A STUDY OF MANGANESE IN THE DIET OF THE DEVELOPING PULLET

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ABSTRACT

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DIET OF THE DEVELOPING PULLET

Four hundred single comb White Leghorn pullets, eight and one-half weeks old, were used in a study to determine the effects of the concentration of manganese in the growing diet on pullet performance to twenty and one-half weeks of age. Seventy-five of these pullets were later used to study manganese nutrition during the reproductive cycle as influenced by the dietary regime of the growing period. It was found that the concentration of manganese in the growing ration; over the range 17.8 to 57.8 p.p.m., had no apparent effect on the growth and development of pullets from eight and one-half weeks to twenty and one-half weeks of age. During the twenty-week reproductive period studied, with birds from 36 to 56 weeks of age, the concentration of manganese in the various growing and laying rations did not have a significant effect on feed efficiency, shell weight per unit area, hatchability of fertile-eggs, or the feed efficiency of chicks hatched from those eggs. Concentrations of manganese in the laying rations varied from 14 to 54 p.p.m. Hen-day production was significantly greater ($P<0.01$) when the growing diet contained 47.8 p.p.m. manganese and the laying diet contained 34 p.p.m. Egg weight was significantly greater ($P<0.01$) when pullets were fed 37.8 p.p.m. manganese during the growing period and either 14 or 54 p.p.m. during the reproductive cycle. Fertility of eggs was significantly ($P<0.01$) affected by the concentration of dietary manganese with lower concentrations in the growing ration and higher

concentrations in the laying ration producing eggs of higher fertility. Highly significant differences ($P<0.01$) were found in initial chick weight according to the dietary treatment of the parent pullet. Chicks having the highest initial weight were produced by pullets fed lower levels of manganese during both the growing and laying cycles. Chick weight gain was significantly ($P<0.01$) affected by the dietary regime to which the parent pullets were subjected during the growing period while the concentration of manganese in the laying diet had little effect on later weight gains of the chicks.

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INTRODUCTION

Past work concerning manganese and its relation to the animal organism is quite controversial. Diametrically opposite results and conclusions can be found in the literature. Growth rate, bone development, reproduction, and eggshell quality have been found to be both dependent upon and not dependent upon manganese intake. These differences are due, undoubtedly, to variations in species or strains being considered, environment, manganese level in the experimental diets, unidentified factors, and interactions between the foregoing. This paper presents data on previously unexplored areas as indicated below.

The author did not find any reports in the literature to date concerning the effects of varying manganese concentrations in the diet of the growing pullet (8 to 20 weeks of age) and the effects of such variation on later performance in the reproductive cycle. Nor does the National Research Council of the United States give recommendations for practical manganese levels for growing pullets or laying hens. To date it has been customary to use the same levels of manganese in the rations of growing birds as are used in the rations of chicks (55 p.p.m.). Laying hens have been arbitrarily assigned an equal level or some lower level which more nearly corresponds to the known requirement of 33 p.p.m. for the breeding hen (National Research Council, 1966). The supplementation of rations with manganese in this manner has produced apparently good results. However, it is well known that the nutrition of growing chickens differs in many respects from that of female chickens in

reproduction, and research about manganese nutriture of the former is lacking. Therefore, the current investigation was designed to determine the effects of manganese nutrition on growing pullets and, subsequently, to determine the influence early manganese nutrition has on the manganese requirement of the hen during reproduction. These two phases of the chicken's life cycle were researched separately and the results reported and discussed accordingly.

LITERATURE REVIEW

The Relationship of Manganese to Growth

Osborne and Mendel (1913) were among the first investigators to find that the supplementation of a ration with small quantities of trace elements improved growth. They had raised rats for many generations on a diet composed of 60% whole milk powder, 12% starch, and 28% lard, and found the diet entirely adequate for growth, maintenance and reproduction. Rats had grown from infancy, given birth to normal litters, and the litters, in turn, had thrived on the food. They concluded that the aforementioned "milk food contains all that is essential for both growth and maintenance." When they tried to synthesize this food, however, using purified protein, lard, starch, and "protein-free milk" they met with uncertain success. The artificial diet promoted growth for a period of 60 to 100 days. After this period the animals began to decline and eventually died unless a change of diet took place. The addition of traces of iodine, manganese, flourine, and aluminum improved the artificial diet but not to the extent that it was equivalent to the original "milk food".

Levine and Sohm (1924) found that rats given manganese in the drinking water were more active than controls, had sleeker hair coats, and had longer and thicker hair. The authors reported that the rats on the supplemented diet proved fertile and that the offspring grew somewhat faster than the average young rat. The authors stated that the results of their experiments indicated that manganese had a marked catalytic effect on growth.

McHargue (1926) demonstrated the growth-stimulating effects of some trace elements using a basal ration practically devoid of vitamins. Using the rat as the experimental animal, the author added 25 p.p.m. copper, 100 p.p.m. manganese, 25 p.p.m. zinc, or a combination of the three to the basal ration. Although nearly all rats attained their maximum weight by the end of the ninth week and then declined rapidly in weight due to the lack of vitamins, weight gains in excess of controls were recorded as follows: copper supplement, 28.0%; manganese supplement, 50.2%; zinc supplement, 27.6%; and the combination of copper, manganese, and zinc, 54.0%. He concluded that compounds of manganese and possibly those of copper and zinc have important biological functions in animal metabolism.

Richards (1930), after an 11-month period, found no differences as regards growth and general health between groups of pigs fed different levels of manganese citrate. The basal diet contained 1 part of manganese in 180,000 of ration and additions were made to provide levels of .4 to 3.5 grams of manganese citrate daily. The author did not say whether the pigs were housed on concrete or had access to bare earth. She felt that it was impractical to feed farm animals on synthetic diets and that the relatively large amounts of manganese in ordinary feedstuffs would preclude the development of manganese deficiency symptoms under ordinary experimental conditions.

Waddell, Steenbock, and Hart (1931) found that an all cow's-milk diet supplemented with copper and iron was apparently adequate for growth. Although growth was not optimum, good weights were eventually secured and no anemia was observed. The authors were not studying manganese per se in these experiments, but their work, as well as other work to be reviewed subsequently, is interesting in view of the fact

that milk is known to contain a low concentration of manganese and, in fact, is used in the dried form as a major ingredient in low manganese experimental diets.

Kemmerer, Elvehjem and Hart (1931) then used a milk diet supplemented with copper and iron as a basal diet in further studies in the nutrition of the mouse. The diet was chosen because it furnished all of the "recognized nutritional factors" and at the same time was low in manganese. The authors took every precaution to isolate the experimental animals from any environmental source of manganese contamination. They then added manganese supplements (0.01 mg. manganese per mouse daily) to the basal diet for young mice and found that both male and female mice grew faster on the supplemented diet. These data, and unpublished data from their laboratory, led them to conclude that "manganese has a measurable effect upon growth and that the effect is sufficiently consistent to warrant the belief that manganese is indispensable for normal growth."

Orent and McCollum (1931), however, found that the growth rates of rats fed a manganese-free basal diet did not differ from those of well-nourished rats in the stock laboratory colony. Also, growth rates and general appearance did not differ noticeably between rats fed the manganese-free basal diet, those groups which received the basal ration supplemented with 0.005% or 0.05% manganese, or the control groups.

Schaible, Bandemer, and Davidson (1938) added manganese, in several chemical forms, to a basal diet containing 11 p.p.m. of manganese. They found that, at the end of 5 weeks, chicks fed the supplemented rations had greater average weights than the controls in most instances.

While investigating the manganese requirement of the New Hampshire chick, Gallup and Norris (1939a) found that the average weight

of chicks, consuming a supplemented basal diet containing 50 p.p.m. manganese, was greater than that of chicks consuming the basal diet containing 10 p.p.m. manganese. The length of the test period was six weeks. The authors concluded that a manganese deficiency prevented optimum growth of the chicks.

Mussehl and Ackerson (1939) reported that the addition of 35 p.p.m. or 350 p.p.m. manganese to a stock diet containing 35 p.p.m. manganese had no significant effect on the growth of turkey poults. The rate of growth was very satisfactory for all treatments including the controls.

Smith and co-workers (1944) found the growth of manganese-deficient rabbits to be significantly less than that of a manganese-supplemented group at 15 weeks of age. They fed a diet composed of whole milk powder supplemented with trace amounts of iron and copper plus 0.1 mg. pyridoxine per day. Manganese supplements were administered by mouth twice weekly and amounted to 5 mg. of manganese per day. The authors stated that, although the supplemented diet produced better growth than the basal diet, growth on the supplemented diet was below that which could be expected on a stock laboratory diet.

Grummer, et al (1950) confined 9-week old pigs on concrete to show that supplements of manganese could affect their growth rate. The daily rate of gain on the supplemented ration containing 52 p.p.m. manganese was significantly greater than that attained on the basal ration containing 12 p.p.m. manganese or on those supplemented rations containing 92 or 172 p.p.m. manganese. Feed efficiency appeared to be improved, also, although not significantly.

Bentley and Phillips (1951) found, in two different experiments, that feeding a ration containing only 7-10 p.p.m. manganese had no adverse effect upon growth of dairy heifers during the first 12 month period. This basal ration, composed of feedstuffs easily available to Wisconsin dairymen, was supplemented with feeding grade manganese sulfate to provide levels of 30, 40, and 60 p.p.m. total manganese as opposing treatments.

The Relationship of Manganese to Anatomical Abnormalities

Landauer and Dunn (1925-26) were probably the first to describe, in chicken embryos, a condition similar to Chondrodystrophia foetalis (Kaufman) in mammals. It was found to occur in 50 of 4000 embryos from several strains and varieties and could be seen as early as the 12th-14th day of incubation. It was observed that the embryos usually died around the 18th-20th day, but, on occasion, some had been found living, but unable to emerge, on the 23rd day. The characteristics of the abnormality were found to be as follows:

1. Shortening and thickening of the leg bones.
2. Abnormal conformation of the head.
3. Little or no abnormality of the wings.
4. The spinal column could be curved or not.
5. Histologically, the cartilage of the epiphyses showed many irregularities.
6. The chicks were apparently normal otherwise.

The authors were unable to say what the etiological factors were.

Payne (1930) described a condition characterized by malformed leg bones in young chickens. He stated that the condition was

aggravated by intensive methods of production and differed from rickets in that it developed in the presence of vitamin D and well balanced mineral mixtures. The deformity appeared between 3 and 6 weeks of age when the hock joint became swollen. It was found that the gastrocnemius tendon would occasionally slip from its condyle and, thereafter, the afflicted leg became stiffened in the malposition. Either or both the femur or tibia could become enlarged and develop a curvature. One or both legs could become involved. The author found that the deformity was not confined to any one group of conditions but occurred among slow and rapid growing chicks fed all-mash or mash and grain rations of high or low mineral content. The birds could be housed either on wire or on board-bottom runways.

Titus and Ginn (1931) first referred to this leg malformation as perosis and provided a more complete description of it. They were attempting to formulate a "uniform" diet for chicks and found that this diet produced a high percentage of birds which exhibited typical symptoms. The authors recognized that perosis was a condition of dietary origin. The condition could be partially prevented by adjusting the dietary calcium-phosphorus ratio or by adding 6 to 10 percent of rice bran to the diet. The authors stated that perosis differed from rickets in that the ash content of the bones was nearly always normal. The calcium and inorganic phosphorus content of the blood serum also fell in a normal range.

Hunter and Funk (1931) produced slipped tendon by feeding a diet containing a high level of corn meal to Single Comb White Leghorn chicks housed on wire. The ration did not, however, produce slipped tendon if the birds were raised on a solid platform covered with sand.

It was further observed that, if the chicks were raised for the first four weeks of their lives on the solid floor covered with sand and then transferred to wire floors for the duration of the experiment, no slipped tendon was observed. This fact indicated to the authors that the mechanism of the deformity manifested itself early in the life of the chick. Further experiments led the authors to believe that the "addition of minerals in several different forms greatly increased the production of 'slipped tendon'".

Titus (1932), and Payne, Hughes, and Leinhardt (1932) supported the finding that the percent ash in the bones of perotic chicks did not differ from that found in the bones of healthy chicks. The calcium and phosphorus contents of the blood were also found to fall within normal ranges. It was observed by Titus that Rhode Island Red chicks were more susceptible to perosis than White Leghorns.

Herner and Robinson (1932) believed that perosis was caused by feeding a ration with a high mineral content. In their experiments, perosis did not occur when casein was used as the protein supplement. When meat meal was used as the protein supplement or when meat meal ash was added to the ration perosis occurred. No rickets was found among surviving birds. The calcium and phosphorus content of the bones of normal and abnormal birds was found to be the same.

Byerly, et al (1934-35) wrote of a disease "not heretofore described" which caused the death of many chick embryos during the third week of incubation. It was found to be characterized by a shortness of the anteroposterior axis of the skull and shortness of the tarso-metatarsi. The shafts of the leg bones were extremely osteoporotic. Some affected embryos occassionally hatched. The chicks so produced

behaved in an abnormal manner similar to polyneuritic chicks. The authors were successful in rearing some of these abnormal chicks on standard chick diets. They observed that the chicks lost all abnormal appearance in the course of 10 weeks time. It was found that the addition of wheat germ and liver, or wheat germ and dried whey, to the diet of the hens prevented the occurrence of the abnormality. Permitting the parent stock access to sunshine and green range reduced the occurrence of this disorder.

Hammond (1936) reported, after statistical studies of 191 lots of Rhode Island Red chicks, that the highest correlation was found between percentage of perosis and percentage of inorganic phosphorus in the diet. The author also reached the conclusion that there was a factor in rice bran which assisted in the regulation of calcium and phosphorus metabolism in the chicken. Chicks reared on diets containing rice bran showed a lower incidence of perosis.

While studying perosis, Wilgus, Norris and Heuser (1936) found that the addition of a technical grade monocalcium phosphate to a perosis producing ration reduced the occurrence of the abnormality. Chemical analysis of the monocalcium phosphate revealed considerable manganese and traces of iron and aluminum. In subsequent experiments the addition of 25 p.p.m. manganese to a basal diet containing 10 p.p.m. manganese demonstrated the preventive action of this element. The addition of 25 p.p.m. each of manganese, aluminum, and iron was entirely preventive when the basal ration contained 1.0 percent calcium and 0.8 percent total phosphorus.

Lyons and Insko (1937) found that hens consuming a ration containing 2.6 percent calcium, 1.4 percent phosphorus and 5.5 p.p.m.

manganese laid eggs from which embryos, that died from the 10th through the 21st day of incubation, showed chondrodystrophy. Eggs from hens consuming the basal ration supplemented with 50 p.p.m. manganese and zinc and 100 p.p.m. iron showed normal bone development among embryos dying during the same time span. Furthermore, the injection of 0.03 mg. of manganese into the albumen of eggs laid by hens on the basal diet totally prevented the occurrence of the abnormality. The authors showed further that the eggs of hens fed the basal diet contained less manganese than the eggs from hens fed the supplemented diet.

Gallup and Norris (1938) studied the relation of manganese to bone development. They observed a high incidence of deformities among chicks consuming a manganese deficient diet. The calcification of the bones appeared normal, but the bones were shorter and thicker when taken from birds on the deficient diet. Tibiae and metatarsi were 7 percent shorter than those of controls at 4 weeks of age while other bones were also found to be shortened by varying amounts. The manganese content of the leg bones of chicks consuming a deficient diet was found to be approximately 30 percent of that found in the leg bones of chicks consuming a diet adequate in manganese content.

A peculiar type of stiffness or lameness in pigs was described by Miller et al (1940). The disorder was found to differ from rickets in that the mineral content of the bones was normal and the condition was not affected or alleviated by the addition of calcium, phosphorus, cod liver oil, or irradiated yeast to the diet. X-rays of the distal extremities of the ulna and radius showed no major changes that would indicate rickets. The occurrence was first noted in pigs housed on concrete and fed high mineral rations. The diets were analyzed and

found to contain 11-14 p.p.m. manganese. The addition of 50-60 p.p.m. manganese to the basal ration prevented the stiffness when compared to pigs consuming the unsupplemented basal ration.

Johnson (1943), in contrast to Miller et al, observed no lameness or stiffness among pigs in early life when they consumed a ration containing 0.27 p.p.m. manganese.

Amdur, Norris, and Heuser (1945) found that rats have slightly longer bones when fed a manganese supplemented ration. The density of fresh bones from rats on a deficient diet was significantly lower than that of controls although the percentage of ash was apparently the same for both groups. The breaking strength of the bones taken from rats fed a manganese deficient diet was also found to be significantly lower.

Smith, Medlicott, and Ellis (1944) found that rabbits fed a manganese supplemented diet had normal appearing legs at the end of the experiment. On the other hand, 6 of 7 rabbits on a deficient diet developed gross deformities of the front legs in 7 to 14 weeks. When the leg bones were dissected out, it was observed that the deformity was a bowing confined to the radius and ulna. The density and length of the humeri of rabbits on the deficient diet were found to be significantly less than those on the supplemented diet. The breaking strength of the ulnae and the manganese content of fresh liver of animals fed the deficient diet were found to be significantly less than those of animals on the supplemented ration.

In further studies, Ellis, Smith, and Gates (1947) found the density and length of humeri of manganese deficient rabbits to be again significantly less than those on a supplemented diet. In both studies the percent ash of dry, fat-free bone was found to be significantly

less in manganese deficient animals even though X-ray studies showed the disorder not to be rickets.

Grummer, et al (1950) found that the addition of 40, 80, or 160 p.p.m. of manganese to a basal ration containing 12 p.p.m. manganese had no effect upon the percent ash found in the 3rd rib of swine. In fact, the authors report that hogs on the basal ration showed the highest bone ash, although not significantly so, of any treatment.

Tal and Guggenheim (1965) reared mice from 3 weeks of age on a diet of ox muscle. They reported that the effects of manganese additions to the meat diet were observable at the end of 2 weeks by the increasing levels of calcium and phosphorus in the bone. However, high concentrations of manganese in the diet decreased the calcium and phosphorus content of the bone.

The Relationship of Manganese to Reproduction

The deficiencies of a milk diet were investigated by Daniels and Hutton (1925). It had been their experience that rats fed exclusively on a whole milk diet very seldom reproduced. Any young that were born were born late and only a small percentage survived until weaning. The authors investigated the possibility that the large percentage of water in whole milk restricted the amount of nutrients which the animal could ingest. To insure an adequate intake of nutrients, a powdered whole milk diet was used. The results obtained were quite similar to those obtained on the diet of whole liquid milk. Growth in the first generation was normal. Few young of the second generation were ever raised and these never reproduced. The addition of cooked soybean flour to the diet produced excellent results, while the addition of

soybean ash produced results which were nearly comparable to those obtained with soybean flour. The authors concluded that the inorganic constituents of milk were inadequate for reproduction. The authors found that the addition of aluminum potassium sulfate ($AlK(SO_4)_2$-12 H_2O), sodium fluoride (NaF), sodium silicate (Na_2SiO_3), and manganese sulfate ($MnSO_4.4H_2O$) to the basal powdered milk diet permitted excellent growth and reproduction.

