# DEVELOPMENT OF SECONDARY BONE IN THE DOMESTIC LAYING HEN

by

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A Dissertation Submitted to the
Graduate Faculty in Partial Fulfillment of
The Requirements for the Degree of
DOCTOR OF PHILOSOPHY

Major Subject: Poultry Nutrition

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1969

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# INTRODUCTION

At the onset of oviparous reproductive activity marked changes occur in calcium metabolism by the female. One of these changes was first observed in the pigeon in which the level of blood calcium doubled when the non-reproductive female entered her reproductive cycle. A subsequent change observed was the ossification of the femur bone marrow cavity which was observed to be cyclic and coincident with the development of the ovarian follicle. These changes are now accepted as necessary concomitants for successful oviparous reproduction.

In the domestic laying hen, a part of the calcium present in every normal egg has been derived from the skeleton of the hen. Radio-tracer estimates reported range from 25% to 65% of the total calcium in the egg. The hen must withdraw calcium from her body stores to produce a well-calcified eggshell because she is unable to obtain calcium from her diet at the rate it is secreted during eggshell formation. The maximum 24 hour calcium retention recorded in the literature is 1.83 grams which equals an average of 76 milligrams per hour. An average eggshell contains 2.00 grams of calcium, which is secreted in 16 hours at an average rate of 125 milligrams per hour. The difference between the amount of calcium obtained from the diet during the formation of the eggshell and the amount secreted in the eggshell must be derived from her body stores.

It has been estimated that 5% of the eggs produced commercially in the United States are lost due to cracking and/or breakage as a result of poor shells. This means that approximately 98 million dollars are lost

through egg breakage by the commercial-egg producers. Also, a substantial amount of high-quality food is not available to the consumer. Any reduction in the above percentage of broken eggs through changes in pullet and/or laying-hen diet management could conceivably result in a considerable reduction in food lost and increase the monetary returns to the commercial-egg producer. Poor eggshells are not due only to faulty nutrition, but may be because of genetics, disease, environment, or physiology of the hen.

Considerable time, energy and money has been spent on research attempting to establish the dietary requirements of the domestic hen for maximum eggshell strength while maximizing egg production. No reports have been found of experiments studying what factor(s) affect the development and maintenance of the medullary bone, which is necessary for successful oviparous reproduction.

The following experiments were conducted in an attempt to delineate what factors and to what extent these factors affect the development and maintenance of medullary bone. Also, an experiment was conducted to study which bone sample(s) might serve as the best indicator of skeletal mineralization.

## REVIEW OF LITERATURE

At the onset of reproductive activity marked changes occur in the calcium metabolism of the female bird. These changes were first observed in the blood of pigeons by Riddle and Reinhart (1926), who observed a rise in the level of blood calcium from 9.3 milligrams/100 milliliters in the non-reproductive female to a value greater than 20 milligrams/100 milliliters at the time of ovulation.

Common (1932) conducted balance experiments on pullets entering their production cycles. He observed that approximately ten days prior to the onset of production there was a large increase in the quantities of calcium and phosphorus retained from the diet. These changes were not due to a change in food intake, but rather to a decrease in the excretion of calcium.

Kyes and Potter (1934) independently discovered a 'physiological marrow ossification' in pigeons which in its most extreme form completely filled the cavity of the femur bone. They pointed out that the deposition of this extra mineral in the long bones of the leg was, first, a cyclical event; secondly, restricted to the female bird; and thirdly, coincident with the maturation of the ovarian follicle.

This phenomenon was referred to as medullary bone formation since it occurred in the medullary cavities of the bones, and it soon became apparent that it provided the structural basis for the extra store of minerals which had been observed during balance experiments.

## Development of Secondary Bone

The deposition of this medullary bone starts about two weeks prior to the onset of production which corresponds with the time when the bird starts to secrete increased amounts of sex hormones and to retain extra calcium and phosphorus (Simkiss, 1961).

Factors affecting the calcium and/or phosphorus requirements of laying-strain pullets from 18 weeks until the onset of production for "normal" development and maximum productive performance during the egg laying cycle have received very little attention.

Studies with rats (Hansard and Plumlee, 1954), (Henry and Kon, 1953), dogs, (Gershoff et al., 1958), and observations on man (Hegsted et al., 1952), have shown efficient metabolic adaptation to low-calcium diets over a long lifetime when these diets are consumed early in life. Henry and Kon (1953) observed that rats maintained on low-calcium diets remained in calcium equilibrium over a long feeding period while animals fed high dietary calcium showed a decrease in retention and a loss of calcium soon after growth was completed.

