calculated at 20 and 16 percent respectively as shown in Table 2. At the end of the ninth

a. Lipamone, 14 percent dienestrol diacetate in corn distillers dried grains, manufactured and distributed by White Laboratories, Inc., Kenilworth, N.J. (Now, American Scientific Laboratories, Inc., Madison, Wis.).

b. Quietrol, containing 50 grams of perphenazine, 1-(2-hydroxyethyl)-4[3-(2-chloro-10-phenothiazinyl)-propyl]-piperazine, per pound, manufactured and distributed by White Laboratories, Inc., Kenilworth, N.J.

week individual body weights, and feather, fleshing and pigment scores were recorded.

Table 2 - Grower ration (basal from 5 to 9 weeks)

Ingredient	High protein lbs. or gms./ 100 lbs.	Low protein lbs. or gms./ 100 lbs.
Corn, grd. No. 2 yellow	54.88	72.78
Soybean oil meal, Solv. 44 percent	30.00	16.00
Fat, No. 2 yellow grease	5.00	1.00
Alfalfa leaf meal, 20 percent protein	2.50	2.50
Meat and bone scraps, 50 percent "	2.50	2.50
Fish meal (Menhaden 60 percent protein	.50	.50
Whey, delactosed product	.50	.50
Yeast, dried brewers	.50	.50
Salt, iodized	.50	.50
Dicalcium phosphate	2.00	2.00
Limestone, grd. (98 percent $CaCO_3$)	1.00	1.00
Delamix	.10	.10
Vitamin suppl. 249C	.05	.05
Choline Chloride (25 percent dry mix)	- --	.10
Nicarbazin (25 percent dry mix)	.05	.05
Vitamin D_3 (3,000 ICU/gm.)	10 grams	10 grams
Vitamin A (10,000 IU/gm.)	10 grams	10 grams
Calculated analysis:		
Crude protein percent	20.2	15.6
Crude fat percent	7.7	4.3
Crude fiber percent	3.5	3.3
Productive energy (Cal/lb.)	998	984

Feathering was scored by the author, as suggested by Arscott (1958), as follows:

Feather score:	Description:
0	Back completely bare
1	Large bare areas
2	Numerous pin feathers and small bare areas
3	Numerous pin feathers
4	Few pin feathers
5	No pin feathers

Fleshing score:

Fleshing was scored by the author on an arbitrary basis. The bird was suspended by the legs so as to determine the degree of fleshing on the legs, thigh and breast. A score of five was highest, zero the lowest (indicating poor fleshing).

Pigmentation score:

Pigmentation was determined visually by the author by comparing the shanks to discs on the Heiman-Carver Yolk Color Rotor.

One-half of the birds from each pen were sacrificed at 9 weeks of age for dressed carcass grade, abdominal fat weights and serum xanthophyll determinations.

The remaining birds in each pen continued on experiment until the thirteenth week of age with the following changes: the high protein basal was reduced from 20 percent to 16 percent protein; the low protein basal was reduced from 16 percent to 13 percent protein, both rations remaining isocaloric

(1000 ≠ calories per pound). The finisher formula is given in Table 3. At the end of the thirteenth week the individual weights and scores were recorded and the birds sacrificed for serum xanthophyll, carcass grade and abdominal fat weights.

Serum xanthophyll determination:

In this experiment, the procedure for determination of serum xanthophyll outlined by Davis and Kratzer (1958) was modified. Approximately 10 ml. of whole blood was collected without an anti-coagulent, from each bird at the time of slaughter. The blood samples were placed in a 35°F. cooler until all birds were slaughtered (approximately 7 hours). Each tube was ringed to allow the clot to withdraw and free the serum. Some samples were centrifuged to facilitate serum collection. The next day the serum was poured off into small vials and frozen.

On the day of serum xanthophyll determination, the samples were separated by treatment and sex and allowed to thaw. A sufficient volume of serum was needed to run duplicate samples for comparison of technique. This necessitated pooling, 2, 3, and in some cases, 4 serum samples from different birds on the same treatment. These pooled samples were then centrifuged to separate any cells that may have been present, and the serum decanted. To each 1 ml. of serum was added 10 ml. of acetone. This solution was mixed by bubbling with air and then centrifuged to remove the precipitate. The supernatant of each mixture was poured into a clean dry spectrophotometer cuvette and the optical density compared to a blank (a cuvette containing acetone).

Table 3 - Finisher ration (basal from 9 to 13 weeks)

Ingredients	High Protein lbs. or gms./ 100 lbs.	Low Protein lbs. or gms./ 100 lbs.
Corn, No. 2 grd. yellow	68.59	78.63
Soybean oil meal, dehulled (50% protein)	13.60	6.00
No. 2 yellow grease	3.50	1.00
Wheat, middling flour	5.00	5.00
Meat and bone scraps	2.50	2.50
Alfalfa leaf meal (20% protein)	2.50	2.50
Whey, delactosed prod.	.50	.50
Yeast, dried brewers	.50	.50
Fish meal, Menhaden	.50	.50
Limestone, grd.	1.00	1.00
Dicalcium phosphate	1.00	1.00
Salt, iodized	.50	.50
Delamix	.10	.10
Vitamin Suppl. 249C	.05	.05
Vitamin B_{12} suppl. (6mg/lb)	.05	.05
Nicarbazin (25 percent mix)	.05	.05
Choline chloride (25 percent dry mix)	10 gram	40 gram
Vitamin A suppl. (10,000 USP/gm.)	10 gram	10 gram
Vitamin D_3 Suppl. (3.000 ICU/gm.)	10 gram	10 gram
	100 lbs.	100 lbs.
Calculated analysis:		
Crude protein percent	15.96	13.02
Crude fat percent	6.64	4.47
Crude fiber percent	2.66	2.69
Prod. energy (Cal/lb.)	1041.30	1038.30

Dressed carcass grading criteria:

The New York dressed birds were graded according to U.S.D.A. Standards for fleshing, conformation, pigmentation and defects. The following scores were then allocated to allow the determination of a percentage figure.

Score:	Description:
10	Grade A
9	Grade A with a defect
8	Grade A with pigmentation or fleshing deficiency
7	Grade B
6	Grade B with a defect
5	Grade B with pigmentation or fleshing deficiency
4	Grade C
0	Rejected

Abdominal fat weight, gonad and oviduct weight determinations:

The carcasses were placed in a freezer until further measurements could be made. A limited number of birds were thawed each day to allow the determination of abdominal fat weight, oviduct weight and gonad weight. The fat surrounding the proventriculus, gizzard and the pad of fat found in the abdominal area was removed and weighed in grams. The testes were removed, trimmed of extraneous material and the weight recorded in milligrams. With the females, a similar procedure was followed for determining the weight of the oviduct.