It was shown by Richards (1930) that the manganese content of plant female reproductive organs increases during the progression of reproduction. After making this discovery, the author suggested that the same thing might happen in animals. By analyzing hen's eggs from the smallest ova to the fully formed egg, it was shown that such was the case. The author could not, however, determine any connection between manganese content of the eggs and their fertility. Fertile and infertile eggs, and "dead in shell" embryos showed approximately the same average manganese content.

Poor reproductive success was reported by Waddell, Steenbock and Hart (1931) while studying whole milk diets with rats. Young were born but many litters were not suckled and died within a few days. Estrous cycles were very irregular, mammary function was poor, and the time of opening of the vagina was late. Marked testicular degeneration was noted among males. The addition of 0.5 mg. manganese per 100 cc. of milk, 0.2 mg. iodine per 100 cc., or a combination of these to the experimental diets caused the females to ovulate at regular intervals. The authors assumed that the effect of the milk diet was due to the low caloric intake caused by the large amount of fluid that had to be ingested during the consumption of such a diet.

Waddell (1931) further elaborated on the male sterility found in the preceding study. He reported that the testes of rats consuming a whole milk diet supplemented with copper, iron, or a combination of copper and iron were small and extremely edematous. Smears from the epididymis showed an absence of sperm. Histological sections of the testes showed a complete absence of the germinal epithelium. The tubules were greatly reduced in size and flattened or otherwise misshapen. In some cases the testes from first generation rats were not completely degenerated. In such instances the tubules were found to contain multinucleated "giant cells", the significance of which is not elaborated upon by the author. However, his results indicated that after as little as 10 weeks on a milk-copper-iron diet, sperm were not motile and were incapable of impregnating the female. The effect was intensified by an unidentified action of ferric chloride.

Kemmerer, Elvehjem and Hart (1931) found the number of estrous cycles in mice, limited to a diet of low manganese content, to be greatly reduced. No litters were born to manganese deficient females and males while "some" litters were born to mice consuming a diet supplemented with manganese.

Orent and McCollum (1931) achieved only partial success in their study with rats. Using essentially purified-type diets, rather than whole milk diets, they found that the estrous cycles of rats consuming a manganese-free diet were similar to and compared favorably with those of control animals or those of animals receiving diets supplemented with manganese. There was, however, a high death rate among offspring from rats consuming the unsupplemented diet. Some were born dead and others died soon after birth from what the authors call neglect and/or

lack of nourishment. Those young that did survive were reduced in size and inferior in appearance due to inadequate development of the mammary tissue of the dam. Dams consuming manganese-free diets also appeared indifferent to their young, while females on a diet supplemented with manganese did not differ from stock colony rats in their attitude toward caring for their young. The authors stated that male rats grew normally on a manganese-free diet for 90-100 days, at which time they became indifferent to females. They then became sterile due to immotility to their sperm cells. From this point the germinal epithelium degenerated, the testes decreased in size, and the epididymis finally degenerated completely until only vestiges remained. However, diets supplemented with manganese preserved the sexual potency of males.

Gallup and Norris (1939b) found that a deficiency of manganese in the diet of the hen resulted in decreased egg production, slightly decreased fertility, and low hatchability. Hens consuming a diet containing 200 p.p.m. manganese laid 83.4 eggs per hen during the experimental period, while a comparable group receiving 13 p.p.m. manganese in the ration laid only 40 eggs per hen. Hatchability of eggs from the low manganese group was approximately 50% that of the manganese supplemented group. The authors also found that the level of manganese in the egg could be varied by adjusting the manganese content of the diet. Embryos dying due to an apparent lack of manganese did so during the latter stages of incubation. They were unable to initiate or to complete the process of hatching.

A breed difference in the manganese requirement of laying hens was found by Golding, Schaible, and Davidson (1940). The authors found that Barred Rocks were more sensitive to a deficiency of manganese than

were White Leghorns. Good hatchability was obtained with the eggs of White Leghorns consuming a ration containing 9 p.p.m. manganese. However, Barred Rocks consuming a ration containing 9.6 p.p.m. manganese produced eggs giving approximately 59 percent hatchability as opposed to 81 percent hatchability when the ration was supplemented with 50 p.p.m. manganese. It was found that those birds consuming a manganese supplemented ration consistently produced significantly more eggs. Fertility was not shown to be correlated with the manganese content of the diet.

Gutowska and Parkhurst (1942) studied the manganese requirement of laying Rhode Island Red pullets. The authors do not state in what manner or under what dietary regime the pullets were raised before housing. Neither do they mention at what age the birds were housed. However, at the end of twelve 28-day periods, the authors had seen but one sign of manganese deficiency and concluded that levels of 17 to 24 p.p.m. manganese in a practical laying diet were sufficient for all practical purposes. The authors were unable to find significant differences in egg production, average egg weight, feed efficiency, fertility, or hatchability between dietary treatments containing low (17-24 p.p.m.) levels or high (61-76 p.p.m.) levels of manganese. Egg shell breaking strength, however, was greater among the supplemented groups.

Smith, Medlicott and Ellis (1944) could reach no definite conclusions regarding the effects of manganese deficiency on the testicular development of rabbits. The authors stated that the number of observations was too small to attach any significance to the results. However, in light of the work quoted above on mice and rats, their results are

interesting enough to report. The testes of one manganese deficient male showed extensive tubular degeneration. The lumina of the epididymides were filled with degenerating cells. In two other males, the testes were in the process of degeneration. Many multinucleate giant cells were evident and the more mature spermatids were sloughing off. The ovaries and uteri of manganese deficient females were significantly smaller than those of controls.

In a further study, Ellis, Smith, and Gates (1947) found that the testes in 2 of 9 manganese deficient rabbits showed only mild tubular degeneration after 12 to 22 weeks on the experimental diet. The authors concluded that manganese deficiency does not lead to testicular degeneration in rabbits as it does in rats.

Bentley and Phillips (1951) reported that heifers consuming a manganese-supplemented ration came into heat earlier than heifers consuming a more deficient diet. Breeding services per conception were slightly higher for the animals fed the low-manganese ration, but otherwise the reproductive performances of the heifers on the various treatments were similar. The number of calves born or the number of calves born dead was not altered by feeding the low-manganese ration although there appeared to be an increase in the number of calves born with weak pasterns on this diet. The level of manganese in the diet had no effect upon milk production.

Chubb (1954) reported increased female infertility among hens consuming a basal diet containing 10 p.p.m. manganese. A high rate of late embryonic mortality was also observed during the 19th and 20th day. Hatchability of fertile eggs was 71.2 percent versus 90.0 percent for

eggs from hens consuming the basal diet supplemented with manganese oxide to provide a level of 50 p.p.m. manganese.

Plumlee, et al (1956) gave evidence that low-manganese gilts exhibited irregular estrous cycles while those on a supplemented ration did not. Ovarian examination of the gilts that did not show estrous disclosed that ovulation was occurring. The low-manganese gilts showed very little udder development and did not come into milk normally. All pigs born were small and weak, and showed poor balance and coordination. They died soon after birth due to lack of milk and inability to nurse.

Cooper, Chubb, and Rowell (1963) fed rations containing 10 and 50 p.p.m. manganese to Light Sussex pullets. The birds were housed individually, at point of lay, in cages and artifically inseminated once weekly with semen from groups of Rhode Island Red cockerels maintained on the same rations. At the end of the 15-week experimental period there were no significant differences found for food consumption, egg production, fertility, or embryonic survival. Embryonic survival was not significantly correlated with any semen characteristic studied. Total daily intake of manganese was relatively high, however, since daily feed intake varied from 187-198 grams.

Atkinson, et al (1967) fed a high energy, 18 percent practical diet to 4 groups of 11 Broad Breasted Bronze turkey hens. The ration was supplemented with 27, 54, 108 or 162 p.p.m. manganese respectively to obtain 4 diet treatments. After a 14 day pre-experimental period, laying tests were conducted for four 25-day periods. Hen-day production was significantly reduced when only 27 p.p.m. manganese was added to the basal diet. All other levels of supplementation gave essentially the same egg production. Dietary treatments produced no effect on fertility.

Hatchability of fertile eggs was depressed significantly at both the lower and higher levels of supplementation. Feed efficiency appeared to be best at the 54 and 108 p.p.m. levels of supplementation. No effect was seen in egg weight or eggshell thickness.

Cox and Balloun (1969) conducted a test to determine the practical manganese requirement of White Leghorn hens for egg production. They housed two hundred and sixteen 22-week-old pullets using a 3X3 factorial experiment in a randomized block design. Dietary treatments consisted of 2.5, 3.0, and 3.5 percent calcium and 20, 53, and 86 p.p.m. manganese. They found that the best egg production was obtained at the higher levels of calcium concentration. If calcium was adequate, the basal diet containing 20 p.p.m. manganese was adequate for high egg production, high feed efficiency, and maintenance of egg size. Eggshell thickness was affected by the level of calcium in the diet but the level of manganese showed no tangible effect upon this trait. They concluded that a level of 20 p.p.m. manganese was sufficient, in a laying ration containing 2.5 percent to 3.5 percent calcium. for maintenance of high egg production, egg size, and good shell quality.

The Relationship of Manganese to Eggshell Quality

Caskey and Norris (1938) reported that the breaking strength and ash content of eggshells increased as the quantity of manganese in the diet increased.

Lyons (1939) found that eggshell quality was adversely affected when the manganese content of the hen's diet was low. The eggs showed large areas of poor calcification as evidenced by areas of roughness and areas of translucency to transmitted light. Shell breaking strength

increased 27.6 percent when hens were fed a ration containing 57 p.p.m. manganese as opposed to one containing 7 p.p.m. manganese.

Gutowska and Parkhurst (1942) observed that the shell quality, as observed by transmitted light, of hens fed high (61-74 p.p.m.) manganese levels was comparable to that of hens fed low (17-24 p.p.m.) manganese levels. However, shell breaking strength was found to be significantly greater among eggs from hens fed the higher levels of manganese.

Cooper, Chubb and Rowell (1963) studied the effects of manganese on reproduction. Using groups of hens fed rations containing 10 or 50 p.p.m. manganese, they observed no significant differences between diets for the number of eggs rejected for incubation because of poor shell quality. Rejection was based on the percentages of hairline cracks or weak ends. No information is given regarding the methods of determining weak ends.

Atkinson et al (1967) reported no significant differences in shell thickness of eggs from turkeys fed levels of 27, 54, 108, or 162 p.p.m. manganese added to a basal ration.

Hill and Mathers (1968) reported variable results in a rather extensive study. In experiment one they found that shell thickness was significantly reduced below controls when a low-manganese diet was fed to crossbred pullets from 19 weeks of age to about 29 weeks of age. However, in another experiment, when they used pullets of the reverse cross, no differences were seen at the end of 6 weeks. In a third experiment, birds were fed rations containing high or low levels of manganese from 18 weeks of age for a period of 6-7 months. It was found that shell thickness was significantly reduced by the lower manganese level both at the start of lay, 7 weeks after the experimental diets were first

offered, and at the end of the experiment. The experimental diets were fed from 4 weeks before lay in the fourth experiment. The experiment lasted only until 30 eggs had been collected from each hen. The differences in shell thickness between experimental groups just failed to reach significance.

Cox and Balloun (1969) observed that the effect of different levels of calcium in the diet of the pullet produced a greater effect on eggshell thickness and strength than did the level of manganese when the diet contained 20 p.p.m. or more of manganese.

Manganese Availability

Schaible and associates (1933) reported that feeding high levels of bone meal, bone ash, C.P. tricalcium phosphate, magnesium carbonate, or certain other minerals, could produce perosis when fed in conjunction with a specific basal ration.

In further experiments, Schaible et al (1938) tested the availability of manganese from several ores by supplementing a ration, known to produce perosis, with various levels of the ores. They then observed the incidence of perosis and translated these results into a scale of relative availability. Manganese from the sulfate, chloride, carbonate, dioxide, and from potassium permanganate were found to be equally prophylactic when supplied at a level of 30 p.p.m. manganese in the basal ration. Oxide ores of manganese were comparable in effect to the foregoing compounds if compared on the basis of equivalent amounts of manganese. The silicate ore (rhodonite) and the carbonate ore (rhodochrosite) were not considered satisfactory.

Wilgus and Patton (1939) reported that manganese in the diet was inefficiently utilized, particularly in the presence of high levels of

calcium and phosphorus. The authors suggest that manganese may become unavailable in the digestive tract due to chemical reactions there. In in vitro studies it was observed that manganese is carried down by tricalcium phosphate when it precipitates. In vivo experiments showed that excessive steamed bone meal rendered the manganese in the digestive tract, excreta, and ration less diffusible. The authors proposed that the precipitation of tricalcium phosphate in the gut, due to an excess of mineral in the ration, caused the removal of part of the manganese from the gut.

Caskey and Norris (1939) found that a level of 1.5 milligrams of manganese per 100 grams of diet containing 1 percent calcium and 0.5 percent phosphorus was just as effective in preventing perosis as a level of 14 milligrams of manganese per 100 grams of diet containing 3 percent calcium and 1.5 percent phosphorus. They found further that the injection into chicks of 10 milligrams of manganese over the length of the experimental period completely prevented perosis in chicks fed a high calcium-phosphorus diet, whereas, the ingestion of 141.7 milligrams in the same period by other chicks was only partially effective.

Bandemer et al (1940) found that the carbonate ore rhodochrosite failed to prevent perosis at levels up to 125 p.p.m. but precipitated manganese carbonate prevented perosis at the level of 30 p.p.m. Decreasing the particle size of the ore had no effect on its ability to prevent perosis.

Gutowska and associates (1941) found that an average of 28.4 to 44.0 percent of the manganese injected into intestinal loops of living chickens was absorbed in two hours, the amount absorbed being proportional to the concentration in the solution.

Mohamed and Greenburg (1943) reported that, in chicks, the largest part of the manganese is excreted whether the dose is oral or injected.

Manganese Toxicity

Few references directly related to manganese toxicity are found in the literature. Among those available, perhaps the following are the most notable.

Richards (1930) found that the tolerance of animals for manganese is apparently great provided the manganese is ingested. The author fed as much as 3.5 grams of manganese citrate daily for 9 months to growing swine without the appearance of symptoms of toxicity.

Skinner (1932) found that female rats which received 10 milligrams of manganese per day in excess of the stock ration containing 13.4 p.p.m. manganese could successfully rear their young. He also observed that the addition of 0.20 percent manganese, as the quadrihydrated sulfate, to a basal ration did not depress the weight of rats after a 12-week growth period immediately following weaning.

Heller and Penquite (1937), in a study of perosis, reported that a basal diet plus 1 percent of manganese carbonate (approximately 0.48 percent manganese) resulted in 52 percent dead chicks and, apparently, no cases of perosis. The authors do not state what the probable cause of death was. They conclude that "high percentages of manganese seem to be detrimental to growth".

Gallup and Norris (1937) reported that an increase in the manganese content of a chick diet to the level of 0.10 percent did not cause the development of toxicity symptoms.

In another study, Gallup and Norris (1939b) reported feeding manganese at levels as high as 0.10 percent in the diet of 5-month-old New Hampshire pullets. The authors do not state whether or not toxicity symptoms were observed. From the data presented, however, one might assume that symptoms of toxicity did not occur.

THE GROWING PERIOD

Experimental Procedure

Four hundred commercial single comb White Leghorn Hybrid pullets, eight and one-half weeks old, were used in this test. All birds had been raised on a commercial ration and debeaked at eight weeks of age. None of these birds showed outward manifestations of manganese deficiency. The pullets were allotted into ten pens of forty birds each, thus permitting five diet treatments and two replicates of each in a randomized block design. The pens were of the floor type, 1.52 meters by 3.66 meters in a forced ventilated, windowless building. Approximately 40 percent of the pen area was raised wire floor. The floor of the remaining 60 percent was covered with wheat straw or wood shavings.

The photoperiod was of 14 hours duration from 8 1/2 to 12 weeks, and 10 hours from 13 weeks until the end of the experiment at 20 1/2 weeks. The light source per pen consisted of one 60-watt white incandescent frosted bulb, without reflector, suspended at a height of 2.1 meters. The light intensity thus achieved varied from 2 to 5 foot-candles at the level of the feeder as measured by a General Electric Type 213 light meter. The variation of light intensity was due to horizontal placement of the light meter, within the pen, with respect to the light source.

Daytime ambient temperature in the home varied from 21.1^{o} to 29.4^{o} centigrade. Relative humidity was not controlled or recorded.

Ration Formulation

A low-manganese basal ration, in the form of a dry mash, otherwise formulated to meet National Research Council (1966) recommendations for nutrient requirements for growing chickens, was offered ad libitum. The composition of the basal ration is shown in TABLE 1. Water was constantly available in automatic cup-type waterers.

Five ration treatments were obtained by making additions of 0, 10, 20, 30, and 40 p.p.m. (weight/weight) of manganese, in the form of

TABLE 1.--COMPOSITION OF THE BASAL GROWING RATION

Ingredient	Per Cent
Corn Meal	58.00
Blood Meal	4.50
Alfalfa Meal (17% Protein)	1.00
Meat and Bone Meal (50% Protein)	3.00
Dried Skim Milk	16.00
Ground Limestone	1.00
Dicalcium Phosphate	2.00
Vitamin Premix*	1.00
Salt, iodized	0.25
Purified Cellulose	11.25
Animal Fat	2.00
	100.00

*--Vitamin Premix supplied the following per kilogram of ration: Vitamin A - 8250.0 I.U., Vitamin D-3 - 818.4 I.C.U., Vitamin E - 5.5 I.U., Vitamin B-12 - 11.0 mcg., Menadione Sodium Bisulfite Complex - 1.1 mg., dl-Methionine - 499.0 mg., Ethoxyquin - 249.5 g., Riboflavin - 5.5 mg., Calcium Pantothenate - 11.0 mg., Niacin - 16.5 mg., Choline Chloride - 275.0 mg.

Mineral Analysis of the Basal Growing Ration
(Average of three samples)

Calcium	1.43%
Total Phosphorus	.96%
Manganese	17.8 p.p.m.

the carbonate, to the basal ration. On the day of mixing, the appropriate amount of manganese was initially incorporated into a premix with the alfalfa. This premix was, in turn, incorporated into 45.5 kg. of corn meal. The corn meal-alfalfa-manganese premix was then added to the other ingredients in a 454.5 kg. capacity vertical central screw-type mixer. Feed was mixed in such quantities that it was never stored more than 30 days before being consumed. Feed consumption and weight records were begun immediately upon housing.

Selection of Birds for Analysis

Initially five representative birds were selected and sacrificed for analysis. These five birds were selected from the remainder of the population and did not disturb the distribution of 40 birds per pen. At the conclusion of the experiment, three birds per pen were also selected and sacrificed for analysis. The left leg was retained from each of the birds. These were placed in individual plastic bags with the flesh intact and stored at -17.8° C. until analyzed. The whole liver of each bird, without the gall bladder, was freed of all adhering fat and mensentaries, placed in a plastic bag and stored at -17.8° C. until analyzed.