Berg et al. (1947) fed dietary calcium levels of 0.68%, 0.90% and 3.02% to pullets for approximately 53 days prior to the onset of production and reported no differential effects on shell quality or production during the first three months of lay.

Young et al. (1962) conducted two experiments to determine if the efficiency of calcium metabolism in the laying hen could be influenced by the amount of calcium fed during the pre-laying period. They reported that calcium levels fed during rearing (0.2% to 1.4%) had no effect on

sexual maturity, rate of production or shell characteristics. A higher mortality was observed early in the production cycle, however, with pullets reared on the high calcium diet. Young et al. (1964) again reported that pullets fed a 3% calcium diet from 8 weeks showed a higher mortality early after the onset of production and that feeding a 5% calcium laying diet had an additive effect on mortality. A significantly higher incidence of nephritis was observed where the pullets were fed a 3% calcium-0.38% phosphorus diet during the growing period. In many cases there was an atrophy of one kidney and the accumulation of urates in the tubules. Also, this incidence of nephritis was higher when a 5% calcium laying diet was fed to pullets reared with a 3% calcium diet.

## Utilization of Secondary Bone

Production of the first few eggs is usually accompanied by a negative calcium balance, (Morgan and Mitchell, 1938), regardless of the dietary calcium level (Hurwitz and Griminger, 1960).

Taylor and Morris (1964) studied the effects of early and late maturity on the skeletons of pullets. At the onset of production the two groups had similar skeletal weights and all bones were well calcified, indicating satisfactory pre-laying storage of calcium in both cases. The skeletons of the precocious birds (average age at first egg was 140 days), after laying an average of 42.2 eggs per bird, were 26% lighter than the samples taken at the onset of production and one bird had collapsed with "cage layer fatigue". The skeletons of the retarded birds (average age at first egg was 176 days), after laying an average of 45 eggs per bird, also declined in weight after the onset of production but in these pullets

the decreased skeletal weight was only 6%. The data presented show that the early maturing pullets failed to balance calcium input and output and consequently suffered very heavy losses of bone calcium. This demonstrates the importance of age at onset of production on skeletal development.

Anderson (1966) reported a highly significant ($P < .005$) negative regression of eggs per bird on eggshell thickness. He concluded that this emphasized the influence of altered egg production potential on the utilization of dietary calcium and/or phosphorus for the maintenance of maximum shell thickness and skeletal mineralization.

In the femurs of young pullets the ends of the bones were found to be the principal site of storage of available calcium which may be utilized during the early period of egg production (Hurwitz, 1965). The ends were found to lose calcium during the production of the first five eggs but the calcium content of the cortical and medullary segments did not change significantly.

Hurwitz and Bar (1966) reported that when one-year-old S. C. W. Leghorn hens were fed a calcium-deficient diet there was a progressive decrease in blood and eggshell calcium. Also, both ends and medullary segments of the femur were markedly depleted of calcium during restriction, while the cortical segment was hardly affected. Following a 5-day depletion with 1.7%-calcium diets, eggshell calcium returned to normal after 6-8 days on a high-calcium repletion regime. After 3 weeks on the repletion regime, the femurs completely recovered their calcium.

A number of estimates have been made of the amount of calcium in the eggshell which has been derived from the skeleton compared with that

obtained directly from the diet. Driggers and Comar (1949) fed a standard laying mash diet, with oystershell and grit free choice, to 18-month-old S. C. W. Leghorn hens and reported, based on $Ca^{45}$ determinations, that 25 to 40% of the calcium in the egg was derived from the skeleton. Jowsey et al. (1956) fed two laying hens $Ca^{45}$ for 26 days and, based on the eggs laid during this time, reported that a hen depositing calcium on the shell of an egg will obtain about 35% of the calcium for the shell from the food in the gut. Hence, 65% of the calcium in the shell must have been derived from the skeleton.

The maximum absolute calcium retention reported in the literature for a laying hen is 1.83 grams for a 24-hour period (Hurwitz and Griminger, 1961). If an egg contains an average of 2 grams of calcium, the difference between calcium "retained" and that in the egg must be derived from body stores. Also, it may be calculated that, during the period (16 hours) of calcification of a shell containing 2 grams of calcium, calcium must be withdrawn from the blood at a rate of 125 milligrams per hour. A daily retention of 1.83 grams would provide calcium at a mean rate of only 76 milligrams per hour. This again indicates a deficit between calcium obtained from the diet and that secreted during eggshell formation which must be covered by withdrawal from body stores.