Statistics:

All data were statistically analyzed using the Duncan Multiple Range and Multiple F Test. Duncan (1955) and the Analysis of Variance and "t" Method. Snedecor (1946).

RESULTS

Body weight and feed conversion:

Statistical analysis of the body weight groupings of the 1,024 birds selected at the end of the 35 day control period showed no significant differences except that of sex. Accordingly, weight differences at the end of the ninth week can be interpreted without reference to differences in the pre-treatment period.

At the ninth week, the body weights were still highly uniform. No differences between replicates had appeared nor was there any interaction between sex and treatment. The initial sex difference was somewhat larger. Now, however, a highly significant effect of treatment had appeared, Table 4.

The birds on the 16 percent protein ration were from 5 — 8 percent lighter than those in corresponding groups on the 20 percent protein ration. Table 5. These differences were not always significant, however, when the body weights are separated according to protein level. as in Table 5, no effect of the hormone or tranquilizer supplements on body weight can be demonstrated. However, when the body weights are separated by sex, Table 6, it appears that the oral administration of Lipamone either to the males or females brought body weights of the low protein recipients up to the body weights of the high protein control fed birds. When the

tranquilizer was orally administered in combination with Lipamone, this effect was noted only in the males.

A statistical analysis of the body weights at 13 weeks of age for both males and females receiving all eight dietary treatments is found in Table 7. These data indicate a highly significant difference in weight gain due to sex while there was no statistical difference between the treatments; thus, the addition of Lipamone or Quietrol had little or no benefit on weight gain, Table 8.

A statistical analysis of body weights at 13 weeks of age between the males receiving the eight dietary treatments (Table 9) showed the males receiving high protein (16 percent) basal plus Lipamone and Quietrol in combination and Lipamone alone were slightly heavier than the other treatments. This would infer that Lipamone plus a protein intake of 16 percent is as efficient at this age as any other combination investigated. An analysis of the body weights of the females indicates a significant difference between the two levels of protein administered and apparently the additives had little or no influence on body weight between 9 and 13 weeks of age.

The effect of treatment on mean body weights of males and females is given in Table 10. This table is devoid of statistical analysis in an attempt to show the influence of the dietary treatments on mean body weights for a simple comparison of the results at both ages.

A comparison of the weight gain and feed efficiency of the males during the growing period, 5 to 9 weeks of age,

Table 4 - Analysis of variance for 9-week body weights as affected by sex, treatment, replication and interaction.

Source of Variance	d.f.	m.s.	F[1]	F value P < 0.05
Total variance	1,016			
Sub-class	31	539,267	27.80**	1.47
Sex	1	14,676,838	756.58**	3.85
Treatment	7	269,938	13.92**	2.02
Interaction	7	21,552	1.11	2.02
Replication	1	560	0.0285	3.85
Error	985	19,399		

[1] F value with two asterisks indicates significance at P < 0.01 level using the Analysis of Variance.

Table 5 -- The effects of protein level on body weight of chickens at 9 weeks of age

Males and females, replicates pooled, receiving high protein (20 percent)

Means (in grams) ranked in descending order (std. error of mean = ± 24.7 grams)[1]

Treatment	HPLQ	HPL	HPQ	HPC	HPL	HPLQ	HPC	HPQ	F value[2]
Sex	M	M	M	M	F	F	F	F	
Number of birds	64	64	63	64	64	64	64	64	
Mean	1386	1385	1357	1342	1134	1122	1118	1110	27.5**

Males and females, replicates pooled, receiving low protein (16 percent)

Means (in grams) ranked in descending order (std. error of mean = ± 24.8 grams)[1]

Treatment	LPL	LPQ	LPLQ	LPC	LPL	LPQ	LPLQ	LPC	F value[2]
Sex	M	M	M	M	F	F	F	F	
Number of birds	64	64	64	63	63	64	63	62	
Mean	1294	1290	1280	1273	1075	1048	1042	1033	24.9**

[1]Means not underscored by the same line are significantly different at $P < 0.01$ level by the Duncan Multiple Range and Multiple F Tests.

[2]F value with two asterisks indicate a significance at $P < 0.01$ level using the Analysis of Variance.

Table 6 - The effects of treatment on body weight of males and females at 9 weeks of age.

Males with replicates pooles, treatment indicated

Means (in grams) ranked in descending order (std. error of mean= ± 19.65 grams)[1]

Treatment	HPLQ	HPL	HPR	HPC	LPL	LPQ	LPLQ	LPC	F value[2]
Number of birds	64	64	63	64	64	64	64	63	
Mean	1,386	1,385	1,357	1,342	1,294	1,290	1,280	1,273	5.73**

Females with replicates pooled, treatment indicated

Mean (in grams) ranked in descending order (std. error of mean= ± 14.91 grams)[1]

Treatment	HPL	HPLQ	HPC	HPQ	LPL	LPQ	LPLQ	LPC	F value[2]
Number of birds	64	63	64	64	63	64	63	62	
Mean	1,134	1,122	1,118	1,110	1,075	1,048	1,042	1,033	7.29**

[1] Means not underscored by the same line are significantly different at $P < 0.01$ level by the Duncan Multiple Range and Multiple F Tests.

[2] F value with two asterisks indicate significance at $P < 0.01$ level using the Analysis of Variance.

Table 7 - Analysis of variance for 13-week body weights as affected by sex, treatment, replication and interaction.

Source of variance	d.f.	m.s.	F[1]	F value P 0.05
Total variance	501			
Sub-class	31	1,020,090	2.93**	1.49
Treatment	7	920,373	2.65*	2.03
Sex	1	23,762,271	68.43**	3.86
Replication	1	13,516	0.03	3.86
T x S	7	90,685		
T x R	7	49,167		
R x S	1	347,248		
T x R x S	7	11,170		
Error	470	53,239		

[1] F value with one asterisk indicates significance at $P < 0.05$ level, two asterisks indicate significance at $P < 0.01$ level using the Analysis of Variance.

Table 8 - The effects of protein level on body weight at 13 weeks of age.