Bone Length and Percent Bone Ash

For analysis, the legs were allowed to thaw and adhering flesh and cartilage were removed from the bones. Extreme tibial and femoral length was determined with the aid of a micrometer caliper accurate to 0.01 centimeters. The femorae were pooled according to sacrifice date and pen number, and dried in a steam cabinet for seven days at 53° C. They were then crushed in a mortar and extracted for six hours on a

Goldfisch apparatus with diethyl ether. The defatted samples were ground to pass a 1-millimeter screen.

Silica ashing dishes were rinsed in 20% aqueous nitric acid and dried at a temperature of 150° C. for a period of two hours. They were then placed in a vacuum desiccator and allowed to cool to ambient temperature. Duplicate samples of dry, fat-free bone, comprising approximately one-half the available sample each, were ashed for 12 hours at 575° C. The samples were removed from the muffle, placed in a vacuum desiccator, and allowed to cool to ambient temperature. Femoral ash was then determined by weighing.

Liver Analysis

In preparation for analysis, the whole livers were removed from the bags, pooled according to date of sacrifice and pen number, and dried for seven days at 53°C. The samples were then broken and crushed in a mortar to facilitate fat extraction. Initial extraction was for a period of two hours on a Goldfisch apparatus using diethyl ether as the solvent. After drying at 53° C. for a period of two hours, the samples were ground to pass a 1-mm. screen. Thereafter, extraction was again carried out for a period of two hours. The samples were then permitted to dry at 53° C. for another period of two hours, placed in a vacuum desiccator, and allowed to cool to ambient temperature.

Duplicate 4 g. samples of dry, fat-free liver were digested with 2 ml. of concentrated sulfuric acid and sufficient concentrated nitric acid to complete digestion. The final solution was filtered into a 50-ml. volumetric flask through quantitative filter paper. To the flask were added 0.3 g. of potassium periodate and sufficient re-distilled water to make a final volume of 30 ml. The mixture was allowed to boil

for 15 minutes on an electric hot plate, cooled and brought to volume with re-distilled water.

Standards were prepared by adding 2-ml. of concentrated sulfuric acid and 20-ml. of concentrated nitric acid to a 50-ml. volumetric flask. The solution was boiled until all traces of nitrous oxide fumes were removed. The appropriate amount of manganese, as potassium permanganate solution, and 0.3 g. of potassium periodate were added and the color allowed to develop for 15 minutes. The samples and standards were read on a Beckman model DU spectrophotometer using 1-cm. square pyrex cells. The color was read at a wave length of 526 millimicrons.

Egg Weight and Per Cent Eggshell Ash

The first 48 eggs layed in each pen were weighed to an accuracy of 0.1 g. and identified. From these 48, a sample of six was selected through the use of a table of random numbers for the determination of initial egg weight. The eggs were then broken out. The membranes were rinsed with distilled water and the shells, with membranes intact, were dried for 24 hours at 53° C. The shells and membranes were then ground to pass a 1-mm. screen.

Silica ashing dishes were dried at a temperature of 150° C. for a period of two hours. They were placed in a vacuum desiccator, allowed to cool to ambient temperature and weighed. Duplicate 2 g. samples of shell were ashed for a period of 72 hours at 600° C. The samples were removed from the muffle furnace, placed in a vacuum desiccator, and allowed to cool to ambient temperature before weighing.

Mineral Analysis of Feed

Calcium in feed was determined with the Perkin-Elmer model 303

Atomic Absorption Spectrophotometer according to the latest revision of "Analytical Methods for Atomic Absorption Spectrophotometry".[1]

Phosphorus in feed was determined according to the method of Parks and Dunn (1963) as modified by Heckman (1965). A Bausch and Lomb Spectronic 20 colorimeter-spectrophotometer with standard cells was used in the determination.

Manganese in feed was determined by a modification of the method of Skinner and Peterson (1930). Duplicate samples of feed were ashed for 12 hours at 575° C. The ash was dissolved in 5 ml. of syrupy phosphoric acid and 30 ml. of re-distilled water. The acid-ash-water mixture was heated on an electric hot plate for 20 minutes and filtered through quantitative filter paper directly into 50-ml. volumetric flasks. To the filtrate, 0.3 g. of potassium periodate was added and the color allowed to develop as above. The solution was cooled and brought to volume with re-distilled water. A set of standards was prepared by adding 0.3 g. of potassium periodate, 5 ml. of syrupy phosphoric acid, the appropriate amount of manganese as potassium permanganate solution, and 30 ml. of re-distilled water to a 50-ml. volumetric flask. The contents were boiled for 15 minutes, cooled, and brought to volume with re-distilled water. The samples and standards were read on the Beckman model DU spectrophotometer. All glassware used during analyses was rinsed in hot 1:4 nitric acid.

Analysis of Data

Data, where applicable, were subjected to analysis of variance according to the method of Snedecor (1956).

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[1] Analytical Methods for Atomic Absorption Spectrophotometry, Perkin-Elmer Corporation, Norwalk, Connecticut, 1965.

Results and Discussion

Varying levels of manganese in the diet of the growing White Leghorn pullet, over the range studied, were found to have no effect on the overall performance of the birds from 8 1/2 weeks of age to 20 1/2 weeks of age as evidenced by several criteria. Those criteria discussed in this section are as follows: final weight of pullets, weight gain, feed efficiency, mortality, bone length, bone ash, weight of first egg, per cent eggshell ash, age at maturity and liver manganese concentration.

Weight Gain

Mean weight gain (TABLE 2) was highly variable between replicates and prevented the discernment of any clear-cut trend. It would appear, however, that a level of 57.8 p.p.m. manganese produced the greatest weight gains in this experiment. Analysis of variance of mean weight gain disclosed no significant differences between treatments or replicates (TABLE A.1, Appendix). An analysis of variance of mean weight at 20 1/2 weeks would be meaningless since no attempt was made to equilibrate weights between pens at the beginning of the experiment. Initial mean replicate weights varied from 694 g. to 735 g.

Final Weight

Morgan (1957), using New Hampshire pullets, found the 5 month weight of control birds to be 3.93 pounds (1,783 g.). Fuller and Dunahoo (1962) reported a weight of 3.61 pounds (1,637 g.) for full-fed White Leghorn pullets at 24 weeks of age in an experiment designed to determine the effects of restricted feeding. They fed a yellow corn-soybean oil meal based ration containing 937 calories of productive energy per pound of ration and 21 percent protein as calculated by the authors.

TABLE 2.--MEAN WEIGHT OF PULLETS AT 20 1/2 WEEKS OF AGE AND MEAN WEIGHT GAIN FOR THE PERIOD FROM 8 1/2 WEEKS TO 20 1/2 WEEKS OF AGE

Treatment (p.p.m. Mn)	Replicate	Mean 20 1/2 week wt. (g.)		Mean weight gain (g.)	
		Replicate	Treatment	Replicate	Treatment
17.8	1	1533	1536	798	814
	2	1538		830	
27.8	1	1524	1522	798	792
	2	1520		785	
37.8	1	1533	1510	821	798
	2	1488		776	
47.8	1	1524	1497	816	789
	2	1470		762	
57.8	1	1651	1592	934	886
	2	1533		839	

Mean final weight of all birds in the present experiment, at 20 1/2 weeks, was 1531 g. with a range of 181 g. between replicates. This is somewhat more than the 1360 g. suggested by the breeder of this strain as being a desirable 21 week weight. However, this additional weight appeared to have no later adverse effects on egg production.

The lack of growth stimulation by manganese in the ration is in accord with the work of Richards (1930), Orent and McCollum (1931), Mussehl and Ackerson (1939), and Bentley and Phillips (1951) who also could detect no growth stimulating effect. It is in contrast, however, to the work of McHargue (1926), Kemmerer, Elvehjem and Hart (1931),

Gallup and Norris (1939a), Smith and co-workers (1944), and others who have demonstrated that an adequate intake of manganese improved growth.

Feed Efficiency

Feed efficiencies (TABLE 3) for the period from 8 1/2 weeks to 20 1/2 weeks of age roughly parallel weight gain as would be expected. They appear to be low, however, since Slinger et al (1962) reported a feed efficiency of 7.99 for debeaked White Leghorn pullets from 9 to 20 weeks of age when fed a corn-wheat-oats diet containing 50 percent whole grain. The 20 week weight of these birds was reported as 1,358 g.

TABLE 3.--FEED EFFICIENCY (KG. FEED/KG. GAIN) AND MORTALITY FOR THE PERIOD FROM 8 1/2 WEEKS TO 20 1/2 WEEKS OF AGE

Treatment (p.p.m. Mn)	Replicate	Feed Efficiency		Mortality per Replicate
		Replicate	Treatment	
17.8	1	6.84	6.64	2
	2	6.43		6
27.8	1	6.66	6.70	3
	2	6.74		3
37.8	1	7.27	7.05	1
	2	6.83		3
47.8	1	6.76	6.80	3
	2	6.84		4
57.8	1	6.23	6.34	3
	2	6.45		2

By this author's calculations the diet contained 15.1 percent protein and 2,865.5 kcal. metabolizable energy per kg. of diet.

According to information printed in 1967, the breeder of the pullets used in this test advises an average feed consumption of 11.3 pounds (5.12 kg.) for the period from 8 1/2 to 20 1/2 weeks to achieve a feed efficiency of 7.53 on a restricted feeding program. The diet used in this program reportedly should contain 15-16 percent protein and 2,860 kcal. metabolizable energy per kg. of ration. Pullets in this present experiment were full-fed the experimental diet in hanging tube-type feeders which were serviced every second day and at no time was any experimental unit without feed. Feed that was billed out was not, and could not be, returned to the feeder. Differences in feed efficiency were not found to be significant (TABLE A.2, Appendix).

Mortality

Mortality (TABLE 3) varied from 5 percent to 10 percent per treatment. Differences in mortality were not shown to be significant (TABLE A.3, Appendix). Necropsies were performed on all birds that died during the experiment at the provincial veterinary laboratory. The results in all cases indicated that Marek's disease was the cause of death. Since differences in mortality were not significant, one may assume that dietary treatment had no effect on mortality and did not weaken and thus predispose the birds to any pathological condition which triggered the Marek's disease.

Bone Length

It has been reported by Gallup and Norris (1938), Amdur, Norris and Heuser (1945), and others that, among other symptoms, the long bones

of the legs are shortened by a deficiency of manganese in the diet. Each replicate value on tibial and femoral length (TABLE 4) represents the mean of individual determinations on 3 bones removed from birds sacrificed at 20 1/2 weeks of age. Differences were found to be small and non-significant (TABLES A.4, A.5, Appendix).

Bone Ash

Manganese apparently has no relation to rickets as bone ash in birds consuming a manganese deficient diet has been reported by Titus and Ginn (1931), Titus (1932), Payne, Hughes and Leinhardt (1932), and others, to be the equivalent of that of birds on a well balanced diet. Replicate femoral ash values (TABLE 5) represent the mean of duplicate

TABLE 4.--MEAN TIBIAL AND FEMORAL LENGTH AT 20 1/2 WEEKS OF AGE.

Treatment (p.p.m. Mn)	Replicate	Tibia (cm.)		Femora (cm.)	
		Replicate	Treatment	Replicate	Treatment
17.8	1	13.04	12.72	8.99	8.82
	2	12.40		8.66	
27.8	1	12.65	12.68	8.60	8.66
	2	12.72		8.72	
37.8	1	12.79	12.64	8.85	8.75
	2	12.49		8.65	
47.8	1	12.63	12.64	8.67	8.73
	2	12.66		8.79	
57.8	1	12.72	12.59	8.75	8.66
	2	12.46		8.57	

TABLE 5.--PER CENT FEMORAL ASH OF DRY FAT-FREE BONE TAKEN FROM BIRDS AT 20 1/2 WEEKS OF AGE

Treatment (p.p.m. Mn)	Replicate	Per cent femoral ash	
		Replicate	Treatment
17.8	1	55.21	55.19
	2	55.17	
27.8	1	56.33	58.16
	2	60.00	
37.8	1	56.53	57.84
	2	59.16	
47.8	1	57.06	57.17
	2	57.28	
57.8	1	52.18	53.68
	2	55.19	

determinations on a composite sample of 3 bones. The bones used were those from which mean femoral length was determined. A level of 27.8 p.p.m. manganese appears to produce bones with the highest percent ash, but analysis of variance (TABLE A.6, Appendix) revealed that percent ash of this treatment was not significantly different from that of other treatments. Neither perosis nor any other known deficiency sympton was found among birds on these tests at any time. Visual examination of the tibiae or femorae used in the compilation of data in tables 4 and 5 revealed no twisting of the bones.

These observations agree with those of Hunter and Funk (1931) who found that the mechanism of slipped tendon (perosis) manifested itself early in the life of the chick. Their findings imply that perosis probably would not occur if the chicks were fed an adequate diet before 4 weeks of age and a perosis-producing diet thereafter. These results also agree with those of Titus and Ginn (1931), and others referred to above, in that bone ash was virtually the same on all treatments. The above results are in contrast to those of Smith, Medlicott, and Ellis (1944) and Ellis, Smith and Gates (1947) who reported that the per cent ash of the dry, fat-free bones of manganese deficient rabbits was significantly less than that of animals on an adequate diet.

Gallup and Norris (1938) and others quoted previously found that a manganese deficiency resulted in shortened and thickened bones. No such observation was evident in the present tests.

Egg Weight

Mean weight (TABLE 6) of a random sample of six eggs, drawn from the first 48 eggs layed in each pen, was quite variable. Mean weight varied from 40.2 g. to 47.4 g. or a difference of 6.8 g. It can be seen from the table that there was no gradual increase or decrease in mean weight from one dietary treatment to the next. Differences between means were found not to be significant at the 5 percent level of probability (TABLE A.7, Appendix). Gutowska and Parkhurst (1942) reported that changes in the manganese content of the ration, over the range that they studied, did not affect egg weight. Cox and Balloun (1969) apparently found no significant differences in egg size due to the manganese level of the diet.

TABLE 6.--MEAN WEIGHT OF FIRST EGG AND PERCENT SHELL ASH

Treatment (p.p.m. Mn)	Replicate	Egg weight (g.)		Eggshell ash (%)	
		Replicate	Treatment	Replicate	Treatment
17.8	1	41.8	42.1	47.5	47.5
	2	42.4		47.5	
27.8	1	41.2	41.2	47.5	47.4
	2	41.2		47.2	
37.8	1	47.4	43.9	46.9	47.2
	2	40.4		47.4	
47.8	1	43.5	41.8	47.6	47.8
	2	40.2		48.0	
57.8	1	46.0	45.1	47.6	47.7
	2	44.2		47.8	

Eggshell Ash

Percent eggshell ash of these first eggs (TABLE 6) was found to vary only slightly and differences between means were found to be non-significant (TABLE A.8, Appendix). Wilgus, Norris and Heuser (1939) reported the percent ash of eggshells from manganese deficient hens to be significantly less than that of eggs from hens on an adequate diet. Gutowska and Parkhurst (1942) observed that the shell breaking strength of eggs laid by pullets on high manganese intakes was significantly greater than that from birds on a low manganese intake. Hill and Mathers (1968) reported variable results on eggshell thickness. In two experiments they found significant differences in shell thickness due

to manganese level in the diet and in two experiments they failed to find significant differences. Cox and Balloun (1969) found that additions of manganese to a basal diet did not significantly affect shell thickness or shell strength.

Age at Maturity

Age at 40 percent lay, described as the first day that 40 percent of the birds in a pen laid an egg, is presented in TABLE 7. Table A.9 (Appendix) reveals that the differences among ages were not significant. The mean age, at 40 percent lay, of all birds on this experiment was 167.8 days. The Agricultural Research Service, United States Department of Agriculture, (1970) gives the regressed mean for age at 50 percent production, for birds of the same breed as those used in this experiment, as 173 days with 170-176 days being the 80 percent confidence limits. It would seem from the data in TABLE 7 that age at 40 percent lay was increased by both the highest and lowest levels of supplementation. Differences between treatments are too small, however, to draw valid conclusions.

Liver Manganese Concentration

Liver manganese concentration, in p.p.m. manganese on a dry, fat-free basis, is presented in TABLE 8. These values represent one analysis each of composite samples of livers from 3 birds in each replicate. No statistical analysis was made since there were no duplicate samples. However, it is apparent that the concentration of manganese in the liver tends to increase as the manganese content of the ration is increased. It is evident that a manganese concentration of 47.8 p.p.m. in the ration afforded maximum liver retention under the conditions of this experiment.

TABLE 7.--AGE OF PULLETS, IN DAYS, AT 40 PERCENT LAY

Treatment (p.p.m. Mn)	Replicate	Age in days	
		Replicate	Treatment
17.8	1	170	168
	2	167	
27.8	1	168	168
	2	167	
37.8	1	( )[1]	166[2]
	2	166	
47.8	1	167	167
	2	167	
57.8	1	170	169
	2	168	

1 Record Missing
2 One replicate only available

The author is not aware of any published work indicating an optimum level of liver storage of manganese.

Orent and McCollum (1931) demonstrated that the organs of rats fed a manganese-containing diet exhibited a higher concentration of manganese than those from rats fed a deficient diet. Johnson (1943) presented evidence that the livers of swine fed a high manganese content diet contained a higher concentration of manganese than those from swine fed a ration containing a low concentration of manganese. Smith and Ellis (1947) found that the manganese concentration of the liver of

TABLE 8.--LIVER MANGANESE CONCENTRATION[1]

Treatment (p.p.m. Mn)	Replicate	Manganese (p.p.m.) Replicate	Manganese (p.p.m.) Treatment
17.8	1	4.96	5.28
	2	5.60	
27.8	1	( )[3]	9.33[2]
	2	9.33	
37.8	1	9.20	9.23
	2	9.26	
47.8	1	18.15	20.46
	2	22.78	
57.8	1	14.65	13.36
	2	12.06	

1 Dry fat-free basis
2 One replicate only available
3 Sample lost in laboratory accident

rabbits increased as the daily consumption of manganese increased. The data of Smith and Ellis (1947) appeared to show a level of supplementation beyond which the liver manganese concentration decreases. A similar observation was made in the present experiment. Feeding 57.8 p.p.m. manganese apparently resulted in less liver retention than when 47.8 p.p.m. was fed. Unfortunately, in the present experiment and in that of Smith and Ellis, the reduction in liver manganese retention occurred at the highest level of supplementation. Since diet treatments containing higher levels of concentration were not used, further

substantiating evidence of the validity of this trend could not be obtained. Mathers and Hill (1968) demonstrated that the livers of chickens consuming a high manganese diet showed higher concentrations of manganese than did those from birds fed a diet low in manganese.

Position Effect

A re-examination of the data in TABLE A.6 (Appendix) showed that, although differences among treatment means were not significant at the 5 percent level of probability, replicates were significant at the 10 percent level. A re-examination of data in TABLES 1 to 8 disclosed evidence of a small but ever present numerical position effect in this experiment which appeared to favor replicate 1. Replicate 1 was positioned on the east side of the house while Replicate 2 was positioned on the west side of the house. It should be remembered, however, that this experiment was conducted in a windowless building. Therefore one would not expect that sunlight had any effect on the experimental results.