Direct evidence that skeletal reserves of calcium are mobilized for shell formation has been provided by an experiment in which autoradiographs were prepared of tangential sections of the shells of egg laid by hens fed $Ca^{45}$ continuously for a week (Tyler, 1954). In the first few days after introducing the isotope into the diet, concentric

dark rings on the films represented areas of shell which had been calcified by radiocalcium from the food, and light rings, areas calcified by skeletal calcium. The transition from dark to light rings was gradual, the intermediate zones representing areas of shell calcified with calcium from food and skeletal stores.

## Cage Layer Fatigue

The cause and sequence of events that result in the syndrome called cage layer fatigue have not been elucidated. It is accepted, however, that leg weakness is an outstanding symptom of the disease. It is also recognized that deficient dietary calcium and/or phosphorus levels can affect bone structure and serum calcium levels. Martin (1937) fed calcium-deficient diets to dogs and observed widespread hemorrhage, prolongation of coagulation time, inflammation of the gastrointestinal tract and osteoporosis. Boelter and Greenberg (1941a, b, 1943) fed calcium-deficient diets to rats and observed growth retardation in 4-5 weeks. In 7-10 weeks they observed a general decrease in reactivity and sensitivity coincident with a fall of serum calcium level to about 5 milligrams per 100 milliliters. Paralysis of the hind legs was noted and when deficient animals were stimulated by galvanic shocks they collapsed. Sixty percent of their rats died by 23 weeks and at autopsy widespread hemorrhages were found in the tissues, extravasation of blood was prominent in the nervous system especially in those animals which exhibited paralysis before death. Also, hemorrhage and paralysis were common observations in the young born of calcium-deficient females.

Harms et al. (1961) reported high mortality in caged hens fed diets containing 0.34% total phosphorus. These birds developed leg weakness several days prior to death, and many of the pullets laid 2 or 3 eggs after the development of leg weakness. Addition of phosphorus to the diet caused a significant reduction in mortality and leg weakness. Harms (1962) also reported that caged pullets fed a diet containing 0.55% phosphorus and showing typical symptoms of cage layer fatigue were benefited by increasing the level of phosphorus in the diet to 0.82%.

Marr et al. (1961), Singsen et al. (1961) and Harms et al. (1961) reported that caged hens have a higher phosphorus requirement than floor-housed hens.

Simpson et al. (1964) studied the relationship of dietary calcium and phosphorus levels to the cage layer fatigue syndrome. They reported that pullets fed a diet containing 0.34% total phosphorus with 3.0% calcium developed leg weakness and had definite histologic evidence of bone damage while no leg weakness was observed in hens fed a 2% calcium diet with 0.70% total phosphorus.

Cox and Balloun (1968) fed diets containing 2.5%, 3.0% and 3.5% calcium with a 0.65% phosphorus level and reported 8 of 10 cases of cage layer fatigue observed were from the 2.5% calcium diet.

Riddell et al. (1967) reported 19 of 22 vertebral columns of caged laying birds displaying a syndrome which resembled cage layer fatigue were fractured in the region of the fourth and fifth thoracic vertebrae. Also, with all fractures there was associated compression and demyelinization of the spinal cord which the authors considered led to the typical

picture of cage layer fatigue.

## Prediction of Skeletal Mineralization

Metabolism of calcium in the chicken has been studied by determining the ash percent, calcium content and distribution of administered radio-calcium present in some individual bones. These procedures have also been applied in the biological assay for vitamin D. In most cases, the tibia has been used for analysis (Baird and MacMillan, 1942), (Migicovsky et al., 1950), (Itoh and Hatano, 1964), (Martin and Patrick, 1962a) and many others cited in this literature review. The humerus and sternum have been used by Martin and Patrick (1962a) while some investigations for a biological vitamin D assay have been developed using toe bones (Baird and MacMillan, 1942), (Evans and Carver, 1944) and (Campbell et al., 1945a, b) or beak (Wei et al., 1954). Individual skeletal bones may grow differently according to the form and function of each, and consequently, there may be differences in their calcium metabolism. There are very few studies of the inorganic content in the individual bones of the laying hen (Taylor et al., 1954, 1956, 1960), and little is known concerning the differences in ash or calcium content and the effects of vitamin D on the calcium metabolism of individual bones.