Males and females, replicates pooled, receiving high protein (16 percent)

Means (in grams) ranked in descending order (std. error of mean = ± 53.36 grams)[1]

Treatment	HPLQ	HPL	HPQ	HPC	HPL	HPLQ	HPC	HPQ	F value[2]
Sex	M	M	M	M	F	F	F	F	
Number of birds	32	32	31	32	32	31	30	32	
Mean	2388	2335	2252	2215	1840	1836	1807	1791	23.78**

Males and females, replicates pooled, receiving low protein (13 percent)

Means (in grams) ranked in descending order (std. error of mean = ± 60.72 grams)[1]

Treatment	LPQ	LPC	LPLQ	LPL	LPL	LPQ	LPLQ	LPC	F value[2]
Sex	M	M	M	M	F	F	F	F	
Number of birds	32	31	32	32	31	32	30	30	
Mean	2077	2044	2041	1983	1661	1665	1653	1606	12.39**

[1] Means not underscored by the same line are significantly different at $P < 0.01$ level by the Duncan Multiple Range and Multiple F Tests.

[2] F value with two asterisks indicate significance at $P < 0.01$ level using the Analysis of Variance.

Table 9 - The effects of treatment on body weight of males and females at 13 weeks of age.

Males with replicates pooled, treatment indicated

Means (in grams) ranked in descending order (std. error of mean = ± 47.67 grams)[1]

Treatment	HPLQ	HPL	HPQ	HPC	LPQ	LPC	LPLQ	LPL	F value[2]
Number of birds	32	32	31	32	32	31	32	32	
Mean	2338	2335	2252	2215	2077	2044	2041	1983	10.05**

Females with replicates pooled, treatment indicated

Means (in grams) ranked in descending order (std. error of mean = ± 32.95 grams)[1]

Treatment	HPL	HPLQ	HPC	HPQ	LPL	LPQ	LPLQ	LPC	F value[2]
Number of birds	32	31	30	32	31	32	30	30	
Mean	1840	1836	1807	1791	1661	1655	1653	1606	8.49**

[1] Means not underscored by the same line are significantly different at $P < 0.01$ level by the Duncan Multiple Range and Multiple F Tests.

[2] F value with two asterisks indicate significance at $P < 0.01$ level using the Analysis of Variance.

Table 10 - Effect of treatment on the mean body weight of males and females at 9 and 13 weeks of age

Treatment	At 9 weeks of age		At 13 weeks of age	
	Males (grams)	Females (grams)	Males (grams)	Females (grams)
HPC	1342	1118	2215	1807
HPL	1385	1134	2335	1840
HPLQ	1386	1122	2388	1836
HPQ	1357	1110	2252	1791
LPC	1273	1033	2044	1606
LPL	1294	1075	1983	1661
LPLQ	1280	1042	2041	1653
LPQ	1290	1048	2077	1665

Table 11 - The effect of treatment on weight gain and feed efficiency of males and females during the growing period (5 to 9 weeks of age)

Treatment	Average gain in grams*	Feed/Gain	Percent improvement
Males (in descending order of efficiency)			
HPLQ	755	3.10	18.6
HPL	758	3.21	15.7
HPQ	722	3.29	13.6
HPC	716	3.30	13.4
LPQ	668	3.54	7.1
LPL	672	3.60	5.5
LPLQ	661	3.61	5.2
LPC	645	3.81	0
Females (in descending order of efficiency)			
HPQ	579	3.44	17.5
HPL	587	3.47	16.8
HPC	575	3.59	13.9
HPLQ	570	3.63	12.9
LPL	524	3.91	6.2
LPLQ	496	4.09	1.9
LPQ	501	4.12	1.2
LPC	486	4.17	0

* Extremely small birds (runts) were eliminated in the calculation of average gain but included in the calculation of feed efficiency.

Table 12 - The effect of treatment on weight gain and feed efficiency of males and females during the finishing period (9 to 13 weeks of age)

Treatment	Average gain in grams*	Feed/Gain	Percent improvement
Males (in descending order of efficiency)			
HPL	945	3.76	19.7
HPQ	913	3.86	17.5
HPC	905	3.90	16.7
HPLQ	957	3.94	15.8
LPLQ	777	4.38	6.4
LPQ	788	4.50	3.8
LPL	723	4.64	0.9
LPC	783	4.68	0
Females (in descending order of efficiency)			
HPL	703	4.22	19.3
HPQ	678	4.30	17.8
HPC	693	4.53	13.4
HPLQ	709	4.61	11.9
LPL	619	4.87	6.9
LPQ	614	5.09	2.7
LPC	570	5.15	1.5
LPLQ	578	5.23	0

* Extremely small birds (runts) were eliminated in the calculation of average gain but included in the calculation of feed efficiency.

is shown in Table 11. The high protein (20 percent) produced a 6 to 11 percent improvement in feed conversion compared to males receiving the low protein (16 percent) basal. The addition of Lipamone in combination with Quietrol may be advantageous when added to a high protein ration. The addition of Quietrol to a low protein (16 percent) ration suggests a protein sparing effect as evidenced by a 7 percent improvement in feed conversion over that exhibited by the low protein basal control.

A similar comparison of the weight gain and feed efficiency of the females during the growing period, 5 to 9 weeks of age, is shown in Table 11. Again the higher protein intake produced poultry meat more efficiently and the addition of Quietrol administered singly proved the most efficient. The addition of Lipamone appears to be slightly less efficient and the combination of Lipamone and Quietrol less efficient than either administered alone as an additive to a high protein ration for females. When a 16 percent protein ration is fed with Lipamone, feed efficiency is improved 6 percent over the low protein basal control. Quietrol alone and in combination with Lipamone did not improve feed efficiency much over that demonstrated by the low protein basal control.

A comparison of the weight gain and feed conversion of the males during the finishing period, 9 to 13 weeks of age, is shown in Table 12. The high protein basal proved to be 9 to 13 percent more efficient than any treatment with the low protein basal. The oral administration of Lipamone and/

or Quietrol as an additive to a high protein basal improved feed efficiency to a greater extent than the high protein basal control and the latter was slightly more efficient than the combination of Lipamone and Quietrol. Again the addition of Quietrol to a lower protein basal ration seemed to exert a protein sparing effect. This is evidenced even in combination with Lipamone which in this particular study was the most efficient when added to a low protein basal and fed to males.

A similar comparison of the feed efficiency of females during the finishing period, 9 to 13 weeks of age, indicated the high protein level produced poultry meat with less feed than a lower protein level. Lipamone as an additive to the higher protein level was slightly more efficient than the addition of Quietrol but the combination was less efficient than the high protein basal control. The lower protein level (13 percent) presented the same pattern as the higher protein ration but to a lesser extent. The Lipamone additive improved feed efficiency approximately 7 percent over that recorded by the females receiving the combination of Lipamone and Quietrol.