TABLE 9.--EVIDENCE OF A NUMERICAL POSITION EFFECT

Item	Mean value per treatment	
	Replicate 1	Replicate 2
Final weight of pullets (g.)	1553	1510
Weight gain (g.)	833	798
Feed efficiency	6.75	6.66
Mortality (number, treatment average)	2.4	3.6
Tibial length (cm.)	12.77	12.55
Femoral length (cm.)	8.77	8.68
Femoral ash (%)	55.46	57.36
First egg weight (g.)	44.0	41.7
Eggshell ash (%)	47.4	47.6
Age at 40% lay (days)	168.8 *	167.0
Liver Mn (p.p.m.)	11.74 *	11.81

* Mean of 4 values only

THE REPRODUCTIVE CYCLE

Experimental Procedure

During the time interval between the growing period and the beginning of the experiment relative to the reproductive cycle, the pullets were maintained on their respective diets as outlined in the growing period. At 25 weeks of age the replicates from the preceding experiment were pooled according to previous ration treatment. Three groups of 5 pullets each were then selected from each treatment making a total of 15 groups and 75 birds. Selection was at random with the exception that any bird chosen had to exhibit external evidences of being in production. The only criteria for selection were those used in good culling practice; a soft, pliable abdomen, increased depth of abdomen, 2 to 3 fingers spread between pubic bones, and a general healthy appearance.

The birds were individually housed in 20.3 cm. by 40.6 cm. wire laying cages in a controlled environment house. The members of a particular diet treatment were housed in adjacent cages and permitted access to the same feeder. Placement of diet treatments with respect to each other was at random. Water was freely circulated in troughs. Feed was available ad libitum. Temperature was cyclic and varied from 14.4° C. (nighttime) to 18.3° C. (daytime). Relative humidity was maintained at approximately 75%. The photoperiod was of 14 hours

duration. The light source consisted of ten 60-watt white incandescent frosted bulbs, with reflectors, suspended at a height of 1.7 meters above feeder level. The bulbs were spaced 1 meter apart in each direction. The light intensity thus achieved was 2 to 5 foot candles at the level of the feeder as measured by a General Electric Type 213 light meter. Variation in light intensity was again due to horizontal placement of the light meter with respect to the light source.

Ration Formulation

A low manganese basal ration, in the form of a dry mash, was formulated to meet or exceed National Research Council (1966) recommendations for minimum nutrient requirements of breeding hens. The composition of the basal ration was similar to that used during the growing period and is given in TABLE 10. Feed was mixed in such quantities that it was stored for no longer than a month before being consumed. Three ration treatments were obtained by making additions of 0, 20, and 40 p.p.m. (weight/weight) of manganese, in the form of the carbonate, to the basal ration. This, then, permitted 3 laying period diet treatments x 5 growing period diet treatments x 5 periods of time in a 3x5x5 factorial experiment completely random in design.

Egg production was allowed to build past its peak and start the decline. At 36 weeks of age the laying test commenced and continued for five 28-day periods. Monthly records were maintained showing feed consumption and percent hen-day production. To assure that an adequate level of nutrients was included in the ration, monthly records of body weight were maintained.

TABLE 10.--COMPOSITION OF THE BASAL LAYING RATION

Ingredient	Per Cent
Corn Meal	58.00
Blood Meal	4.50
Alfalfa Meal (17% Protein)	1.00
Meat and Bone Meal (50% Protein)	3.00
Dried Skim Milk	16.00
C.P. Calcium Carbonate *	4.00
Ground Limestone	1.00
Dicalcium Phosphate	1.00
Vitamin Premix **	1.00
Salt, Iodized	.25
Purified Cellulose	8.25
Animal Fat	2.00
	100.00

*--For the final mix, a bagged precipitated calcium carbonate replaced the reagent grade chemical.
**-Vitamin Premix supplied the following per kilogram of ration: Vitamin A - 8250.0 I.U., Vitamin D-3 - 818.4 I.C.U., Vitamin E - 5.5 I.U., Vitamin B-12 - 11.0 mcg., dl-Methionine - 499.0 mg., Ethoxyquin - 249.5 g., Riboflavin - 2.2 mg., Calcium Pantothenate - 4.4 mg., Niacin - 6.6 mg., Choline Chloride - 110.0 mg.

Mineral analysis of the basal laying ration
(Average of six samples)

Calcium	2.78%
Total Phosphorus	.75%
Manganese	14.0 p.p.m.

Insemination

Artificial insemination was conducted at 7-day intervals throughout the course of the experiment using 0.05 ml. of undiluted semen per insemination. Eight cockerels of the same breed were separately main-

tained on a commercial ration. Semen from all cockerels was pooled for insemination. The lapse of time between the collection of semen and the end of the insemination process was never more than 45 minutes.

Egg Collection

Eggs were collected the last 7 consecutive days of each period and pedigreed by diet treatment. Those eggs obtained during the first 3 days of the collection period were used in the determination of mean egg weight and mean shell weight. Eggs collected during the last 4 days of the collection period were used for fertility, hatchability, and chick growth studies.

Shell Weight Determination

Mean shell weight, as described by Tyler and Geake (1961), was used as an expression of mean shell thickness. Specific gravity was determined according to Archimede's principle. The equation M=1.07G-13.5, as set forth by Tyler and Geake (1961) (page 286) was used to determine the true shell weight (M) in milligrams per square centimeter from the specific gravity (G). The mean true shell weight was then determined for each treatment. Eggs used for specific gravity measurements were collected every two hours during the day beginning at 8:00 o'clock a.m. and ending at 4:00 o'clock p.m. The eggs were allowed to cool to ambient temperature and specific gravity was then determined immediately through the use of a laboratory balance accurate to 0.0001 grams. Eggs appearing at 8:00 o'clock a.m. were discarded, unless they were still warm to the touch, on the theory that they could have been laid immediately after the 4:00 o'clock p.m. collection the preceding day.

Eggshell Ash

Eggshells used for percent ash determination were emptied of their contents, rinsed with distilled water, and dried, with membranes intact, for 24 hours at a temperature of 105°C. The shells were then pooled by ration treatment and ground to pass a 1 mm. screen. Silica ashing dishes were dried at a temperature of 150° C. for a period of two hours. They were then placed in a vacuum desiccator and allowed to cool to ambient temperature. Duplicate 2 g. aliquots of the ground shell were ashed for 72 hours at 600° C. The samples were removed from the muffle furnace, placed in a vacuum desiccator, and allowed to cool to ambient temperature before weighing.

Hatching Eggs

Eggs to be retained for hatching were stored in a walk-in type cooler at 10°-13° C. and 70% relative humidity for a maximum period of 10 days and were rotated 90° once daily during storage. The eggs were incubated in an 18,000 egg capacity commercial incubator which provided automatic temperature and humidity control and periodic turning of the eggs. Temperature and humidity were maintained according to accepted practice. All eggs were candled before being set and were not handled again until the 22nd day except to transfer them to the hatcher. All eggs were hatched in separate compartments according to ration treatment. Eggs remaining on the 22nd day were broken out to determine lack of fertility and abnormal embryos.

Chick Growth

Immediately after removal from the hatcher, all viable chicks were group weighed and placed by treatment, at random, in electrically

heated batteries for 28-day growth studies. Feed consumption and weight were recorded at 28 days. All groups were fed the same commercial starter ration in the form of crumbles. Feed and water were available ad libitum. Ambient temperature in the battery room was maintained at 26.7° C. Brooding temperatures in the batteries were regulated according to accepted practice. Humidity was uncontrolled and unrecorded. The photo-period was continuous for the entire 28-day period. The light intensity was a minimum of 5 foot candles at the level of the feeder during the night. This was supplemented by natural light, during the daytime, giving a maximum light intensity of 40 foot candles.

Analysis of Data

Data, where applicable, were subjected to analysis of variance according to the method of Snedecor (1956). Means were separated by the use of Duncan's New Multiple Range Test. Missing plots, if any, were calculated by the method of Yates (1933) as quoted in Cochran and Cox (1957).

Results and Discussion

Manifestations of nutritional adequacy during the reproductive period include not only those relating to the dam but those relating to the offspring as well. Those criteria which are discussed in this section include the following: Hen-day production, pullet feed efficiency, egg weight, eggshell thickness, fertility, hatchability, initial chick weight, chick weight gain, and chick feed efficiency.

Hen-Day Production

TABLE 11 presents data pertaining to the percent hen-day production

TABLE 11. PERCENT HEN-DAY PRODUCTION

LAYING LEVEL (PPM)	GROWING LEVEL (PPM)	REPLICATES 1	2	3	4	5	LAYING LEVEL MEANS **	GROWING LEVEL MEANS **	LAYING x GROWING MEANS *
14	17.8	96.43	92.86	96.43	75.00	85.71	87.64^{ab}		90.11^{ab}
		92.86	89.29	78.57	78.57	82.14			
		92.86	96.43	92.86	96.43	92.86			
		92.86	92.86	82.14	89.29	89.29			
	27.8	82.14	82.14	78.57	71.43	78.57			83.95^{abcd}
		92.86	92.86	85.71	85.71	85.71			
		85.71	85.71	82.14	78.57	85.71			
	37.8	92.86	85.71	82.14	75.00	78.57			82.69^{bcd}
		82.14	78.57	82.14	75.00	75.00			
		85.71	82.14	75.00	64.29	10.71			
		89.29	100.00	89.29	100.00	96.43			
	47.8	85.71	85.71	85.71	78.57	75.00			90.48^{ab}
		100.00	100.00	96.43	92.86	92.86			
		92.86	89.29	85.71	89.29	78.57			
	57.8	92.86	85.71	85.71	85.71	85.71			89.58^{abc}
		100.00	85.71	92.86	89.29	89.29			
		89.29	92.86	89.29	92.86	89.29			
		85.71	89.29	82.14	71.43	82.14			
		100.00	96.43	92.86	85.71	75.00			

TABLE 11. Continued

LAYING LEVEL (PPM)	GROWING LEVEL (PPM)	REPLICATES 1	2	3	4	5	LAYING LEVEL MEANS **	GROWING LEVEL MEANS **	LAYING x GROWING MEANS *
		96.43	96.43	92.86	85.71	89.29			
		92.86	82.14	85.71	78.57	85.71			
	17.8	96.43	89.29	92.86	78.57	92.86		84.92^{bc}	87.22^{abc}
		96.43	82.14	75.00	57.14	71.43			
		96.43	96.43	100.00	92.86	100.00			
		89.29	85.71	78.57	82.14	53.57			
	27.8	85.71	78.57	71.43	64.29	60.71		83.32^{c}	85.82^{abcd}
		96.43	96.43	85.71	89.29	75.00			
		100.00	96.43	100.00	92.86	96.43			
34	37.8	85.71	82.14	85.71	85.71	85.71	90.33^{a}	87.94^{abc}	91.89^{a}
		96.43	85.71	89.29	89.29	92.86			
		100.00	100.00	96.43	92.86	96.43			
		89.29	89.29	85.71	85.71	82.14		91.33^{a}	91.78^{ab}
	47.8	92.86	89.29	75.00	82.14	92.86			
		92.86	89.29	85.71	78.57	85.71			
		85.71	85.71	85.71	82.14	82.14			
		100.00	100.00	100.00	92.86	92.86		90.08^{ab}	92.76^{a}
	57.8	96.43	92.86	96.43	92.86	92.86			
		100.00	92.86	96.43	85.71	89.29			

TABLE 11. Continued

LAYING LEVEL (PPM)	GROWING LEVEL (PPM)	REPLICATES 1	2	3	4	5	LAYING LEVEL MEANS **	GROWING LEVEL MEANS **	LAYING x GROWING MEANS *
		60.71	67.86	21.43	71.43	67.86			
		92.86	89.29	82.14	53.57	25.00			76.07^{d}
	17.8	89.29	82.14	85.71	82.14	85.71			
		92.86	85.71	85.71	85.71	82.14			
		71.43	82.14	75.00	75.00	53.57			
	27.8	85.71	89.29	82.14	82.14	82.14			79.94^{cd}
		89.29	89.29	85.71	78.57	67.86			
		85.71	96.43	96.43	92.86	85.71			
		71.43	82.14	85.71	85.71	78.57			
54	37.8	100.00	96.43	96.43	89.29	100.00	85.22^{b}		88.51^{abc}
		85.71	75.00	78.57	67.86	75.00			
		100.00	96.43	92.86	89.29	96.43			
		96.43	85.71	82.14	82.14	78.57			91.69^{ab}
	47.8	85.71	92.86	92.86	92.86	92.86			
		78.57	82.14	89.29	89.29	82.14			
		89.29	89.29	92.86	92.86	92.86			
	57.8	92.86	89.29	89.29	85.71	78.57			87.58^{abc}
		89.29	89.29	85.71	82.14	78.57			
		100.00	100.00	85.71	42.86	89.29			
REPLICATE MEANS **		92.96^{a}	90.53^{ab}	87.59^{bc}	83.29^{c}	83.35^{c}			

* Means in the same column having different superscript letters differ significantly ($P < 0.05$)
** Means having different superscript letters differ significantly ($P < 0.01$)

of selected pullets. Pullets were selected for study only if they had laid at least one egg during each 28 day period. Pullets that consumed a laying ration containing 34 p.p.m. manganese had the highest rate of production ($P<0.01$) while the lowest rate of production was achieved by those pullets fed a laying ration containing 54 p.p.m. (see TABLE A.10). An intermediate level of production was shown by those pullets consuming a ration containing 14 p.p.m. manganese. These findings are in contrast to those of Gutowska and Parkhurst (1942) who reported a lack of significant differences in egg production between dietary treatments of 17-24 p.p.m. and 61-76 p.p.m. manganese when working with Rhode Island Red pullets. Cox and Balloun (1969), using Hy-Line 934-H pullets, found that if calcium was adequate a laying ration containing 20 p.p.m. manganese was capable of maintaining high egg production.

Pullets which were grown on diets containing the highest levels of manganese, 47.8 p.p.m. and 57.8 p.p.m., laid at a higher rate ($P<0.01$) than did their counterparts which were grown on rations containing lesser concentrations of manganese. The rate of production appeared to decrease directly as the manganese content of the growing diet decreased. Richards (1930) found that the manganese content of plant female reproductive organs and hen's ova increased as the reproductive organs matured. Waddell, Steenbock, and Hart (1931) found that the addition of manganese to a milk diet increased the regularity of ovulation in rats. It would appear from the above findings and the observations of the present study that manganese is required for the development of the ovaries and that higher levels of manganese are therefore required in the rations of pullets during the growing period than during the laying period.

A decrease in the rate of production with time is well known to occur during the reproductive cycle of the pullet (Hendricks, 1934). The rate of egg production decreased with time on experiment in the present study and was reflected by highly significant ($P<0.01$) differences among means.

The interaction between the manganese levels of the growing diet and those of the laying diet proved to be significant ($P<0.05$). FIGURE 1 is a graphical representation of the mean values of those interactions as they relate to hen-day production. No definite trends were clearly discernable. However, with the exception of two values, it can be seen that higher levels of manganese in the growing diet resulted in a higher rate of production regardless of the level of manganese found in the laying diet.

Pullet Feed Efficiency

Data on pullet feed efficiency are presented in TABLE 12. The analysis of variance (TABLE A.11) showed that feed efficiency was unaffected by dietary treatment. It was, however, highly significant ($P<0.01$) across periods, the better feed efficiency appearing during period 1. Gutowska and Parkhurst (1942) found no significant differences between dietary manganese levels in the feed efficiency of heavy breed layers. Cooper, Chubb, and Rowell (1963) reported no significant differences in food consumption when feeding rations containing 10 and 50 p.p.m. manganese to Light Sussex pullets. However, Atkinson, et al (1967), working with Broad Breasted Bronze hens, found that feed efficiency was best at intermediate levels of manganese supplementation.

Egg Weight

Differences between egg weight means (TABLE 13) were found to be

FIGURE 1. INTERACTION OF LAYING LEVELS AND GROWING LEVELS AS THEY AFFECT HEN-DAY PRODUCTION

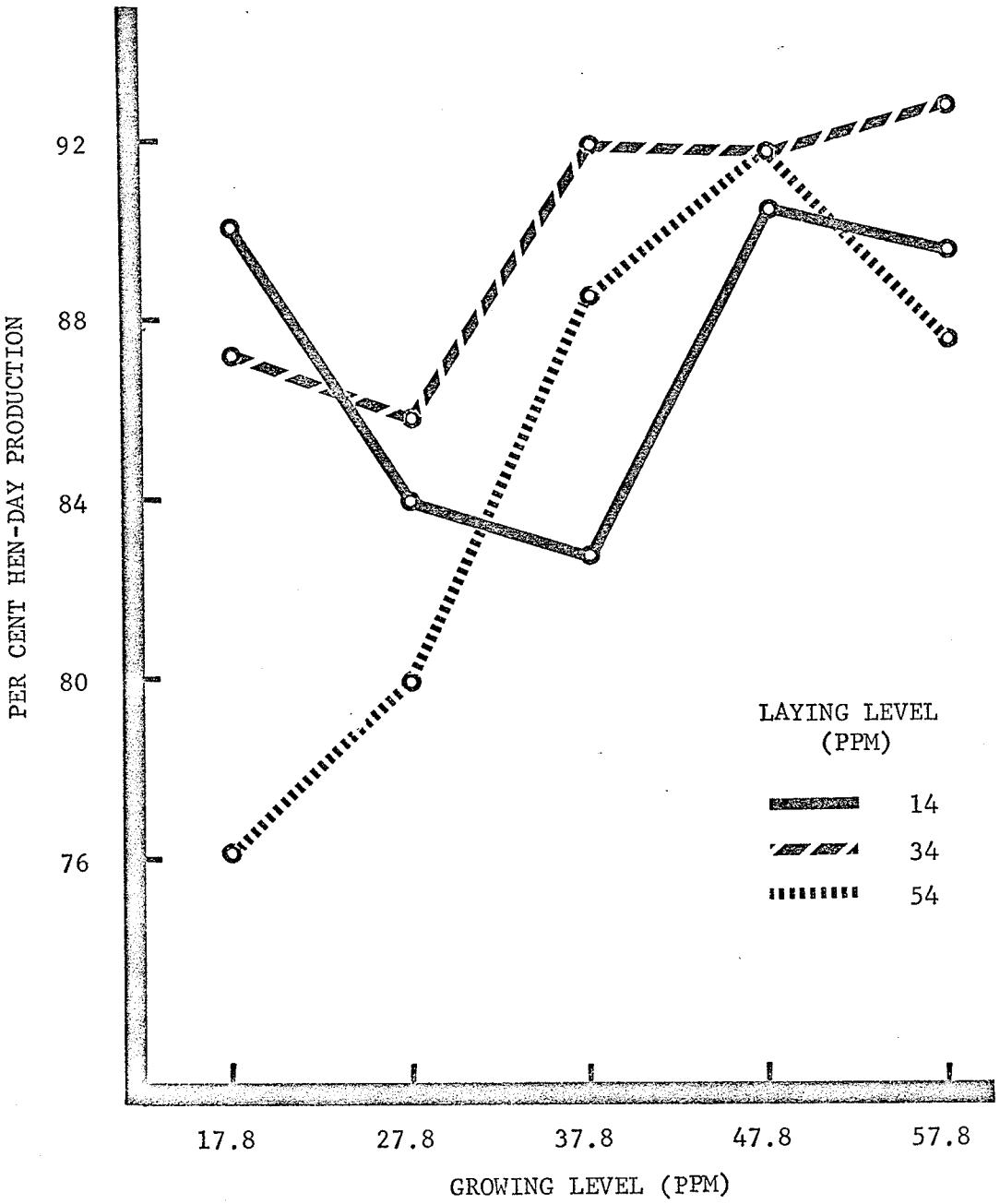


TABLE 12. PULLET FEED EFFICIENCY
(Kilograms feed per dozen eggs)