Itoh and Hatano (1964) studied calcium contents and radio-calcium uptake rates in eight bone samples from the skeleton of chicks and reported calcium turnover rates decreased in the order: femur, tibia and metatarsus. Values for the femur were more similar to the total skeleton than were those from any other bone sample.

Morris et al. (1966) presented a regression equation and reported very good general agreement between the predicted skeletal weight based on the regression of skeletal weight on tibia and coracoid bones weighed separately and observed skeletal weight ($r = +0.98$). They also presented an equation which they proposed would serve as an index of the degree of skeletal depletion. The authors concluded, however, that "the reliability of this index as a quantitative estimate of the amount of bone which had been resorbed from a particular skeleton cannot be very high".

## Parathyroid Hormone

The basic mechanism by which parathyroid hormone exerts its physiological effects have not been elucidated. It has been observed that feeding low-calcium diets lead to a hypertrophy of the parathyroid glands. Stoerk and Carnes (1945) showed an almost perfect correlation between the volume of the parathyroid glands and the serum-calcium level of the rat. Benoit (1950) fed low-calcium diets to domestic ducks and observed up to a six-fold increase in the size of the parathyroid glands. Raisz (1963) cultured 13-day-chick embryonic parathyroid glands in vitro, and reported that the secretory and the mitotic activity of the tissue were under the control of the concentration of ionic calcium. This indirect evidence suggested that secretion and possibly synthesis of parathyroid hormone was stimulated by low levels of serum calcium. Direct evidence confirming this interpretation has been provided by Patt and Luckhardt (1942) who perfused decalcified blood through the parathyroid glands of a dog and showed the effluent contained a substance with the characteristic properties of the

parathyroid hormone. More recently, a radioimmuno assay has been devised for estimating the quantity of parathyroid hormone in the plasma and it has been shown that there is a reciprocal relationship between the amount of the hormone and the concentration of calcium in the blood. Thus the release of the hormone is directly controlled by the concentration of plasma calcium although it has been shown by Sherwood et al. (1966) that magnesium can act like a high calcium level in repressing the release of parathyroid hormone.

The serum calcium level of the hen rises markedly when egg production commences and returns to a non-productive level in the molting period (Macowan, 1932). It is known that these changes are under the influence of the parathyroid gland and estrogen has been considered to have an additive effect in elevating serum calcium (Urist et al., 1960). Hurwitz and Griminger (1961) reported a three-fold increased weight of the parathyroid glands of 9-month-old laying hens fed a .03% calcium diet compared to those fed a 2.75% calcium diet. The recent development of a radioimmuno assay for parathyroid hormone will greatly aid in much needed investigations on its relationship(s) to the metabolism of calcium during eggshell formation.

## Calcitonin

Copp et al. (1962) published a paper which added a significant factor to the understanding of the control of the calcium level of the blood. They showed that the perfusion of the thyroid-parathyroid complex of a dog with blood with a very high calcium content resulted in a fall in the

blood calcium level in the dog and that the rate of decrease was faster than if the thyroid-parathyroid complex was surgically removed from the animal. The surgical removal insured that the complex ceased to release its hormone into the blood, so the more rapid fall in blood calcium level following perfusion could not be explained as simply a cessation of parathyroid activity but suggested a hormone that actively lowered the calcium level of the blood. This new hormone was called calcitonin. The existence of the hormone has been accepted as has its effect of antagonizing some of the effects of parathyroid hormone. There is some doubt, however, as to whether calcitonin is secreted by the parathyroid or the thyroid when it is sometimes called thyrocalcitonin. Bussolati and Pearse (1967) showed that calcitonin is not present in the regular thyroid cells, but is present in the parafollicular C cells. Pearse and Carvalheira (1967) also demonstrated by histological specific staining reactions that these are probably ultimobronchial cells. In fish, amphibians, reptiles and birds these cells exist as a separate gland, but in mammals they become inbedded in the thyroid and inferior parathyroid (Carvalheira and Pearse, 1967). Copp and Cockcroft (1967) and Tauber (1967) demonstrated that the ultimobronchial gland of the fowl contains a potent hypocalcemic agent. The chicken ultimobronchial gland is located in the chest near the bifurcation of the common carotid and axillary artery (Copp and Cockcroft, 1967). No reports have been found of the effect(s) of the calcitonin hormone, its secretion or blood levels during the mobilization of calcium for secretion during egg shell production.