Summary of live scores:

Only the males receiving Quietrol and Lipamone in combination showed any improvement in mean feather score between the ninth and the thirteenth weeks of age. The males fed the remaining treatments and the females, regardless of treatment administered, showed a decrease in mean feather score between

the two periods. Fleshing was improved by the administration of the high protein basal control to the males, and the high protein basal in any combination with the additives improved the fleshing score of the females. Observed pigmentation scores were higher at 13 weeks of age. The males receiving the lower protein level exhibited an increased amount of pigment deposition. This is consistent with serum xanthophyll determinations discussed in another section. Since it is questionable whether any of these trends are statistically significant, the data are not presented.

Dressed carcass grade:

The results of the carcass grading for both sexes and all eight dietary treatments after conversion to a percentage figure is found in Table 13. The dressed grade of chickens receiving a high protein ration apparently can be improved by the addition of Quietrol to the ration. The males receiving the high protein basal plus Quietrol had a dressed grade which was significantly higher than those males fed the high protein basal control. No other differences were observed.

Abdominal fat weight:

A statistical analysis of the abdominal fat, removed and weighed from 12 birds of each sex and representing each of the dietary treatments indicated a non-significant difference, Table 14. The addition of an estrogen or a tranquilizer to either a high or low protein ration did not increase fat deposition in the abdominal area of either sex.

Serum xanthophyll:

The serum xanthophyll content of the blood collected from 9-week old males receiving any combination of the 16 percent protein basal ration was significantly higher than the other groups, Table 15. The females fed the high protein basal displayed the least amount of xanthophyll in the blood serum. At 13 weeks of age, the groups fed the lower protein ration continued to show the higher serum xanthophyll content. The sexual difference in serum xanthophyll was not as pronounced at 13 weeks of age as at the earlier age.

Gonadal, oviducal and comb weights:

The mean weight of the testes removed from the experimental chickens at nine weeks of age is shown in Table 16. The testicular weight of the cockerels receiving the combination of dienestrol diacetate and Quietrol was equivalent to the testicular weight of the control birds. The testicular weight of the cockerels fed either additive singly was significantly lighter than the controls demonstrating a physiological effect of the oral administration of either compound at this age.

As indicated in this table, protein level was omitted and an analysis of the effect of treatment only is observed. A statistical analysis of testes weight at 13 weeks of age indicates a justification of the previous assumption that protein level of the ration has little or no effect on the weight of the gonads. The mean weights of the testes from 12 birds on each of the dietary treatments show little effect

on testes weight due to protein level. Table 17. The addition of the estrogen. however, decreased testes weight approximately 80 percent and the tranquilizer administered singly reduced testes weight 84 percent as compared to the testes of the control fed birds. The combination of the two additives increased testes weight slightly over 100 percent in the high protein basal and almost 50 percent in the low protein basal treatments.

Figure 1 illustrates the histological changes of the testes following dietary additives. Figure 1-A represents a section of a normal testis removed from a male receiving the control ration. Approximately 15 percent of the seminiferous tubules contain spermatids. The other tubules exhibit various stages of spermatozoa. Figure 1-B, at a magnification of 247 times (identical in all photographs) clearly demonstrates the decrease in the size of the tubules and the lack of sperm activity when the male was fed dienestrol diacetate. The majority of seminiferous tubules are in the prepuberal state, showing small tubules with a single layer of cells and no apparent multiplication of the basal layer. The males fed Quietrol, Figure 1-C, are very similar in appearance to the dienestrol diacetate recipient birds in the organization of the tubules and the lack of multiplication and growth of the primary spermatocytes. Figure 1-D aptly demonstrates the increase in size of the seminiferous tubules of the tests removed from males administered the combination of dienestrol diacetate and Quietrol. All of

tubules exhibit multilayered epithelium representing various stages of spermatogenesis. From the wall of the tubule to the lumen are found spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids, the nutritive cells (cells of Sertoli) to which the spermatids are attached, and the spermatozoa. The connective tissue stroma between the tubules contains the interstitial cells (Leydig) and blood vessels.

A statistical analysis of ovarian weight at 9 weeks of age is given in Table 18. A significant difference (5 percent level) is observed between the ovarian weight of birds receiving the tranquilizer compared to the weight of the ovaries removed from birds fed the other treatments. The additives fed singly tend to depress ovarian growth but when fed in combination indicate a lessening of this suppression. These data would suggest that the tranquilizer has more influence on the hypophysis than that exhibited by the administration of kienestrol diacetate.

The effect of the oral administration of Lipamone or Quietrol, fed singly or in combination, on the mean oviducal weight of the 9-week old pullets is shown in Table 19. Either additive, when administered singly, significantly increased oviducal weight in the recipient pullets whereas the combination when fed to pullets caused the oviduct to be very similar in weight to that exhibited by the control fed birds. In this table, protein level was omitted and an analysis of the effect of treatment only is observed. A statistical analysis

of oviducal weight at 13 weeks of age (Table 20) again indicates that protein level of the ration has little or no effect on the weight of the oviduct.

Oral administration of estrogen increased the oviduct weight of 13-week old females 160 percent or more as did the inclusion of the tranquilizer in the high protein basal compared to that observed in females fed a control ration demonstrating the physiological effects of the two additives on oviduct weight. The low protein basal plus the estrogen increased oviduct weight 130 percent or more whereas the tranquilizer increased the weight of the oviduct only 50 percent over that recorded for the low protein basal control. The combination of estrogen and tranquilizer increased oviduct weight approximately 16 percent.

The effect of protein level and treatment on comb weights of males and females at 9 weeks of age is shown in Table 21. These data suggest that protein level has little or no effect on comb weight of either sex at this age. However, the addition of Lipamone or Quietrol to the ration significantly reduced the comb weight of the males below that exhibited by the control birds or those fed the combination. The same trend is apparent in the comb weights of the females at this age but the differences are not as decisive as in the males.

DISCUSSION

Body weight gains and feed conversion:

The results of body weight gains in this study are in agreement with that observed by other workers, that is, that a higher protein intake resulted in improved rate of gain and improved feed efficiency when compared to that obtained with a lower protein ration. However, the birds fed the lower protein basal control had a dressed grade score as high as any group of birds receiving the other dietary treatments. These data do not agree with that reported by Bird (1948), who observed lean, blue birds lacking in finish when fed a lower protein ration. Lorenz (1954) reported a greater response to the various treatments when fed in combination with a lower protein ration. In the experiments reported herein, the dressed grade of male carcasses were slightly improved by the addition of the tranquilizer to a low protein ration indicating a limited response to the treatment.