LAYING LEVEL (PPM)	GROWING LEVEL (PPM)	PERIODS 1 REPLICATES 1	1 REPLICATES 2	2 REPLICATES 3	2 REPLICATES 4	LAYING LEVEL MEANS	GROWING LEVEL MEANS	LAYING x GROWING MEANS
	17.8	1.60	1.57	1.84	1.95			1.74
	27.8	1.64	1.54	1.81	1.80			1.70
14	37.8	1.66	1.55	1.92	1.93			1.77
	47.8	1.46	1.53 +	1.90	1.93			1.70
	57.8	1.53	1.53	1.77	1.90	1.72		1.68
	17.8	1.57	1.47 +	1.70	1.77		1.72	1.63
	27.8	1.65	1.70	1.81	1.95		1.71	1.78
34	37.8	1.45	1.53	1.72	1.74		1.71	1.61
	47.8	1.60	1.40	1.70	1.81		1.67	1.63
	57.8	1.55	1.40	1.70	1.90	1.66	1.65	1.64
	17.8	1.60	1.67	1.85	2.02			1.79
	27.8	1.50	1.49	1.76	1.83			1.64
54	37.8	1.65	1.70	1.83	1.83			1.75
	47.8	1.44	1.37	1.87	1.99			1.67
	57.8	1.49	1.48	1.68	1.92	1.70		1.64
PERIOD MEANS *			1.54[b]		1.84[a]			

* Means having different superscript letters differ significantly ($P < 0.01$)
\+ Calculated missing plot

TABLE 13. EGG WEIGHT (Grams)

LAYING LEVEL (PPM)	GROWING LEVEL (PPM)	REPLICATES 1	2	3	4	5	LAYING LEVEL MEANS *	GROWING LEVEL MEANS	LAYING x GROWING MEANS *
		59.84	61.71	65.66	64.22	62.95			
	17.8	64.63	67.81	65.41	69.00	66.25			62.54^{ab}
		55.49	55.10	60.04	59.45	60.47			
		62.10	62.66	67.54	68.79	67.84			
	27.8	61.29	60.65	64.93	63.53	66.33			64.14^{a}
		61.51	64.34	64.95	63.53	62.05			
		58.06	59.30	59.67	62.43	61.74			
14	37.8	64.51	66.17	69.29	73.15	71.47	61.64^{a}		64.21^{a}
		62.56	67.03	64.27	61.29	62.25			
		55.89	57.79	57.11	59.09	60.07			
	47.8	60.17	60.56	58.59	61.91	58.21			57.52^{c}
		53.89	53.87	55.12	55.71	54.87			
		58.81	60.14	60.19	64.87	64.34			
	57.8	50.51	52.78	55.20	55.09	54.61			60.25^{abc}
		62.68	59.11	59.42	62.49	64.53			
		59.79	64.79	65.42	63.41	66.85			

TABLE 13. Continued

LAYING LEVEL (PPM)	GROWING LEVEL (PPM)	REPLICATES 1	2	3	4	5	LAYING LEVEL MEANS *	GROWING LEVEL MEANS	LAYING x GROWING MEANS *
		59.95	59.94	61.54	63.40	62.33			
	17.8	58.20	61.61	60.44	62.32	59.34		60.54	58.75^{bc}
		51.55	56.20	53.92	52.95	57.62			
		55.97	58.01	55.24	58.20	59.41			
	27.8	57.34	58.56	57.48	57.29	59.26 +		61.26	58.76^{bc}
		61.55	58.18	61.68	61.86	61.44			
		54.69	58.38	57.37	58.95	60.79			
34	37.8	54.42	60.33	62.42	57.25	60.38	59.45^{b}	61.90	58.77^{bc}
		56.51	59.46	59.23	58.35	63.07			
		54.13	58.44	56.44	58.75	55.46			
	47.8	58.55	62.95	63.86	63.01	64.07		59.69	61.46^{abc}
		62.71	61.80	69.20	66.96	65.58			
		62.96	64.32	61.85	68.65	64.90			
	57.8	54.57	56.22	55.28	59.05	59.93		60.70	59.49^{bc}
		52.61	55.57	56.74	57.18	57.48			
		58.40	60.69	59.55	62.00	61.91			

TABLE 13. CONTINUED

LAYING LEVEL (PPM)	GROWING LEVEL (PPM)	REPLICATES 1	2	3	4	5	LAYING LEVEL MEANS *	GROWING LEVEL MEANS	LAYING x GROWING MEANS *
		55.08	53.55	64.84	65.45	68.37			
	17.8	59.24	61.60	62.30	61.81	65.30			60.32^{abc}
		53.50	56.20	55.47	59.55	62.53 +			
		60.22	60.51	65.15	60.91	61.13			
	27.8	58.08	60.83	59.51	63.50	61.92			60.88^{abc}
		58.66	60.34	61.41	61.47	59.58			
		57.80	57.27	61.40	60.99	62.10			
54	37.8	56.79	58.55	59.12	56.36	61.46	61.34^{a}		62.71^{ab}
		66.67	69.45	69.14	72.75	70.86			
		55.65	56.16	55.24	62.46	60.85			
	47.8	61.71	63.07	63.07	65.06	66.30			60.10^{abc}
		58.67	58.30	57.50	59.49	57.99			
		58.21	56.70	55.64	62.85	65.28			
	57.8	62.81	63.61	63.58	64.67	62.79			62.36^{ab}
		57.99	58.10	63.67	63.97	62.86			
		61.97	66.05	62.81	68.25	65.40			
REPLICATE MEANS *		58.52^{c}	60.10^{bc}	60.93^{ab}	62.16^{ab}	62.34^{a}			

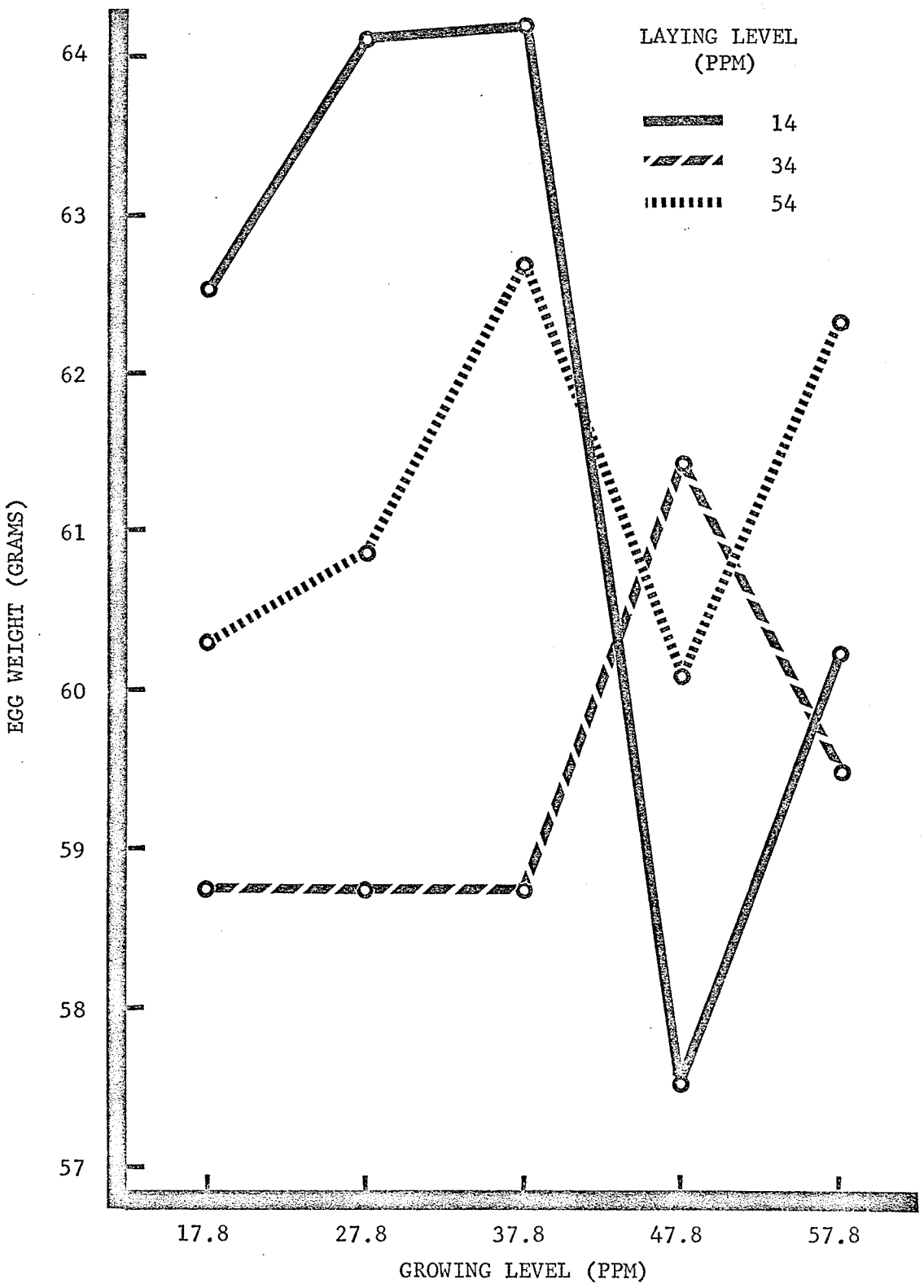
* Means having different superscript letters differ significantly ($P < 0.01$)
\+ Calculated missing plot

significant in three measurements (TABLE A.12). Eggs selected for inclusion in the data were laid by pullets which produced at least one egg during each collection period. The concentration of manganese in the laying diet had a highly significant effect ($P<0.01$) on egg weight. Pullets on both the lowest and highest manganese concentrations produced eggs which were heavier than those laid by pullets on the intermediate concentration. Gutowska and Parkhurst (1942) reported no significant differences in egg weight when heavy breed pullets consumed laying rations of low (17-24 p.p.m) or high (61-76 p.p.m.) manganese concentration. Atkinson, et al (1967) found no significant differences in egg weight in turkeys when supplementing a basal ration with 27, 54, 108, or 162 p.p.m. manganese.

The level of manganese in the growing diet, over the range studied, produced no significant differences in egg weight during five 28-day periods. However, egg weight increased significantly ($P<0.01$) during the experiment. The rate of change varied between replicates but was consistent in that it was always positive. Clark (1940) has previously documented the increase in weight of pullet eggs as the reproductive period progresses and such a phenomena is well known to occur by those individuals associated with the egg industry.

The interactions of laying levels and growing levels were found to have a highly significant effect ($P<0.01$) on egg weight means. A graphical representation (FIGURE 2) shows that the greatest mean egg weight was achieved by those pullets consuming rations with either 27.8 or 37.8 p.p.m. manganese in the growing diet and only 14 p.p.m. manganese in the laying diet. Eggs of lowest weight were produced by hens fed 47.8 p.p.m. manganese during egg production. In this study the

FIGURE 2. INTERACTION OF LAYING LEVELS AND GROWING LEVELS AS THEY AFFECT EGG WEIGHT



mean egg weight was apparently adversely affected by the addition of 10 p.p.m. manganese during the growing period.

Eggshell Thickness

Tyler and Geake (1961) found that shell weight per square centimeter (TABLE 14), as determined from the specific gravity of the egg, was a good measure of eggshell thickness. Eggshell thickness has been used to denote the relative ability of the egg to withstand abuse without cracking or breaking, the thicker eggshell being assumed to be capable of withstanding greater abuse. However, to this author's knowledge, no investigator has shown that eggshell thickness per se is the only factor affecting shell strength. The strength and/or resiliency of the organic matrix, the density of the calcium carbonate deposited, and the brittleness of the shell, which is probably a function of both the density of the calcium carbonate deposition and the strength of the bond between adjoining molecules, must have considerable effect on shell strength. In the present study dietary manganese treatment, either during the growing period or the laying period, was found to have no significant effect (TABLE A.13) on shell thickness. Cox and Balloun (1969) and Atkinson et al (1967) also found that eggshell thickness was unaffected by the level of added manganese in the diet. Gutowska and Parkhurst (1942), on the other hand, found that eggshell breaking strength was greater among heavy breed hens when they consumed a manganese supplemented ration. In the present study there was no significant decrease in shell weight per square centimeter with time. Ordinarily it is found that eggshell quality declines as the reproductive period progresses. This decline is evidenced by increasing numbers of cracked, checked, or otherwise imperfect shells. Perhaps, then, shell

TABLE 14. SHELL WEIGHT
(Milligrams per square centimeter)

LAYING LEVEL (PPM)	GROWING LEVEL (PPM)	REPLICATES 1	2	3	4	5	LAYING LEVEL MEANS	GROWING LEVEL MEANS	LAYING x GROWING MEANS
14	17.8	76.7	73.3	73.4	71.7	72.7	74.5		74.4
		78.3	76.3	72.6	76.2	77.3			
		72.0	70.0	76.8	76.1	73.1			
	27.8	69.7	76.3	72.8	66.9	72.2			73.7
		72.2	66.1	64.0	71.3	66.8			
		82.0	79.6	80.9	81.7	82.7			
	37.8	68.2	71.0	73.9	71.4	69.0			75.6
		91.2	87.0	79.6	80.4	87.6			
		70.6	77.3	68.7	67.4	71.5			
	47.8	75.3	70.4	73.6	67.0	69.8			73.1
		70.9	68.6	66.8	64.9	63.4			
		80.2	87.2	78.2	79.9	80.6			
	57.8	87.2	79.3	83.2	76.8	76.9			75.3
		72.8	66.3	69.8	71.4	68.2			
		71.6	70.2	74.5	70.5	70.3			
		78.2	80.4	79.0	81.4	78.7			

TABLE 14. Continued

LAYING LEVEL (PPM)	GROWING LEVEL (PPM)	REPLICATES 1	2	3	4	5	LAYING LEVEL MEANS	GROWING LEVEL MEANS	LAYING x GROWING MEANS
		79.6	78.8	76.9	78.2	79.0			
	17.8	77.9	74.0	74.0	73.7	73.0		75.9	76.0
		81.4	73.5	71.5	73.4	75.0			
		74.8	72.2	72.8	69.9	73.7			
	27.8	82.1	78.0	80.2	79.8	80.8 +		76.9	78.6
		80.9	84.6	83.6	82.2	83.0			
		73.8	70.8	70.3	66.6	68.1			
34	37.8	83.0	79.8	79.8	78.9	76.5	75.7	74.6	75.3
		77.1	76.6	79.0	76.2	72.6			
		75.5	78.5	75.6	68.5	67.4			
	47.8	75.4	73.3	71.7	75.2	55.1		74.2	72.1
		75.3	72.7	73.4	72.7	71.4			
		73.6	67.9	75.2	68.8	71.9			
	57.8	80.1	78.6	77.2	79.1	78.0		75.4	76.4
		79.2	77.0	74.7	76.2	83.0			
		78.6	77.9	76.8	81.8	72.2			

TABLE 14. Continued

LAYING LEVEL (PPM)	GROWING LEVEL (PPM)	REPLICATES 1	2	3	4	5	LAYING LEVEL MEANS	GROWING LEVEL MEANS	LAYING x GROWING MEANS
		74.2	70.8	73.7	71.9	71.2			
	17.8	85.7	78.7	83.1	81.8	78.8			77.4
		78.4	79.0	81.6	75.5	76.1 +			
		93.6	87.3	83.3	87.6	83.4			
	27.8	77.0	76.7	72.2	70.2	77.1			78.5
		74.1	70.3	72.4	73.6	78.7			
		73.4	74.3	73.6	74.8	76.4			
54	37.8	74.7	71.0	68.4	77.2	72.3	76.1		73.0
		75.1	72.9	71.9	70.4	68.3			
		77.3	81.4	75.3	76.9	79.6			
	47.8	76.6	77.9	76.2	77.2	78.6			77.4
		80.1	75.0	73.8	72.8	81.8			
		74.3	68.7	69.0	67.1	73.7			
	57.8	78.3	72.0	74.5	73.2	77.7			74.6
		72.0	67.8	71.3	68.1	76.8			
		83.9	80.0	78.3	77.9	87.3			
REPLICATE MEANS		77.4	75.4	75.0	74.4	75.0			

+ Calculated missing plot

thickness is not a good indicator of shell quality. However, the duration of this experiment was only five 28-day periods at the end of which time the birds were approximately 56 weeks of age. Ordinarily extreme shell quality problems do not arise until after this age. In addition, close examination of the data in TABLE 14 and TABLE A.13 disclosed considerable variability and a relatively large error term. This variability would have the effect of preventing the attainment of significance.

Fertility

A compilation of fertility data is given in TABLE 15. Since the eggs were not pedigreed, but identified only by treatment, a single mean value appears for each treatment. There was a significant difference ($P<0.05$) between laying level means with the two diets containing the highest concentrations of manganese, 34 and 54 p.p.m. showing the greatest fertility (TABLE A.14). Richards (1930) could find no relation between manganese and fertility. Gallup and Norris (1939b) reported slightly decreased fertility among eggs from New Hampshire pullets consuming a diet containing 13 p.p.m. manganese. Gutowska and Parkhurst (1942) found no significant differences in fertility among eggs from Rhode Island Red pullets fed rations of low (17-24 p.p.m.) or high (61-76 p.p.m.) manganese content. Chubb (1954) reported increased infertility among pullets on a manganese low diet while Cooper, Chubb and Rowell (1963) found no significant differences in fertility of eggs from Light Sussex pullets fed a low manganese diet as compared with those from birds fed a manganese supplement diet or a manganese adequate control diet.