# EXPERIMENTAL PROCEDURE

## General

Physiological factors studied on individual birds during all experiments reported herein were 140 day body weight, terminal body weight, terminal age, age at first egg, number of eggs laid, rate of egg production, fat-extracted dry bone and ash weight of individual bones.

After birds were sacrificed by axial dislocation, the sample bones were removed from the carcass and autoclaved for the minimum amount of time necessary for the removal of soft tissue. The bones were then extracted for 48 hours with hot ethanol and then 48 hours with hot Skelly-B solvent after which they were dried at 80° Centigrade for 24 hours. The fat-free-dry bones were weighed in standardized ashing crucibles and ashed at 600° Centigrade. The ashed samples were then cooled in a dessicator and weighed.

Statistical analyses were conducted by the Iowa State Computation Center. The Omnitab Programming System was used in all experiments. In the results and discussion sections of this thesis, femur ash (grams) and femur ash (grams) per kilogram body weight will be referred to as $Y_1$ and $Y_2$ respectively.

The formulae of the diets used in Experiment IV and Experiments II and III are presented in Table 1 and Table 2 respectively.

## Experiment I

Single Comb White Leghorn female chicks from Line-A that had been developed by selection for a high rate of egg production were used in

Table 1. Formulae of diets for experiment IV

| Ingredient | % of total | | | |
|---|---|---|---|---|
| | Starter | Grower | | Layer |
| Weeks | 0-6 | 6-12 | 12-20 | |
| Ground corn | 69.8 | 61.0 | 66.0 | 60.0 |
| Soybean meal (50%) | 18.0 | 20.0 | 10.0 | 15.0 |
| Fish meal (70%) | 5.0 | --- | --- | 2.5 |
| Meat scrap (50%) | --- | --- | --- | 2.5 |
| Wheat midds | --- | 10.0 | 10.0 | 7.2 |
| Dehy alfalfa (20%) | 2.0 | 5.0 | 10.0 | 5.0 |
| Soybean oil | 1.7 | --- | --- | --- |
| Dicalcium phosphate | 1.5 | 1.5 | 1.5 | 2.0 |
| Oyster shell | 0.5 | 1.0 | 1.0 | 5.0 |
| Mineral premix[a] | 0.5 | 0.5 | 0.5 | 0.3 |
| Vitamin premix | 1.0[b] | 1.0[b] | 1.0[b] | 0.5[c] |
| Total | 100.0 | 100.0 | 100.0 | 100.0 |

[a]Mineral premix when used at 0.5% provides, per kg of diet: sodium chloride 4.49 gms; manganese 60 mgs; zinc 75 mgs; iron 55 mgs; copper 9 gms; iodine 1.1 mgs; cobalt 0.55 mgs.

[b]Vitamin premix when used at 0.5% provides, per kg of diet: vitamin A 7,500 IU; vitamin $D_3$ 1,000 ICU; vitamin E 10 IU; menadione sodium bisulfite 1 mg; vitamin $B_{12}$ 10 mcgs; riboflavin 5 mgs: pantothenic acid 10 mgs; niacin 25 mgs; choline 450 mgs; methionine equivalent 1,000 mgs; Santoquin 125 mgs; penicillin 3.3 mgs; streptomycin 16.5 mgs.

[c]Vitamin premix when used at 0.5% provides, per kg of diet: vitamin A 9,000 IU; vitamin $D_3$ 2,000 ICU; vitamin $B_{12}$ 5 mcgs; riboflavin 5 mgs; pantothenic acid 5 mgs; niacin 15 mgs; choline 100 mgs; methionine equivalent 750 mgs; Santoquin 125 mgs.