Estrogen administered singly tends to decrease metabolism (Bird, 1946) and activity and the fattening effects may be attributed, in part, to these factors. In combination with a tranquilizer, which is alleged to diminish activity and motivation, further reduction in rate of metabolism can be expected (Wolterink et al., 1958). A reduction in metabolic rate and the fattening effects may be expressed in the slight improvement of feed efficiency exhibited when Lipamone

and Quitrol are fed compared to the feed efficiency of the birds not receiving the additives.

Numerous investigators have established that the administration of an estrogenic compound to chickens decreases the secretion of FSH and a balance is therefore maintained depressing the secretion of androgen accounting for the capon-like appearance of hormonized chickens. A smooth "silky" texture is imparted to the dressed carcass by the increased subcutaneous fat and by the effects of estrogen on skin structure (Lorenz, 1945). The relative effect of estrogen treatment on gain in body weight increases with increasing age of birds. Estrogenic substances mixed in the feed in moderate dosages have had no effect. on the average, on rate of gain in growing birds (Lorenz, 1945). The results of this immediate study show a similar response was obtained from the administration of dienestrol diacetate and Quietrol. singly and in combination.

The mechanisms of these effects are undoubtedly complex. Stimulation of lipogenesis may exert either a positive or a negative effect on total growth, depending on whether fat is deposited in addition to, or substituted for, other tissue. The stimulation of lipogenesis will have a net negative effect on feed efficiency in either case, because of the higher caloric content of adipose tissue. The increased feed consumption together with decreased energy expenditure due to the inhibition of aggressiveness, should favor accumulation of weight and higher feed efficiency. A hypothesis suggested

by Bird (1949) is the possible relation of estrogen to the basal metabolism rate; a low rate of "metabolic efficiency" is requisite for the deposition of fat. Another theory is the stimulation of growth-hormone production or an adrogen-like action of estrogens in promoting an increased anabolism of protein, accompanied by loss of fat (Kumaran and Turner. 1949).

Abdominal fat:

Numerous investigators have reported that estrogen increases the amount of fat deposited in the tissue, particularly abdominal fat, and improves the grade of the carcass of the male. Begin and Grainger (1957) observed the amount of abdominal and subcutaneous fat found in the carcass was greatest in the diethylstilbestrol-treated birds. The above authors stated that the fat was more evenly distributed in the birds fed dienestrol diacetate. Warden (1958) reported that DES implants significantly increased the amount of abdominal fat over that exhibited by birds fed dienestrol diacetate. The results reported herein, substantiate the previous findings by the slightly heavier body weight of the birds receiving the estrogenic compound singly and in combination with the tranquilizer and the non-significant difference in abdominal fat weights shown to exist between treatments and between sexes. This even distribution of fat has economic significance by improving carcass grade and appearance besides a slightly improved rate of gain compared to birds not fed either additive for at least a four week

period before slaughter.

Serum xanthophyll:

Blood serum from the male showed a higher concentration of xanthphyll than that found in the serum of the female. independent of the protein content of the feed. Also a lower protein level fed to males resulted in a higher xanthophyll concentration than males fed a slightly higher protein level and slightly higher than that found in the blood serum of the females. The differences are possibly due to the greater amount of yellow corn in the lower protein ration. This gives further evidence that xanthophyll content of the feed is one factor responsible for the amount of pigmentation that appears on the finished product. The sexual differences in serum xanthophyll may be partially explained by the greater amount of feed normally consumed by the males. The males consumed approximately 10 percent more feed than the females during the 5 to 9 week period and 20 percent more feed during the 9 to 13 week period. The percent difference in serum xanthophyll between sexes at 9 weeks of age was 28 percent and 8 percent at 13 weeks of age. Obviously the expression of some other mechanism is involved.

Preliminary research done in this laboratory (unpublished) suggests that capons have approximately the same xanthophyll content as intact males, virtually eliminating androgen as the factor responsible for the sexual differences shown to exist. The oral administration of an estrogenic compound had little or no effect on pigment deposition or

serum xanthophyll suggesting the apparent elimination of an estrogenic influence on the sex differences in xanthophyll. The xanthophyll content of the blood serum remains at a higher concentration in the male compared to female at least to one year of age (unpublished), which would allow sufficient time for the expression of hormonal secretion of either sex. The author concludes from these data that the sexual differences in visual pigmentation and serum xanthophyll concentration are specific and a conclusive explanation for these results cannot be expressed until further experimentation is done on this particular occurrence.

Gonadal and oviducal weights:

Kumaran and Turner (1949a and 1949b) studying the histology of the testes of White Plymouth Rock males at various ages, reported that during the first 5 weeks, the seminiferous tubules are organized, followed by multiplication of the basal layer of cells (spermatogonia). The primary spermatocytes begin to appear about the sixth week. During the next 2 or 3 weeks, growth of the primary spermatocytes takes precedence over the further multiplication of the spermatogonial layer. The secondary spermatocytes begin to appear at about 10 weeks of age as a result of the reduction division of the primary spermatocytes. Spermatids (immature spermatozoa) begin to appear in the seminiferous tubules at about 12 weeks of age, and by the twentieth week are usually present in all the tubules.

Microscopic examination of representative testes

removed from males receiving the control basal ration shows spermatocytes in various stages of development from primary to immature spermatozoa, however, the number of tubules showing spermatozoa was very limited. The oral administration of an estrogenic compound depressed the proliferation of germ cells. The tubules are organized, and very little if any multiplication is observed. In no case was spermatozoa observed. Exogenous estrogen in unphysiological dosages is a potent inhibitor of gonadotrophins produced by the anterior-hypophysis. The dosages of dienestrol diacetate used in this experiment were highly unphysiological, hence complete inhibition was produced and the observed results expected. The result of such treatment is an impaired genital system which simulates that observed in an animal after hypophysectomy.