TABLE 15. FERTILITY
(Percent of eggs set)

LAYING LEVEL (PPM)	GROWING LEVEL (PPM)	REPLICATES 1	2	3	4	5	LAYING LEVEL MEANS *	GROWING LEVEL MEANS **	LAYING x GROWING MEANS *
14	17.8	94.74	94.44	83.33	93.33	80.00	91.58^{b}		89.92^{cd}
	27.8	90.91	93.33	100.00	100.00	88.89			96.74^{abc}
	37.8	85.71	92.31	100.00	100.00	100.00			99.27^{abc}
	47.8	68.75	85.71	71.43	83.33	69.23			76.11^{d}
	57.8	93.33	83.33	88.24	94.44	88.24			89.87^{cd}
34	17.8	100.00	100.00	91.67	100.00	100.00	96.55^{a}	94.94^{abc}	99.66^{a}
	27.8	94.12	100.00	100.00	100.00	100.00		98.39^{a}	99.76^{a}
	37.8	100.00	89.47	94.74	94.44	81.82		97.73^{ab}	93.97^{abc}
	47.8	93.33	100.00	76.92	87.50	88.89		90.11^{c}	91.67^{bcd}
	57.8	89.47	94.44	88.24	100.00	76.47		91.25^{bc}	92.09^{bc}
54	17.8	94.12	86.67	93.33	100.00	66.67	96.27^{a}		91.32^{bcd}
	27.8	100.00	93.33	94.12	93.75	100.00			97.71^{abc}
	37.8	100.00	100.00	100.00	87.50	100.00			99.48^{ab}
	47.8	100.00	100.00	87.50	83.33	100.00			97.57^{abc}
	57.8	88.24	93.75	94.44	88.89	92.31			91.69^{bcd}

* Means in the same column having different superscript letters differ significantly ($P < 0.05$)
** Means having different superscript letters differ significantly ($P < 0.01$)

A highly significant difference ($P<0.01$) was found to exist between growing level means with lower levels of manganese in the growing diet appearing to have improved fertility as compared with higher levels. In actuality, eggs from pullets fed a growing diet containing 27.8 p.p.m. manganese showed the highest fertility. Significant differences ($P<0.05$) were also found to exist between means representing the interactions of laying levels with growing levels. These means are shown graphically in FIGURE 3. Generally speaking, the highest fertility was attained when the laying level containing 34 p.p.m. manganese was combined with either of two growing levels containing 17.8 or 27.8 p.p.m. manganese. Obviously one point in Figure 3, growing level 47.8: laying level 14, is at variance with the other data and should be discounted.

Hatchability

Hatchability data are shown in TABLE 16. The data shown are the mean values of each dietary treatment. The analysis of variance of hatchability (TABLE A.15) shows that there were no significant differences between the means of dietary treatments. There was a great deal of variability within treatment groups and, consequently, a large error term appeared in the analysis of variance. This error term precluded the attainment of significance. It is the author's belief that the hatching eggs were incorrectly handled during storage and/or incubation for while fertility was high and relatively consistent, hatchability was, at times, extremely poor regardless of dietary treatment. Upon breaking out those eggs not hatching, not one case of nutritional chondrodystrophy could be identified.

FIGURE 3. INTERACTION OF LAYING LEVELS AND GROWING LEVELS AS THEY AFFECT FERTILITY

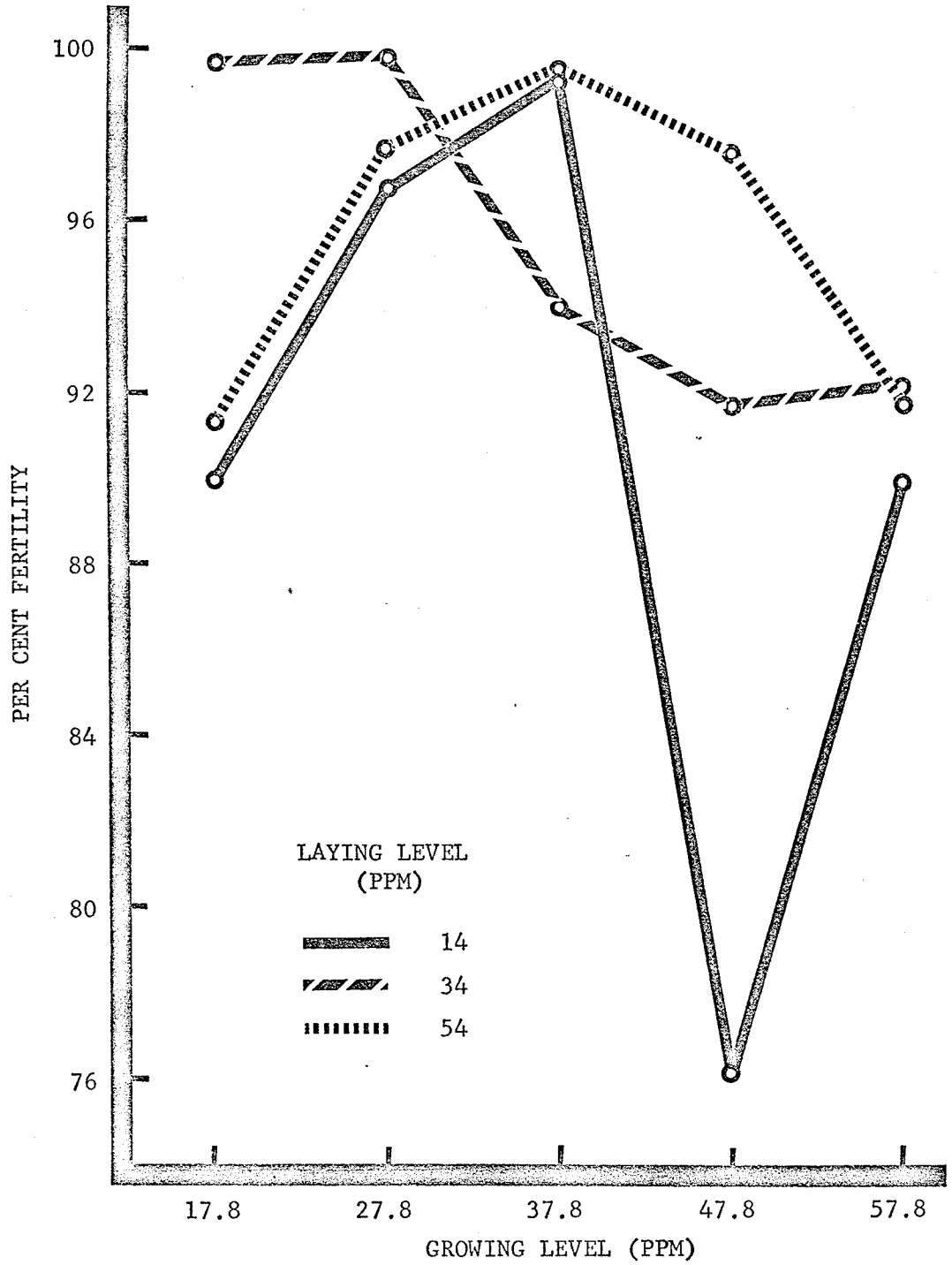


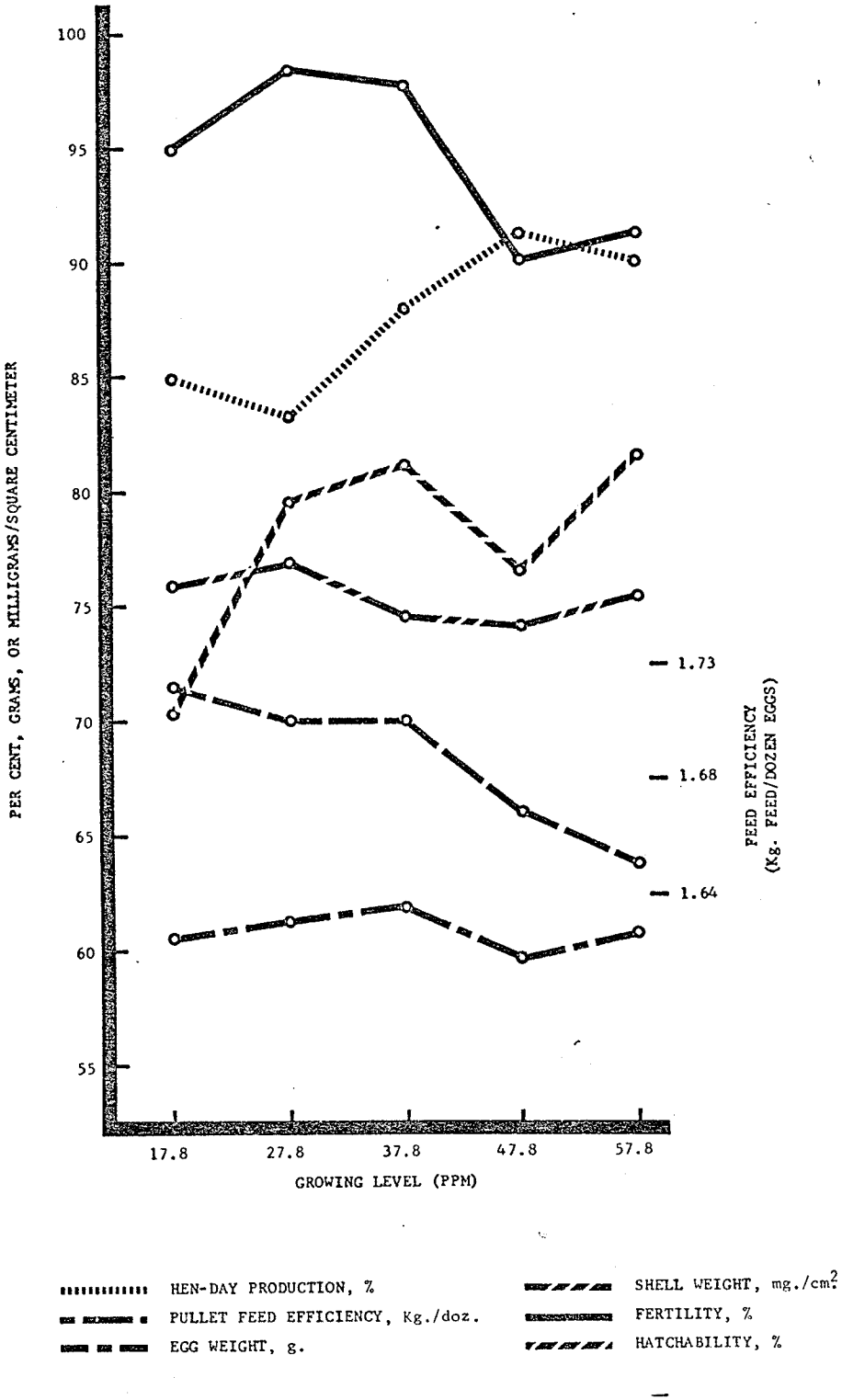
TABLE 16. HATCHABILITY
(Per cent fertile eggs)

LAYING LEVEL (PPM)	GROWING LEVEL (PPM)	REPLICATES 1	2	3	4	5	LAYING LEVEL MEANS	GROWING LEVEL MEANS	LAYING x GROWING MEANS
	17.8	55.56	64.71	60.00	78.57	50.00			62.10
	27.8	80.00	64.29	100.00	71.43	75.00			81.98
14	37.8	66.67	50.00	54.54	63.64	100.00			72.00
	47.8	90.91	66.67	80.00	80.00	88.89			82.03
	57.8	85.71	73.33	66.67	100.00	86.67	77.33		85.98
	17.8	69.23	85.71	90.91	91.67	46.15		70.32	78.71
	27.8	87.50	72.73	66.67	72.73	87.50		79.51	78.06
34	37.8	89.47	94.12	94.44	64.71	88.89		81.17	87.72
	47.8	21.43	90.00	90.00	71.43	75.00		76.63	71.40
	57.8	88.24	94.12	86.67	84.21	84.62	81.16	81.56	87.84
	17.8	68.75	69.23	71.43	62.50	75.00			69.45
	27.8	77.78	92.86	81.25	60.00	75.00			77.39
54	37.8	64.71	94.74	82.35	78.57	86.67			82.54
	47.8	55.56	60.00	71.43	70.00	100.00			76.03
	57.8	60.00	66.67	76.47	81.25	58.33	75.27		68.92

Gallup and Norris (1939b) found that a deficiency of manganese in the diet of New Hampshire pullets resulted in low hatchability. Golding, Schaible, and Davidson (1940) reported that supplementation of a basal diet with manganese improved the hatchability of eggs from heavy breed hens. Gutowska and Parkhurst (1942) could not find significant differences in hatchability between dietary treatments when working with Rhode Island Red pullets. Chubb (1954) reported higher hatchability of eggs from Barred Rock and Brown Leghorn pullets fed a manganese supplemented diet. Cooper, Chubb, and Rowell (1963) found no significant differences in embryonic survival in eggs from Light Sussex pullets fed supplemented and unsupplemented rations. Atkinson, et al (1967) reported significant depression of hatchability at low (27 p.p.m.) and high (162 p.p.m.) levels of supplementation in turkeys.

FIGURE 4 is an aggregate graphical representation of the effects of manganese concentration in the growing diet on all pullet parameters. Hen-day production was lowest at lower concentrations of manganese and then increased as the concentration of manganese in the diet increased. Hen-day production apparently decreased somewhat, although not significantly, at concentrations above 47.8 p.p.m. Feed efficiency varied but little and was only numerically superior at the higher concentrations of manganese. Likewise egg weight and shell weight were little affected by the level of manganese in the growing diet. Fertility was significantly higher at intermediate levels (27.8 and 37.8 p.p.m.) of manganese concentration. Both higher and lower levels of manganese resulted in significantly depressed fertility. Hatchability was not significantly affected by the concentration of manganese in the growing diet in this study.

FIGURE 4. EFFECT OF GROWING LEVELS ON PULLET PARAMETERS



An aggregate graphical representation of the effects of the manganese concentration of the diet during the laying period on all pullet parameters is given in FIGURE 5. Hen-day production was best at the intermediate level (34 p.p.m.) of manganese concentration. Higher or lower concentrations significantly depressed production. Pullet feed efficiency paralleled hen-day production although differences between treatment means were not significant. Egg weight was significantly greater at both the lower and higher levels of concentration while shell weight per square centimeter was not significantly affected by the manganese concentration in the laying diet. Fertility was significantly greater at the higher levels of concentration (34 and 54 p.p.m.). Hatchability was not significantly affected by the level of manganese in the laying diet.

Initial Chick Weight

Eggs were not pedigree hatched. Therefore, each value shown in TABLE 17 represents the mean weight upon hatching of all chicks on a dietary treatment. Four measurements within this parameter were found to show significance (TABLE A.16). Chicks having significantly greater initial weights ($P<0.01$) were hatched from eggs produced by pullets fed the laying diet containing 14 p.p.m. manganese. This was partially in agreement with the data of TABLE 13 where it was shown that eggs from pullets consuming this diet and the diet containing 54 p.p.m. manganese were significantly heavier than eggs produced by pullets on the diet containing 34 p.p.m. manganese. Since no evidence of significant differences in shell weight were found (TABLE 14), one would, therefore, expect an identical egg weight/shell weight ratio with the result that initial chick weight would parallel egg weight.

FIGURE 5. EFFECT OF LAYING LEVELS ON PULLET PARAMETERS

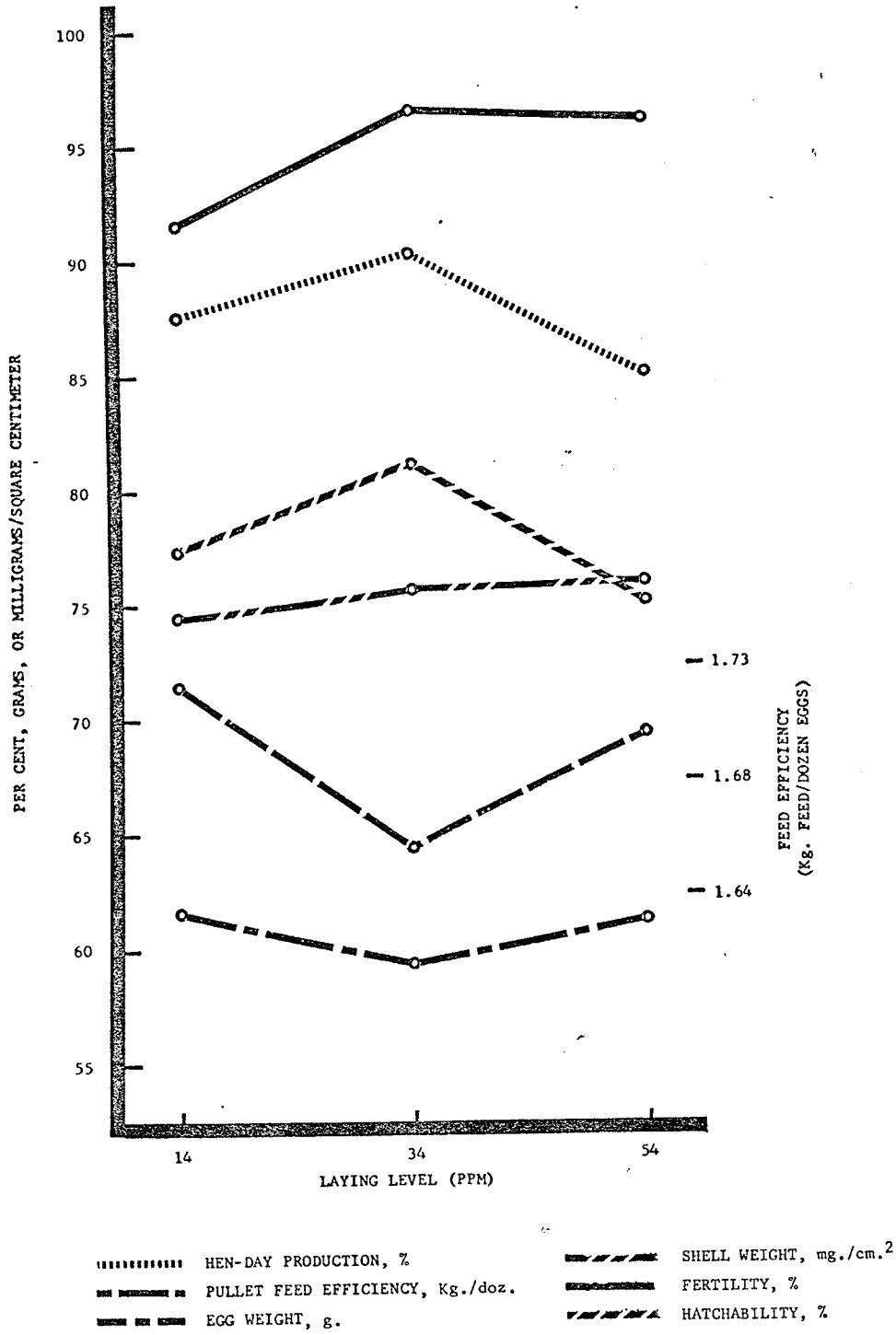


TABLE 17. INITIAL CHICK WEIGHT (Grams)