Experiment I. The chicks were brooded on deep litter floors until eight weeks of age then range-reared. At 20 weeks of age the birds were weighed individually and placed singly into 10 x 16 x 16 inch wire laying cages. Commercial starting, growing and laying rations and water were available ad libitum. The chicks received 20 hours of light until transferred to range where they received natural increasing daylight. At 22 weeks of age

Table 2. Formulae of diets for experiments II and III

| Ingredient | % of total | | | | |
|---|---|---|---|---|---|
| | Starter[a] | Grower | | Pre-layer[a] | Layer[b] |
| Weeks | 0-6 | 6-18[c] | 6-20[a] | 18-22 | |
| Ground corn | 66.5 | 62.5 | 67.0 | 57.0 | 60.0 |
| Soybean meal (50%) | 20.0 | 12.5 | 17.5 | 17.0 | 20.0 |
| Fish meal (70%) | 5.0 | 3.0 | 2.0 | --- | 5.0 |
| Meat scrap (50%) | --- | --- | 5.0 | 5.0 | --- |
| Dehy alfalfa (18%) | 5.0 | 10.0 | 5.0 | 5.0 | --- |
| Solka flock | --- | 8.2 | --- | 4.9 | 3.2 |
| Soybean oil | --- | 1.0 | --- | 3.0 | 2.0 |
| Dicalcium phosphate | 1.0 | 1.0 | 1.0 | 1.0 | 2.1 |
| Oyster shell | 1.0 | 1.0 | 1.0 | 6.3 | 6.9 |
| Mineral premix[d] | 0.5 | 0.5 | 0.5 | 0.3 | 0.3 |
| Vitamin premix | 1.0[e] | 1.0[e] | 1.0[e] | 0.5[f] | 0.5[f] |
| Coccidiostat | --- | +[g] | --- | --- | --- |
| Total | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 |

[a]These rations were used in experiments II and III.

[b]This ration was used in experiment III.

[c]This ration was used in experiment II.

[d]Mineral premix -- listed under Table 1.

[e]Vitamin premix -- listed under Table 1-b.

[f]Vitamin premix -- listed under Table 1-c.

[g]Coccidiostat - Amprol at 0.0125% of the diet.

they were stimulated with 14 hours of artificial light per day.

At 18 weeks of age and weekly thereafter, four non-layers were sacrificed until production commenced in the flock. Subsequently, two non-layers, three birds that had just begun laying, three that had laid an average number of eggs and three that had laid a high number of eggs relative to the surviving birds were sacrificed weekly. This sacrificial procedure was continued as long as birds were available. The tibia and humerus bones were selected as sample bones and were prepared as outlined in the general experimental procedure section.

Experiment II

Single Comb White Leghorn female chicks from Line-A were used in Experiment II. The chicks were brooded and reared on deep-litter floor in confinement until they were weighed individually and placed singly into 10 x 16 x 16 inch wire laying cages at 20 weeks of age. Water and feed were available ad libitum. The formulae of the starter and grower rations are presented in Table 2. The same commercial laying ration fed in Experiment I was used in Experiment II. The chicks received 20 hours of light until six weeks of age then restricted to six hours of light per day until 22 weeks of age at which time they were stimulated with 14 hours of light per day.

At 18 weeks of age and weekly thereafter, four non-layers were sacrificed until production commenced in the flock. Subsequently, two non-layers, three birds that had just begun laying, three that had laid an average number of eggs and three that had laid a high number of eggs

relative to the surviving birds were sacrificed weekly. This sacrificial procedure was continued as long as birds were available. The tibia and humerus bones were selected as sample bones and were prepared as outlined in the general experimental procedure section.

Experiment III

A commercial egg-production strain of S. C. W. Leghorn female chicks were obtained at one day of age for Experiment III. The chicks were brooded in conventional electrically-heated wire-floored starting batteries for six weeks and then transferred to wire-floor growing batteries until 18 weeks of age at which time they were placed singly into individual 10 x 16 x 16 inch wire laying cages. Chicks received 20 hours of light daily to six weeks of age, a six hour daily light regime to 22 weeks of age, then stimulated with 14 hours of light per day. Feed and water were available ad libitum throughout the growing and experimental periods. The starter, grower and layer ration formulae are presented in Table 2.

At 18 weeks of age and weekly thereafter, four non-layers were sacrificed until production commenced in the flock. Subsequently, two non-layers, three birds that had just begun laying, three that had laid an average number of eggs relative to the surviving birds were sacrificed weekly. This sacrificial procedure was continued as long as birds were available. The humerus bone was selected as the sample bone and was prepared as outlined in the general experimental procedure section.

Experiment IV

A commercial strain of broiler female chicks was used in Experiment IV. The chicks were brooded in conventional electrically-heated wire-floor starter batteries and then transferred to wire-floor grower batteries at four weeks of age. Water was available ad libitum throughout the experiment. Feed was available ad libitum until the chicks were six weeks old after which they were restricted to a "skip-a-day" regime until 20 weeks of age and then fed ad libitum. Formulae of the rations are presented in Table 1.