A representative testes from a male receiving the oral administration of Quietrol shows stages of development very similar to that exhibited by the males fed dienestrol diacetate. These results present strong evidence that the tranquilizer used in this study blocks the secretion of the hypophyseal gonadotrophins. Pino and Hudson (1953) reported a similar phenomena when Enheptin (2-amino, 5-nitrothiazole) was fed to White Leghorn pullets and cockerels just prior to sexual maturity. These same authors state that Enheptin completely blocks sexual maturation when fed to immature chickens; and, as long as the drug is continued, the birds fail to overcome its effects. These authors also reported

that a histological examination of testis sections taken at autopsy substantiated the gross observations of sexual involution. While spermatogenesis and testes conditions were normal in the controls, there was a regression in the drug-treated tubules to one layer of cells, presumably spermatogonia, lying next to the tubular wall. Tubule size was reduced and the interstitial tissue appeared fibrous and condensed. In addition, these authors found that not only in hypophysis size reduced significantly in Enheptin-treated chickens, but so is the total hypophysis gonadotrophic hormone potency.

In this experiment, microscopic examination of representative testes removed from males receiving the combination show that almost all of the seminiferous tubules contain spermatids. The tubules and interstitial tissue appear normal indicating that the increased size was not due to just FSH or LH secretion alone but rather a simultaneous intensified elaboration of gonadotrophins. Abnormal spermatogenic cells are found. In the spermatogonia this is manifested by an excessive hypertrophy. Among the spermatocytes, giant forms are also common, as well as cells with two or more nuclei, sometimes of unequal size. They arise from fusion or abnormal mitosis. The spermatids fuse to form multinucleated giant cells (Maximow and Bloom, 1957).

The degenerating and monster spermatogenic cells are carried, with the mature spermia, into the epididyimis, where their substance is perhaps reabsorbed by the epithelium

of the excretory duct. The sex cells of the seminiferous epithelium are sensitive to noxious factors of various kinds. In pathological conditions of general (infectious diseases, alcoholism, dietary deficiencies) or local (injury, inflammation) character, and under the influence of mental depression, the degenerative changes, especially the formation of multinucleated giant cells by the coalescing spermatids, may become prominent (Maximow and Bloom, 1957).

The occurrence of a many-fold differential when two compounds are fed in combination is not uncommon. For example, the administration of FSH at a given dose level is known to increase testes size. A comparable dosage of LH, fed singly, will result in a similar accretion of testicular tissue. When the two compounds, FSH and LH, are fed in combination, an increase above that of an expected two-fold increase in testes size can be found. The significance of the results found in this study are directly the opposite of these conventional observations. When dienestrol diacetate or Quietrol was fed singly, reduction in testes weight was observed. To be specific, the reduction in testes weight compared to control values was 80 percent when dienestrol diacetate was fed and 84 percent for Quietrol. When fed in combination the expected results would be an equivalent reduction or possibly a greater suppression than when either compound was fed singly. The data, however, demonstrate that the additives, fed in combination, counteract each other and in addition, stimulate FSH and LH release, hence

resulting in nearly a two-fold increase in testes size when compared to the testes size of birds fed the control ration.

When the combination of dienestrol diacetate and the perphenazine derivative was administered, it is postulated that the secretion of FSH and LH is stimulated, increasing gonad size and maturation compared to that expressed by either compound fed singly. This may not necessarily be a stimulated FSH and LH release but could be, theoretically, an increased sensitivity of the end target organ, the testes, to respond to the same amount of circulating gonadotrophins. When either compound is fed singly apparently the secretion of hypophyseal gonadotrophins (FSH) are suppressed resulting in the conventional decreased gonad size. The results of comb weights that were recorded at 9 weeks of age indicate that the combination increased the weight of the comb compared to the weight of the comb from birds fed either of the compounds singly or the control birds. This would suggest that the increased secretion of gonadotrophin (LH) stimulated the interstital cells resulting in an increase of androgen secretion, hence, comb growth.

Apparently the combination permitted some sedation approaching mental depression to allow the formation of giant cells that were found in the seminiferous tubules.

The oral administration of dienestrol diacetate increased oviducal weight as expected. This occurrence is presumably due to a reduction of hypophyseal gonadotrophin

secretion (FSH) acting directly on the ovary which was slightly smaller than the ovarian weight recorded for the birds not receiving the estrogenic compound. The estrogenic-like constituent of dienestrol diacetate replaces the natural estrogen liberated by the ovary, hence, stimulating growth of the oviduct. Ovarian weight of the tranquilizer fed birds was significantly less than the ovarian weight of the control fed birds. These data are strikingly similar to that recorded for the birds fed dienestrol diacetate indicating a suppression of FSH and LH secretion reducing ovarian size and hence, decreasing normal estrogen production of the ovary. The oviducal weights, of the females receiving the tranquilizer were heavier than those removed from the birds receiving the control ration. The stimulated oviducal growth must be due to the tranquilizer, the only additive to the basal control in this instance, indicating that the compound has an estrogen-like property.

When the combination of dienestrol diacetate and Quietrol was fed, ovarian weight was very much like that recorded for the birds fed the control ration. This would suggest that the inhibition of hypophyseal gonadotrophin secretion expressed by either compound fed singly was counteracted permitting normal function of the ovary. Since both compounds were fed simultaneously at the same level, the dosage was actually two times that administered when either compound was fed singly. Possibly one compound blocked the expression of the other and permitted the normal secretion of

estrogen from the ovary, giving a similar oviducal size of these birds as compared to those fed the control ration.

These data concerning an estrogen and Quietrol, fed singly and in combination, would offer excellent tools in the study of reproduction in the fowl. Since it is possible to interrupt the reproductive cycle, apparently through endocrine interference, a more thorough investigation of endocrine inter-relationship can be made.

SUMMARY AND CONCLUSIONS

Dienestrol diacetate (a synthetic estrogenic compound incorporated at 21.14 grams per ton of feed) and Quietrol (an oral tranquilizer incorporated at 4 grams per ton of feed) were fed singly and in combination, together with a control at two protein levels.

A higher protein intake permitted improved rate of gain and improved feed efficiency when compared to that obtained with a lower protein ration. A slight increase in body weight gain was observed when dienestrol diacetate and/or Quietrol were administered singly or in combination compared to birds fed a control ration. The addition of dienestrol diacetate and Quietrol improved feed efficiency slightly above that observed when a high or low protein basal control was fed.

Either administered singly or in combination, the additives gave a non-significant difference in abdominal fat weight between treatments and between sexes.

Blood serum from the male showed a higher concentration of xanthophyll than that found in the serum of the female. Also a lower protein level fed to males resulted in a higher xanthophyll concentration than males fed a slightly higher protein level and slightly higher than that found in the blood serum of the females. These differences are further evidence that xanthophyll content of the feed is one factor responsible for the amount of pigmentation that appears on

the finished product and the concentration of xanthophyll found to exist in the blood serum.