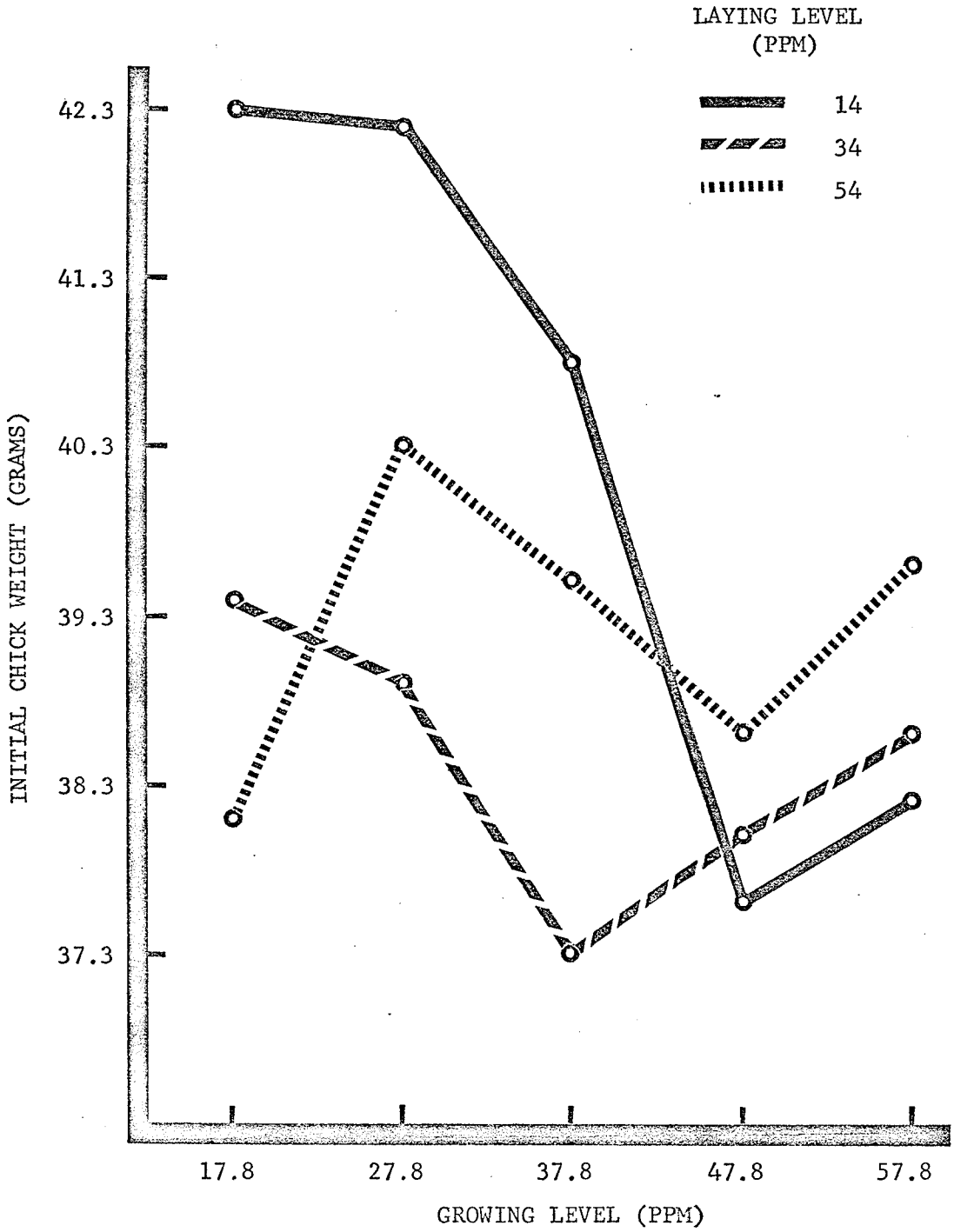
LAYING LEVEL (PPM)	GROWING LEVEL (PPM)	PERIODS 1 REPLICATES 1	1 REPLICATES 2	2 REPLICATES 3	2 REPLICATES 4	LAYING LEVEL MEANS *	GROWING LEVEL MEANS *	LAYING x GROWING MEANS *
14	17.8	42.0	41.6	41.3	44.4	40.2^{a}	40.0^{ab}	42.3^{a}
	27.8	40.6	43.1	43.1	41.8		40.4^{a}	42.2^{ab}
	37.8	40.2	38.0	41.7	43.4		39.2^{abc}	40.8^{abc}
	47.8	36.5	37.9	38.6	37.4		38.1^{c}	37.6^{e}
	57.8	37.4	38.2	39.3	38.1		38.8^{bc}	38.2^{de}
34	17.8	38.9	38.6	40.9	39.4	38.4^{b}		39.4^{cde}
	27.8	38.9	39.0	38.9	38.8			38.9^{cde}
	37.8	36.7	36.8	37.5	38.1			37.3^{e}
	47.8	37.3	35.2	41.4	38.0			38.0^{de}
	57.8	39.0	38.1	38.5	38.6			38.6^{cde}
54	17.8	38.4	36.8	37.3	39.8	39.2^{b}		38.1^{de}
	27.8	38.9	40.5	40.5	41.3			40.3^{abcd}
	37.8	37.9	39.9	39.4	40.9			39.5^{cde}
	47.8	37.6	37.2	39.4	40.3			38.6^{cde}
	57.8	37.9	39.1	40.0	41.5			39.6^{cde}
PERIOD MEANS **		38.6^{b}		40.0^{a}				

* Means in the same column having different superscript letters differ significantly ($P < 0.01$)
** Means having different superscript letters differ significantly ($P < 0.01$)

The differences between period means were highly significant ($P<0.01$) with the greater initial chick weight occurring late in the experimental period. This would normally be expected since it has been shown (TABLE 13) that egg weight increased significantly as the experimental period progressed. Differences between growing level means were also highly significant ($P<0.01$). Lower levels of manganese in the diet of the growing pullet apparently had the effect of producing chicks with the highest initial weight during the later laying cycle of the pullet fed such a diet. Here, however, the parallelism between egg weight and initial chick weight does not appear to hold since neither egg weight nor shell weight were significantly affected by the manganese concentration in the growing diet.

The differences between means of the interactions of laying levels and growing levels, illustrated graphically in FIGURE 6, were highly significant ($P<0.01$). In this experiment, chicks hatched from eggs laid by pullets grown on a diet containing 17.8 p.p.m. manganese and later fed a diet containing 14 p.p.m. manganese during the laying cycle were found to be heavier than those from other dietary treatments. It was also clearly evident that, in general, the more manganese the growing diet contained the smaller was the initial chick weight, regardless of how much manganese was found in the laying diet. This trend was evidently reversed at a growing diet manganese concentration equal to or greater than 37.8 p.p.m. since chick weight thereafter apparently increased. Such trends will be seen to roughly parallel egg weight as illustrated in FIGURE 3. These findings might suggest a toxicity of certain levels of manganese in the growing diet of the pullet with regards to the later production of large eggs and chicks. However, the validity of this observation would require further study. —

FIGURE 6. INTERACTION OF LAYING LEVELS AND GROWING LEVELS AS THEY AFFECT INITIAL CHICK WEIGHT



Chick Weight Gain

Data pertaining to weight gain of chicks over a 28-day experimental period are given in TABLE 18. These data, again, represent the mean value of each dietary treatment. An examination of the analysis of variance of chick weight gain (TABLE A.17) disclosed that the level of manganese in the laying diet had no significant effect on chick weight gain. However, the level of manganese in the growing diet of the pullets did have a highly significant effect ($P<0.01$) on later weight gains of chicks hatched from their eggs. The greatest 28-day mean weight gain, 286.1 grams, occurred when the pullets had been grown on a diet containing 27.8 p.p.m. of manganese. Higher or lower concentrations of manganese in the growing diet of the pullet resulted in significantly decreased 28-day weight gains. It will be noted in TABLE 17 that pullets fed this particular growing diet also produced chicks with the highest mean initial, or hatching, weight of any growing diet. Thus, in this experiment, a dietary treatment not only produced the largest chick at hatching but also produced the most vigorous chick as evidenced by its superior rate of growth during the first 28 days of life.

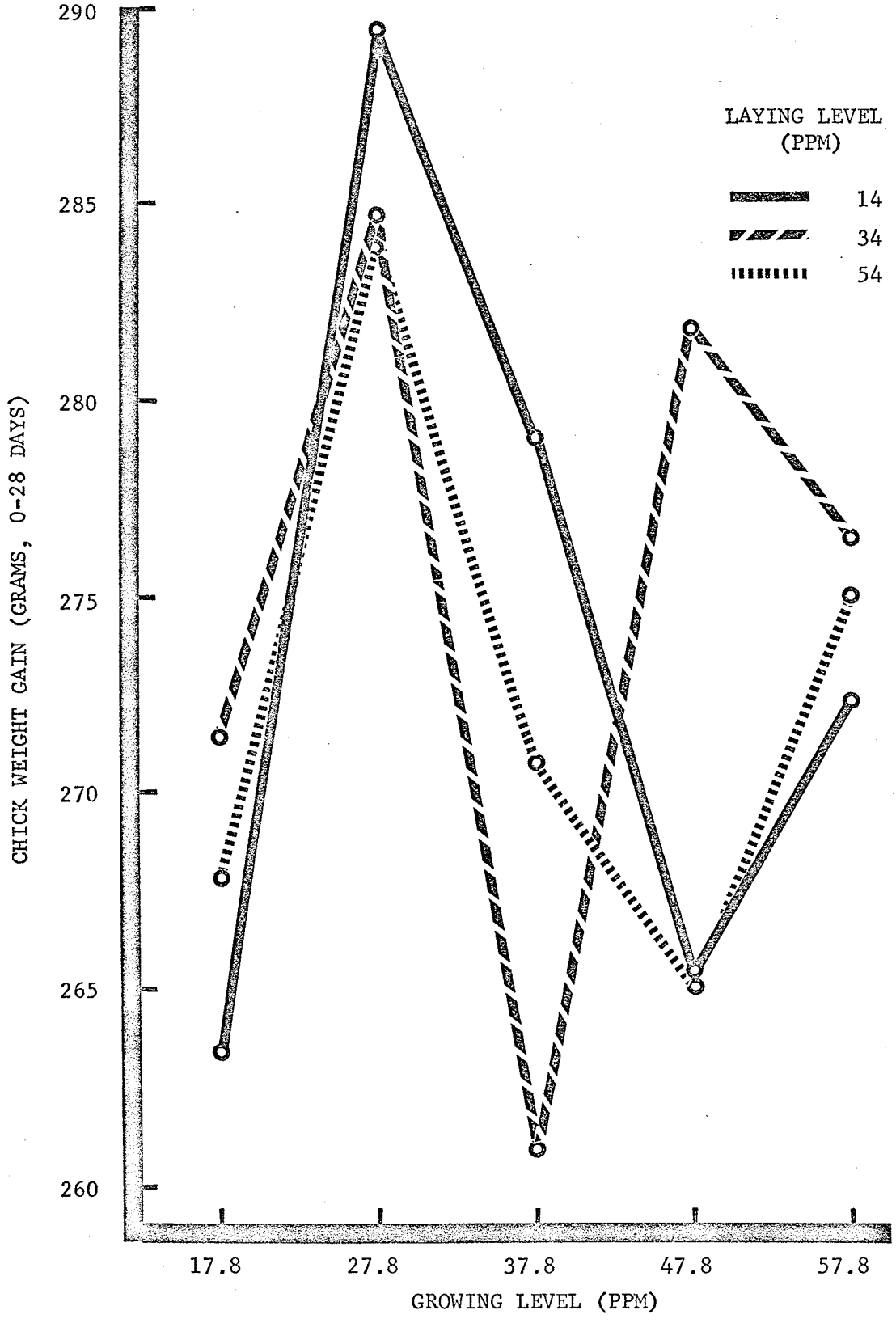
The interactions between laying diets and growing diets did not produce means which were significantly different. FIGURE 7, however, shows the apparent superiority of the pullet growing diet containing 27.8 p.p.m. manganese to produce better chick weight gains regardless of the level of manganese in the laying diet. Apparently, the interaction of diets was such that the better weight gains occurred with lower levels of manganese, 14 p.p.m., in the laying diet. FIGURE 7 also shows the near parallel results obtained on the three laying levels and their interactions with the growing levels shown.

TABLE 18. CHICK WEIGHT GAIN
(Grams, 0-28 days)

LAYING LEVEL (PPM)	GROWING LEVEL (PPM)	PERIODS 1 REPLICATES 1	1 REPLICATES 2	2 REPLICATES 3	2 REPLICATES 4	LAYING LEVEL MEANS	GROWING LEVEL MEANS *	LAYING x GROWING MEANS
	17.8	242.6	247.4	281.0	282.8			263.4
	27.8	286.5	267.3	296.6	307.6			289.5
14	37.8	275.9	261.5 +	287.3	291.1			279.0
	47.8	256.2 +	258.0	272.9	274.4			265.4
	57.8	262.5	248.0	302.1	276.5	273.9		272.3
	17.8	271.4	265.5	261.1	287.6		267.6^{b}	271.4
	27.8	271.5	287.9	291.5	288.0		286.1^{a}	284.7
34	37.8	247.2	242.1	263.1	291.2		270.2^{b}	260.9
	47.8	273.3	268.4	281.4	304.2		270.7^{b}	281.8
	57.8	261.0	262.7	281.9	300.0	275.0	274.5^{ab}	276.4
	17.8	256.2	275.3	266.3	273.6			267.8
	27.8	289.3	276.3	281.4	289.2			284.0
54	37.8	263.0	243.0	284.4	292.3			270.7
	47.8	258.6	258.9	283.3	259.0			265.0
	57.8	264.4	248.1	286.2	301.1	272.5		275.0
PERIOD MEANS **			263.0^{b}		284.6^{a}			

* Means in the same column having different superscript letters differ significantly ($P < 0.01$)
** Means having different superscript letters differ significantly ($P < 0.01$)
\+ Calculated missing plot

FIGURE 7. INTERACTION OF LAYING LEVELS AND GROWING LEVELS AS THEY AFFECT CHICK WEIGHT GAIN



Chick Feed Efficiency

Chick feed efficiency, TABLE 19, was found to be little affected by the maternal diet. The analysis of variance (TABLE A.18) shows that neither the laying diet nor the growing diet of the pullet significantly affected chick feed efficiency. Therefore, those chicks found to have gained at a greater rate (TABLE 18) also consumed more feed and hence did not utilize feed more efficiently. The interactions of laying levels and growing levels, while significant ($P<0.05$), did not follow any definite trends (FIGURE 8) which might be considered meaningful. The data were quite variable and fluctuated widely, both within a diet and between diets. The period during which the egg was laid had no significant effect on chick feed efficiency.

FIGURE 9 graphically presents the effects of the level of manganese in the growing diet of the pullet on the performance of chicks hatched from their eggs. The effects of 27.8 p.p.m. manganese in the growing diet of the pullet on the initial chick weight and on chick weight gain are quite vividly illustrated therein. The chicks with the greatest initial weight had the largest weight gains. It is also quite evident from the graph that there was no relationship between chick weight gain and chick feed efficiency when considered from the point of view of the level of manganese in the growing diet of the pullet.

The effect of the level of manganese in the laying diet of the pullet upon the chick parameters is shown graphically in FIGURE 10. Those chicks from eggs laid by pullets fed the diets containing the highest concentrations of manganese, 34 and 54 p.p.m., had significantly lower initial weights than the chicks from pullets fed the 14 p.p.m. manganese diet. Similar differences, however, did not occur subse-

TABLE 19. CHICK FEED EFFICIENCY
(Grams feed per gram gain)

LAYING LEVEL (PPM)	GROWING LEVEL (PPM)	PERIODS 1 REPLICATES 1	1 REPLICATES 2	2 REPLICATES 3	2 REPLICATES 4	LAYING LEVEL MEANS	GROWING LEVEL MEANS	LAYING x GROWING MEANS *
14	17.8	2.5	2.4	2.4	2.4	2.4		2.4^{abc}
	27.8	2.6	2.4	2.3	2.4			2.4^{abc}
	37.8	2.4	2.5 +	2.4	2.7			2.5^{abc}
	47.8	2.4 +	2.4	2.4	2.4			2.4^{abc}
	57.8	2.5	2.4	2.4	2.4			2.4^{abc}
34	17.8	2.4	2.3	2.4	2.3	2.4	2.4	2.4^{c}
	27.8	2.4	2.7	2.6	2.5		2.4	2.5^{ab}
	37.8	2.4	2.3	2.4	2.6		2.4	2.4^{abc}
	47.8	2.8	2.5	2.5	2.4		2.5	2.5^{ab}
	57.8	2.4	2.3	2.4	2.3		2.4	2.4^{c}
54	17.8	2.6	2.3	2.5	2.8	2.4		2.6^{a}
	27.8	2.4	2.3	2.4	2.4			2.4^{bc}
	37.8	2.6	2.3	2.1	2.3			2.3^{c}
	47.8	2.5	2.5	2.4	2.6			2.5^{abc}
	57.8	2.4	2.4	2.3	2.5			2.4^{abc}
PERIOD MEANS		2.4		2.4				

* Means in the same column having different superscript letters differ significantly ($P < 0.05$)
\+ Calculated missing plot