Four non-layers were sacrificed at 20 weeks of age and weekly thereafter until production commenced in the flock. Subsequently, two non-layers, two that had just begun producing, two that had laid an average number of eggs and two that had laid a high number of eggs relative to the surviving birds were sacrificed weekly. This sacrificial procedure was continued as long as birds were available. The humerus bone was selected as the sample bone and prepared as outlined in the general experimental procedure section.

Experiment V

Single Comb White Leghorn laying hens from two Lines: D - selected for large body weight; and E - selected for small body weight, were used to study the relationship between individual bones within the skeleton and the total skeleton. All birds received the same commercial starter, grower and layer rations and water ad libitum. The chicks were brooded on deep-litter floor then range-reared. At 18 weeks of age they were

housed in slatted breeding pens where they were mated for genetic studies. When these studies were completed the hens were 40 weeks of age at which time they were placed singly into 10 x 16 x 16 inch wire laying cages. Individual egg production records were observed for three weeks and birds that produced approximately the same number of eggs during this period were sacrificed at 43 weeks of age and used in this experiment. The bone samples were prepared as outlined in the general experimental procedure section.

# RESULTS

## Experiment I

Means, standard errors and coefficients of variation for the variables measured on 12 non-layers and 62 layers in Experiment I are presented in Table 3 and Table 4 respectively. Mean terminal body weight, tibia weight and tibia ash weight differed very little between the non-layers and layers. Mean femur weight and femur ash weight of layers were 94.1% and 95.1% respectively of the non-layers. Expressed on the basis of body weight, however, mean layer femur ash was only 43.6% that of the non-layers, indicating that a marked loss of femur ash was associated with egg production.

Expressing the bone and ash variables as a ratio with body weight explained a great deal of the observed variation in these variables as shown by the substantial reduction in the respective standard errors. Also, the reductions of the standard errors were greater in magnitude for the laying birds. The explanation of the observed variation of bone and ash weights was not improved by expressing these weights in terms of metabolic body weight.

The depletion of femur ash associated with egg production is illustrated in Figures 1, 2, 3, 4 and 5. Figure 1 and Figure 2 show the effect of number of eggs laid on $Y_1$ and $Y_2$ respectively and indicate that after the onset of production there was a depletion of femur ash which occurred at an average rate of 4.4 milligrams and 3.7 milligrams per kilogram of body weight respectively. The regression of $Y_1$ on number of eggs laid was not significant (Appendix, Table 17) whereas

Table 3. Statistics of variables measured on 12 non-layers during Experiment I

| | Mean ± S.E. | Coefficient[a] of variation |
|---|---|---|
| Terminal body wt kg | 1.61 ± 0.13 | 11.4 |
| Tibia wt g | 4.51 ± 0.20 | 15.1 |
| Tibia ash g | 2.70 ± 0.12 | 15.1 |
| Femur wt g | 4.07 ± 0.22 | 18.3 |
| Femur ash g | 2.26 ± 0.15 | 23.2 |
| Femur wt g/kg body wt | 2.53 ± 0.10 | 14.2 |
| Femur ash g/kg body wt | 1.40 ± 0.08 | 19.2 |
| Femur ash g/kg body $wt^{3/4}$ | 1.58 ± 0.09 | 19.7 |

[a] $S/\bar{X}$ times 100.

Table 4. Statistics of variables measured on 62 layers during Experiment I

| | Mean ± S.E. | Coefficient of variation |
|---|---|---|
| Terminal body wt kg | 1.58 ± 0.03 | 15.9 |
| Age at first egg | 181 ± 2.9 | 12.7 |
| Number of eggs | 30 ± 3.4 | 88.4 |
| Rate of production %[a] | 72.9 ± 1.7 | 18.7 |
| Tibia wt g | 4.51 ± 0.08 | 16.0 |
| Tibia ash g | 2.68 ± 0.06 | 17.9 |
| Femur wt g | 3.83 ± 0.09 | 19.2 |
| Femur ash g | 2.15 ± 0.06 | 23.1 |
| Femur wt g/kg body wt | 2.42 ± .04 | 15.2 |
| Femur ash g/kg body wt | 1.34 ± .07 | 18.5 |
| Femur ash g/kg body $wt^{3/4}$ | 1.85 ± .04 | 18.8 |

[a] Number of eggs laid/number of days in production x 100.