Dienestrol diacetate physiologically suppresses hypophyseal gonadotrophin release resulting in decreased testicular weight and ultimately comb size in the male and decreased ovarian weight with increased oviducal weight in the female compared to that exhibited by the birds fed the control ration.

No noticeable tranquilization was observed when Quietrol was administered at the given level. However, this level was physiologically active in reducing testicular weight and comb size in the male and decreasing ovarian weight in the female. These data suggest that hypophyseal secretions are depressed when Quietrol is fed. An increase in oviducal weight with a decrease in ovarian weight following Quietrol administration suggests an estrogen-like activity by this compound.

When dienestrol diacetate and Quietrol were fed in combination ovarian weight was very similar to that exhibited by birds fed the control ration, indicating that the suppression of hypophyseal secretions, when either compound was administered singly, was relieved. Oviducal weights indicated that when fed in combination, the two compounds counteracted each other eliminating their estrogenic activity and giving ovarian weights similar to the controls. In contrast testicular weight was increased significantly, when the combination was fed, compared to testicular weight of birds fed the control ration. Microscopic examination of

a representative testis removed from a male receiving the combination shows that almost all of the seminiferous tubules contain spermatids at 13 weeks of age. The tubules and interstitial tissue appear normal indicating an intensified elaboration of the gonadotrophins FSH and LH or an increased sensitivity of the end organ. The number of tubules showing spermatozoa was very limited in a representative testis removed from a male receiving the basal control ration.

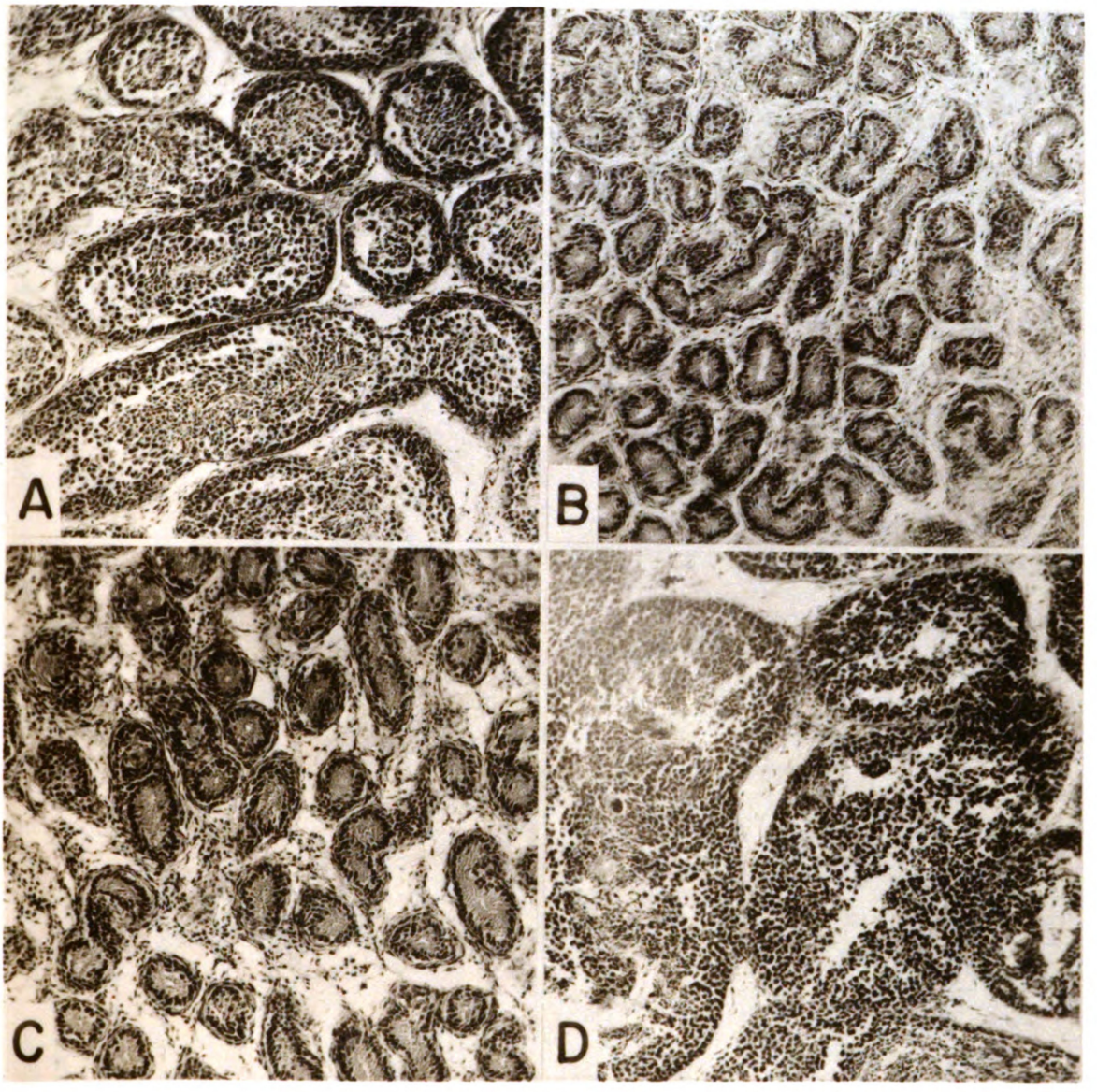

Figure 1. Cross sections of testes of cockerels showing the effect of the oral administration of Lipamone or Quietrol, fed singly or in combination, on testicular development at 13 weeks of age. (A) The normal or section of a testis from a cockerel fed the control ration. (B) The effect of feeding Lipamone on testes development. (C) The effect of the combination, Lipamone and Quietrol, on testes growth and spermatogenesis.

Table 13 – Effect of treatment on dressed grade (a

Table 13 - Effect of treatment on dressed grade (expressed in percentage) of males and females (9 and 13 weeks of age combined)

Average grade in percent in descending order[1]

Males (Std. error of mean = $\pm$ 2.05)

Treatment	HPQ	LPC	LPLQ	HPLQ	HPL	LPQ	HPL	HPC
Sample size	61	62	64	59	64	64	62	64
Mean grade in percent	95.2	92.7	91.7	91.5	91.0	91.0	89.3	87.3

Females (Std. error of mean = $\pm$ 2.66)

Treatment	HPL	HPC	HPQ	HPLQ	LPC	LPL	LPLQ	LPQ
Sample size	53	51	54	55	48	55	57	54
Mean grade in percent	91.1	90.5	90.1	89.4	88.9	88.3	85.6	85.5

[1] Means not underscored by the same line are significantly different at $P < 0.05$ level by the Duncan Multiple Range and Multiple F Tests.