FIGURE 8. INTERACTION OF LAYING LEVELS AND GROWING LEVELS AS THEY AFFECT CHICK FEED EFFICIENCY

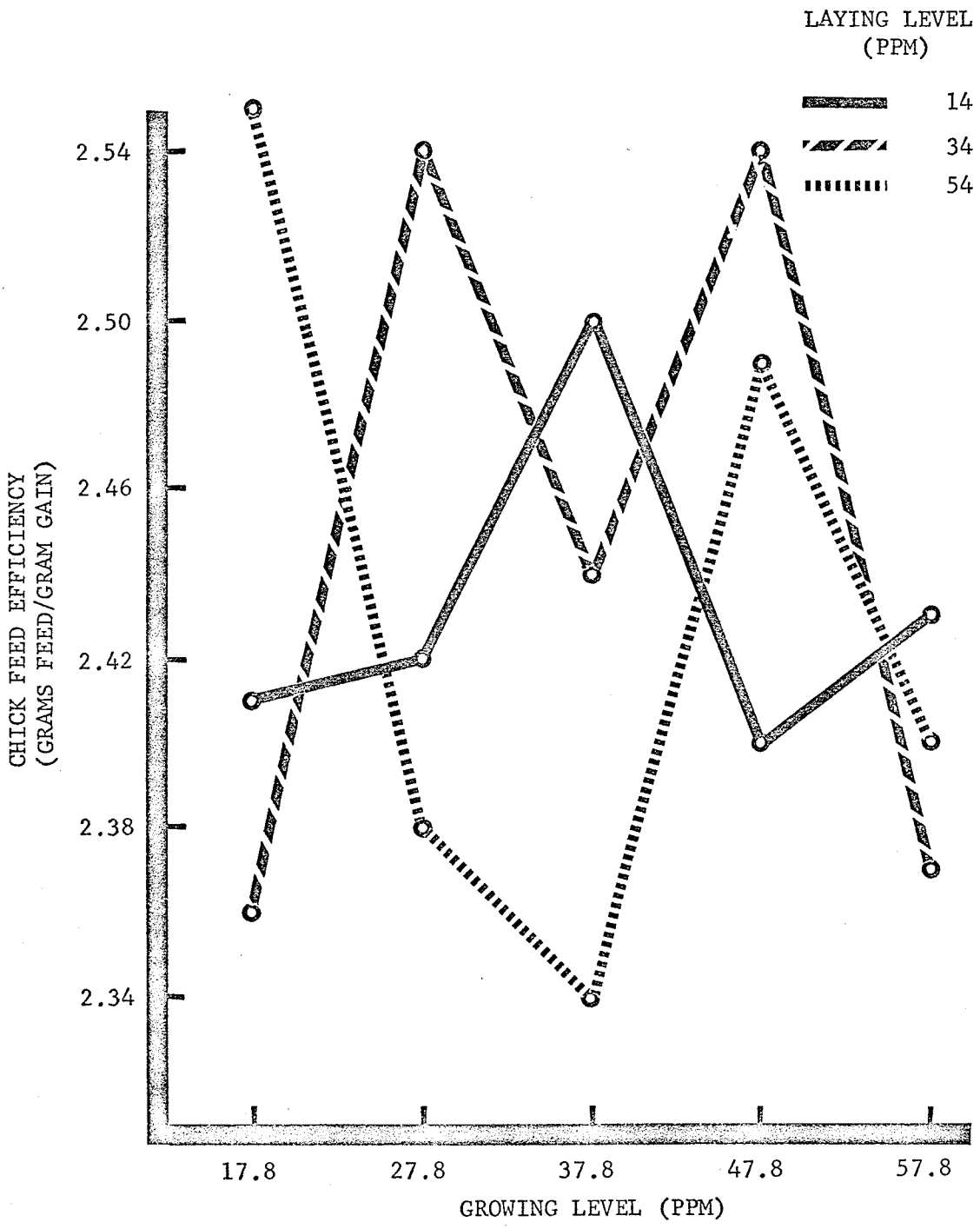


FIGURE 9. EFFECT OF GROWING LEVELS ON CHICK PARAMETERS

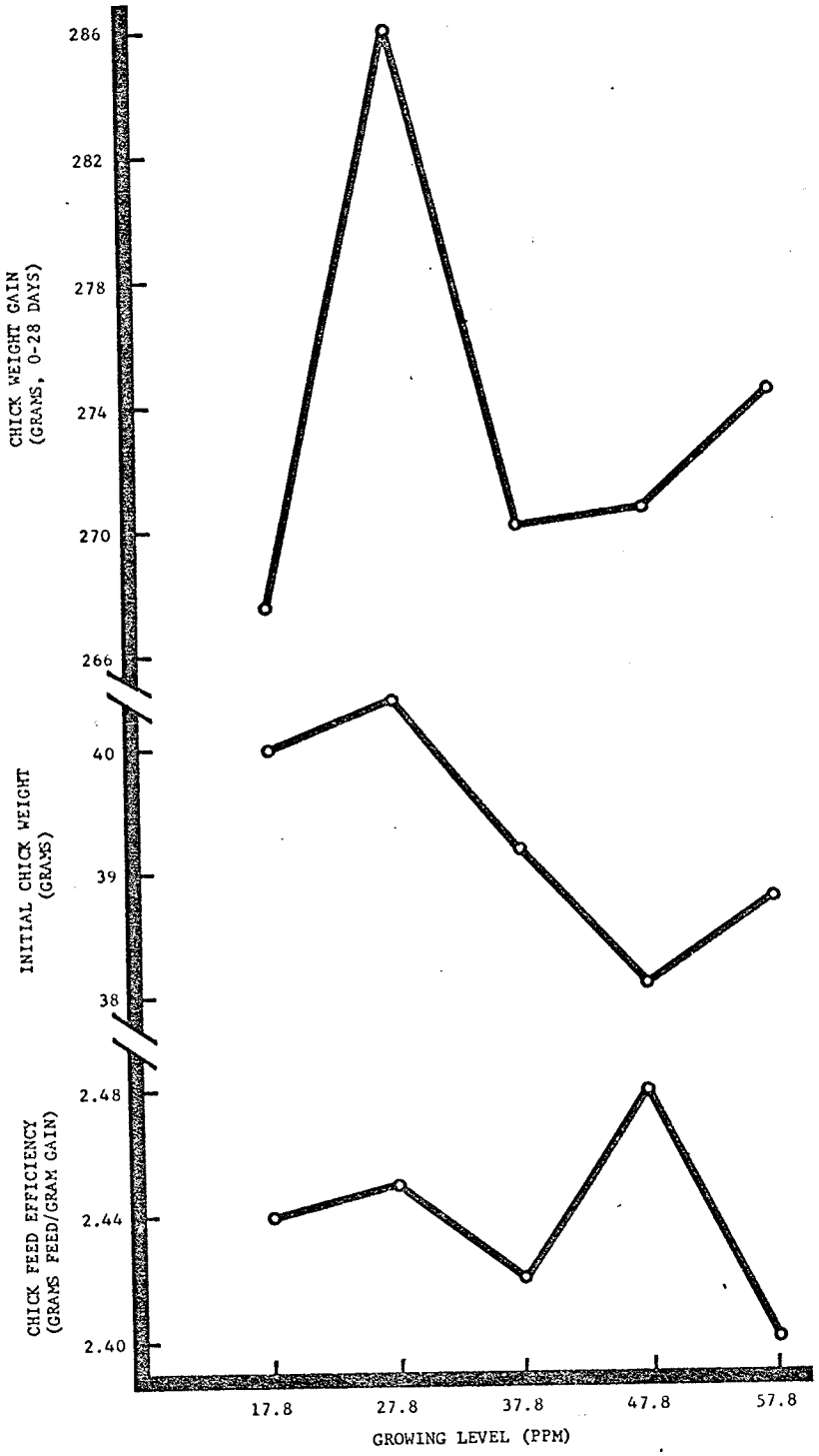
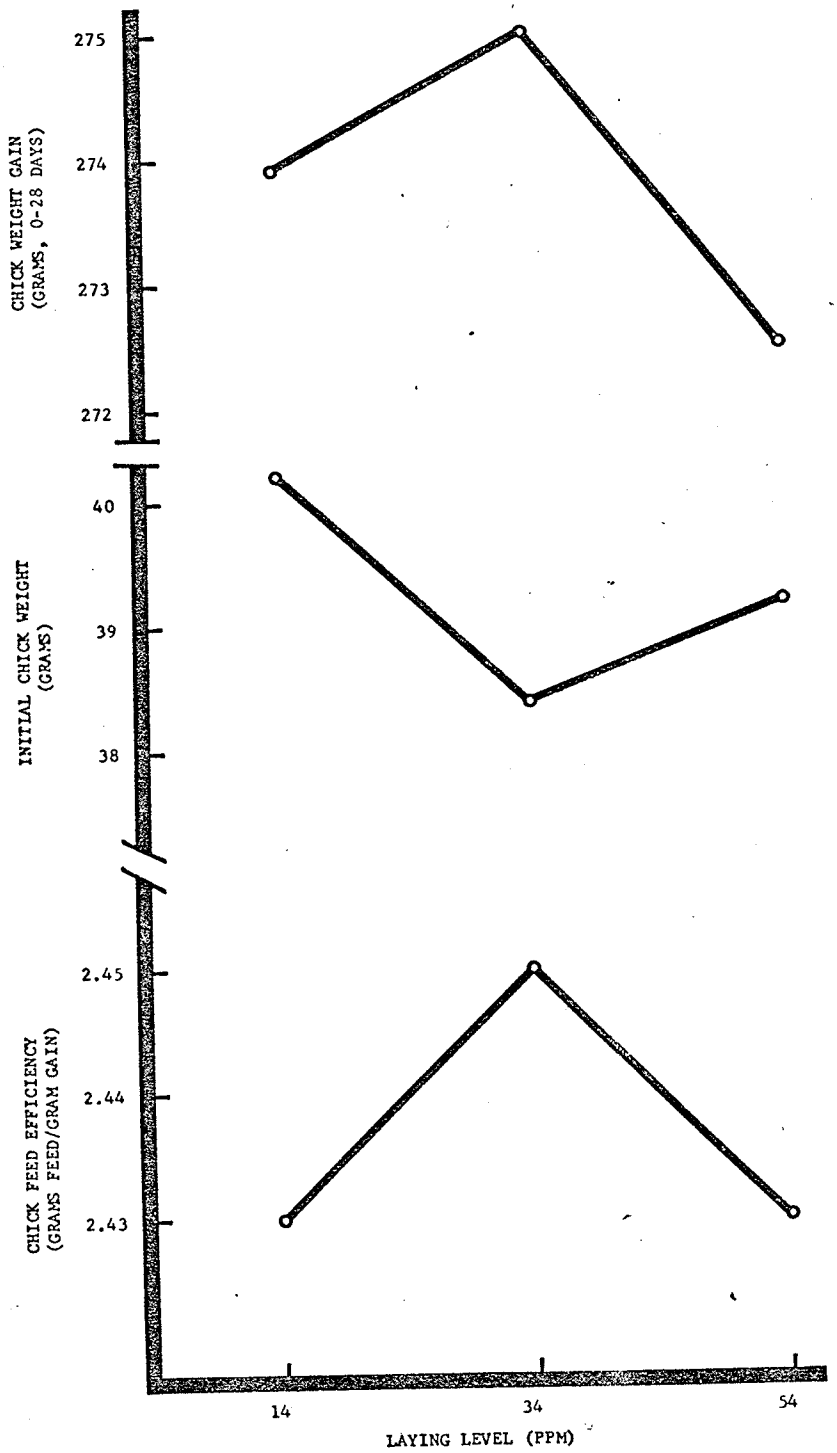


FIGURE 10. EFFECT OF LAYING LEVELS ON CHICK PARAMETERS



quently in weight gain or feed efficiency. It will be noted that, in this test, the chicks with the highest initial weight did not gain at the greatest rate as they did when the level of manganese in the pullet growing diet was considered. There are, therefore, different carry-over effects on the chick from the maternal diet depending upon which maternal diet is being considered, growing or laying.

SUMMARY AND CONCLUSIONS

Four hundred single comb White Leghorn pullets, eight and one-half weeks old, were used in a study to determine the effects of the concentration of manganese in the growing diet on pullet performance to twenty and one-half weeks of age. Seventy-five of these pullets were later used to study manganese nutrition during the reproductive cycle as influenced by the dietary regime of the growing period. Considering the range of manganese concentrations studied, the results of these experiments warrant the following statements:

1. The concentration of manganese in the growing ration had no apparent effect on the growth and development of pullets from eight and one-half weeks to twenty and one-half weeks of age.
2. During the twenty-week reproductive period studied, with birds from 36 to 56 weeks of age, the concentration of manganese in the various growing and laying rations did not have a significant effect on feed efficiency, shell weight per unit area, hatchability of fertile eggs or the feed efficiency of chicks hatched from these eggs.
3. The greatest hen-day production was achieved when the growing diet contained 47.8 p.p.m. manganese and the laying diet 34 p.p.m. The differences in egg production means were highly significant ($P<0.01$).
4. Pullets fed diets containing 37.8 p.p.m. manganese during the

growing period and either 14 or 54 p.p.m. during the laying period produced eggs of significantly greater weight ($P<0.01$) than those produced by pullets fed other dietary treatments.

5. Fertility of eggs was significantly ($P<0.01$) affected by the concentration of dietary manganese with lower concentrations in the growing ration and higher concentrations in the laying ration producing eggs of higher fertility. A manganese level of 27.8 p.p.m. in the growing diet combined with a level of 34 p.p.m. in the laying diet gave a mean fertility of 99.76 percent.
6. Highly significant differences ($P<0.01$) were found in initial chick weight according to the dietary treatment of the parent pullet. Pullets consuming diets containing either 17.8 or 27.8 p.p.m. manganese in the growing period and 14 p.p.m. during the laying cycle produced chicks having the highest initial weight.
7. Chick weight gain was significantly ($P<0.01$) affected by the dietary regime to which the parent pullets were subjected during the growing period while the concentration of manganese in the laying diet had little effect on later weight gains of the chicks. The highest weight gains occurred in chicks from pullets grown on a diet containing 27.8 p.p.m. of manganese.

In conclusion, dietary manganese has been shown in these experiments to affect certain parameters in poultry production while not affecting others. The results of this study both confirms the results of certain past research and contradicts that of other studies.

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APPENDIX

TABLE A.1--ANALYSIS OF VARIANCE OF MEAN WEIGHT GAIN

Source	DF	SS	MS	F
Replicates	1	3,063	3,063	0.75
Treatments	4	1,322	330	0.08
Error	4	16,414	4,104	
Total	9	20,799		

TABLE A.2--ANALYSIS OF VARIANCE OF FEED EFFICIENCY

Source	DF	SS	MS	F
Replicates	1	.02	.02	0.40
Treatments	4	.53	.13	2.60
Error	4	.19	.05	
Total	9	.74		

TABLE A.3--ANALYSIS OF VARIANCE OF MORTALITY

Source	DF	SS	MS	F
Replicates	1	3.6	3.6	2.0
Treatments	4	5.0	1.2	0.7
Error	4	7.4	1.8	
Total	9	16.0		

TABLE A.4--ANALYSIS OF VARIANCE OF MEAN TIBIAL LENGTH

Source	DF	SS	MS	F
Replicates	1	.14	.14	3.50
Treatments	4	.02	.01	0.25
Error	4	.15	.04	
Total	9	.31		

TABLE A.5--ANALYSIS OF VARIANCE OF MEAN FEMORAL LENGTH

Source	DF	SS	MS	F
Replicates	1	.02	.02	1.00
Treatments	4	.03	.01	0.50
Error	4	.09	.02	
Total	9	.14		

TABLE A.6--ANALYSIS OF VARIANCE OF PERCENT FEMORAL ASH

Source	DF	SS	MS	F
Replicates	1	3.09	3.09	6.31
Treatments	4	9.72	2.43	4.96
Error	4	1.95	.49	
Total	9	14.76		

TABLE A.7--ANALYSIS OF VARIANCE OF FIRST EGG WEIGHT

Source	DF	SS	MS	F
Replicates	1	13.2	13.2	2.9
Treatments	4	20.9	5.2	1.1
Error	4	18.5	4.6	
Total	9	52.6		

TABLE A.8--ANALYSIS OF VARIANCE OF PERCENT SHELL ASH

Source	DF	SS	MS	F
Treatments	4	1.5	0.4	0.5
Reps./Treat.	5	4.1	0.8	1.6
Determinations	10	5.1	0.5	
Total	19	10.7		

TABLE A.9--ANALYSIS OF VARIANCE OF AGE AT 40 PERCENT LAY

Source	DF	SS	MS	F
Treatment	4	8.6	2.1	1.2
Error	4	7.0	1.8	
Total	8	15.6		

TABLE A.10--ANALYSIS OF VARIANCE OF HEN-DAY PRODUCTION

Source	DF	SS	MS	F
Laying Levels (L)	2	960.45	480.22	5.27 **
Growing Levels (G)	4	1776.43	444.11	4.87 **
L X G	8	1501.49	187.69	2.06 *
Periods (P)	4	3343.52	835.88	9.17 **
L X P	8	199.48	24.94	0.27
G X P	16	354.92	22.18	0.24
L X G X P	32	854.86	26.71	0.29
Hens/L X G X P	210	19137.60	91.13	
Total	284[1]	28128.75		

TABLE A.11--ANALYSIS OF VARIANCE OF PULLET FEED EFFICIENCY

Source	DF	SS	MS	F
Laying Levels (L)	2	0.04	0.02	2.
Growing Levels (G)	4	0.04	0.01	1.
L X G	8	0.13	0.02	2.
Periods (P)	1	1.30	1.30	130. **
L X P	2	0.01	0	0
G X P	4	0.05	0.01	1.
L X G X P	8	0.05	0.01	1.
Replicates	28	0.17	0.01	
Total	57[2]	1.79		

* Indicates significance at the 5% level
** Indicates significance at the 1% level
1. 285 observations-1=284 DF
2. 60 observations-2 calculated missing plots-1=57 DF

TABLE A.12--ANALYSIS OF VARIANCE OF EGG WEIGHT

Source	DF	SS	MS	F
Laying Levels (L)	2	221.37	110.69	7.27 **
Growing Levels (G)	4	118.86	29.71	1.95
L X G	8	611.56	76.45	5.02 **
Periods (P)	4	437.54	118.39	7.77 **
L X P	8	32.68	4.09	0.27
G X P	16	73.12	4.57	0.30
L X G X P	32	56.62	1.77	0.12
Hens/L X G X P	163	2482.43	15.23	
Total	237[1]	4070.18		

TABLE A.13--ANALYSIS OF VARIANCE OF SHELL WEIGHT

Source	DF	SS	MS	F
Laying Levels (L)	2	107.8	53.9	1.7
Growing Levels (G)	4	95.4	23.8	0.8
L X G	8	633.3	79.2	2.5
Periods (P)	4	249.9	62.5	2.0
L X P	8	119.8	15.0	0.5
G X P	16	270.8	16.9	0.5
L X G X P	32	235.5	7.4	0.2
Hens/L X G X P	163	5122.7	31.4	
Total	237[1]	6835.2		

**Indicates significance at the 1% level
1. 240 observations-2 calculated missing plots-1=237 DF

TABLE A.14--ANALYSIS OF VARIANCE OF FERTILITY

Source	DF	SS	MS	F
Laying Levels (L)	2	594.64	297.32	3.78 *
Growing Levels (G)	4	1459.83	364.96	4.64 **
L X G	8	1589.97	198.75	2.53 *
Replicates	60	4716.78	78.61	
Total	74[1]	8361.22		

TABLE A.15--ANALYSIS OF VARIANCE OF HATCHABILITY

Source	DF	SS	MS	F
Laying Levels (L)	2	216.54	108.27	0.84
Growning Levels (G)	4	583.86	145.96	1.13
L X G	8	1096.34	137.04	1.06
Replicates	60	7758.99	129.32	
Total	74[1]	9655.73		

TABLE A.16--ANALYSIS OF VARIANCE OF INITIAL CHICK WEIGHT

Source	DF	SS	MS	F
Laying Levels (L)	2	32.5	16.2	13.5 **
Growing Levels (G)	4	42.2	10.6	8.8 **
L X G	8	58.5	7.3	6.1 **
Time Periods (T)	1	28.5	28.5	23.8 **
L X T	2	0.6	0.3	0.2
G X T	4	5.5	1.4	1.2
L X G X T	8	10.1	1.3	1.1
Replicates	30	36.7	1.2	
Total	59[2]	214.6		

* Indicates significance at the 5% level
** Indicates significance at the 1% level
1. 75 observations-1=74 DF
2. 60 observations-1=59 DF

TABLE A.17--ANALYSIS OF VARIANCE OF CHICK WEIGHT GAIN

Source	DF	SS	MS	F
Laying Levels (L)	2	65.5	32.8	0.3
Growing Levels (G)	4	2557.6	639.4	5.5 **
L X G	8	1560.9	195.1	1.7
Time Periods (T)	1	7022.2	7022.2	60.4 **
L X T	2	193.6	96.8	0.8
G X T	4	1024.8	256.2	2.2
L X G X T	8	956.3	119.5	1.0
Replicates	28	3252.8	116.2	
Total	57[1]	16633.7		

TABLE A.18--ANALYSIS OF VARIANCE OF CHICK FEED EFFICIENCY

Source	DF	SS	MS	F
Laying Levels (L)	2	0	0	0
Growing Levels (G)	4	0.04	0.01	1.0
L X G	8	0.22	0.03	3.0 *
Time Periods (T)	1	0	0	0
L X T	2	0	0	0
G X T	4	0.02	0.01	1.0
L X G X T	8	0.18	0.02	2.0
Replicates	28	0.39	0.01	
Total	57[1]	0.85		

* Indicates significance at the 5% level
** Indicates significance at the 1% level
1. 60 observations-2 calculated missing plots-1=57 DF