Table 14 - The effect of treatment on abdominal fat weight of males and females at 13 weeks of age.

Mean abdominal fat weight (in grams) in descending order[1]

Males (std. error of mean = ± 7.18 grams)

Treatment	HPL	LPC	LPLQ	LPL	HPLQ	HPQ	LPQ	HPC
Sample size	12	12	12	12	12	12	12	12
Mean weight	62.6	59.7	58.6	55.8	53.3	50.4	48.1	43.1

Females (std. error of mean = ± 6.6 grams)

Treatment	HPQ	LPL	LPLQ	HPL	HPLQ	HPC	LPC	LPQ
Sample size	12	12	12	12	12	12	12	12
Mean weight	61.6	61.5	52.6	51.4	48.9	48.6	42.9	37.2

[1] Means not underscored by the same line are significantly different at $P < 0.01$ level by the Duncan Multiple Range and Multiple F Tests.

Table 15. The effect of treatment and sex on serum xanthophyll expressed as optical density at 9 and 13 weeks of age.

Means ranked in descending order at 9 weeks of age.[1] Std. error of mean = ± 0.0037

Treatment	LPC	LPL	LPQ	LPLQ	LPQ	LPC	LPLQ	HPQ	LPL	HPL	HPC	HPLQ	HPL	HPLQ	HPQ	HPC
Sex	M	M	M	M	F	F	F	M	F	M	M	M	F	F	F	F
Sample size	14	16	18	16	16	18	21	16	16	15	16	12	14	14	18	17
Mean	.152	.148	.142	.142	.125	.116	.115	.110	.107	.100	.098	.096	.077	.076	.075	.074

Means ranked in descending order at 13 weeks of age.[1] Std. error of mean = ± 0.0072

Treatment	LPLQ	LPQ	LPQ	LPL	LPC	LPL	LPLQ	HPL	HPQ	HPLQ	LPC	HPC	HPQ	HPL	HPC	HPLQ
Sex	M	M	F	F	M	M	F	M	M	M	F	M	F	F	F	F
Sample size	10	18	15	18	10	14	18	17	12	11	12	17	12	10	14	10
Mean	.300	.295	.293	.291	.286	.280	.274	.253	.248	.240	.235	.232	.231	.230	.226	.209

[1] Means not underscored by the same line are significantly different at $P < 0.01$ level by the Duncan Multiple Range and Multiple F Tests.

Table 16 - Effect of the oral administration of Lipamone or Quietrol, fed singly or in combination, on the testicular weights of 9-week old cockerels.

Testicular weight (in milligrams) in descending order[1]. (Std. error of mean = ± 30.9 mg.)

Treatment	Combination	Control	Quietrol	Lipamone
Sample size	13	10	13	18
Mean weight	507	429	208	190

[1]Means not underscored by the same line are significantly different at $P < 0.01$ level by the Duncan Multiple Range and Multiple F Tests.

Table 17 - The effects of protein level of the ration and lipamone or quietrol fed singly or in combination on testes weight of 13-week old cockerels.

Testes weight (in milligrams) in descending order[1]

(std. error of mean = 474.4 milligrams)

Treatment	HPLQ	LPLQ	LPC	HPC	HPL	HPQ	LPL	LPQ
Sample size	12	12	12	12	12	12	12	12
Mean weight	4958	3764	2564	2435	485	379	313	304

[1]Means not underscored by the same line are significantly different at $P < 0.01$ level by the Duncan Multiple Range and Multiple F Tests.

Table 18 - Effect of the oral administration of Lipamone or Quietrol, fed singly or in combination, on the ovarian weight of 9-week old pullets.

Ovarian weight (in milligrams) in descending order

(std. error of mean = $\pm$ 15.1 milligrams)[1]

Treatment	Control	Combination	Lipamone	Quietrol
Sample size	9	17	14	13
Mean weight	302	295	277	243

[1] Means not underscored by the same line are significantly different at $P < 0.05$ level by the Duncan Multiple Range and Multiple F Tests.

Table 19. Effect of the oral administration of Lipamone or Quietrol, fed singly or in combination, on the mean oviducal weight of 9-week old pullets.

Oviducal weight (in milligrams) in descending order[1]

(Std. error of mean = ± 35.04 mg.)

Treatment	Lipamone	Quietrol	Control	Combination
Sample size	14	13	9	17
Mean weight	429	355	185	171

[1]Means not underscored by the same line are significantly different at $P < 0.01$ level by the Duncan Multiple Range and Multiple F Tests.

Table 20 - The effects of protein level of the ration and lipamone or quietrol, fed singly or in combination, on oviducal weights of 13-week old pullets.

Oviduct weight (in milligrams) in descending order[1]

(std. error of mean = ± 97.83 milligrams)

Treatment	HPL	HPQ	LPL	LPQ	LPLQ	HPLQ	HPC	LPC
Sample size	12	12	12	12	12	12	12	12
Mean weight	983	965	858	539	463	420	387	360

[1]Means not underscored by the same line are significantly different at $P < 0.01$ level by the Duncan Multiple Range and Multiple F Tests.

Table 21 - The effect of protein level and treatment on comb weights of males and females at 9 weeks of age

Mean comb weight (in milligrams) in descending order[1]

Males (Std. error of mean = ± 81.33 mg.)

Treatment	LPLQ	HPLQ	LPC	HPC	HPQ	LPL	LPL	LPQ
Sample size	16	16	16	16	16	16	14	16
Mean weight	986	840	805	745	370	329	304	299

Females (Std. error of mean = ± 7.68 mg.)

Treatment	HPLQ	LPC	HPC	LPLQ	HPQ	LPQ	HPL	HPQ
Sample size	16	16	15	16	16	16	16	16
Mean weight	114	110	108	94	87	76	76	71

[1]Means not underscored by the same line are significantly different at $P < 0.01$ level by the Duncan Multiple Range and Multiple F Tests.

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EXPERIMENTAL DESIGN

Male Pens			Female Pens	
LPQ	LPLQ		HPLQ	HPL
LPL	HPLQ		HPC	LPQ
HPL	HPQ		LPC	LPLQ
HPC	LPC		HPL	LPL
HPQ	LPQ		LPL	HPLQ
HPLQ	LPL		LPLQ	LPC
LPC	HPL		HPQ	HPC
LPLQ	HPC		LPQ	HPQ