Figure 1. Effect of number of eggs laid on femur ash weight. Experiment I

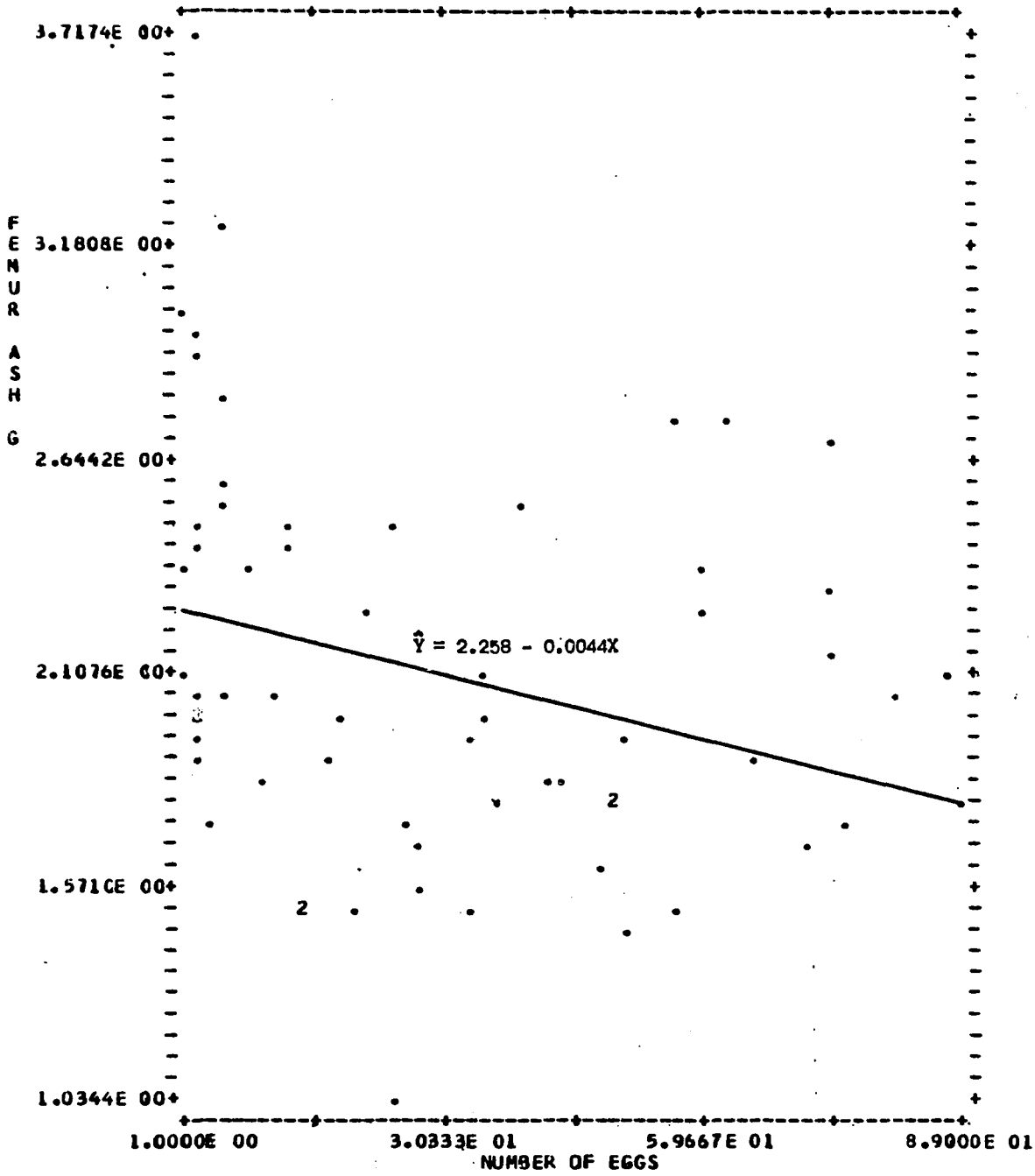

3.7174E 00+
3.1808E 00+
2.6442E 00+
2.1076E 00+
1.571CE 00+
1.0344E 00+
FEMUR ASH G
$\hat{Y}$ = 2.258 - 0.0044X
2
2
1.0000E 00
3.0333E 01
5.9667E 01
8.9000E 01
NUMBER OF EGGS

Figure 2. Effect of number of eggs laid on femur ash in grams per kilogram body weight. Experiment I

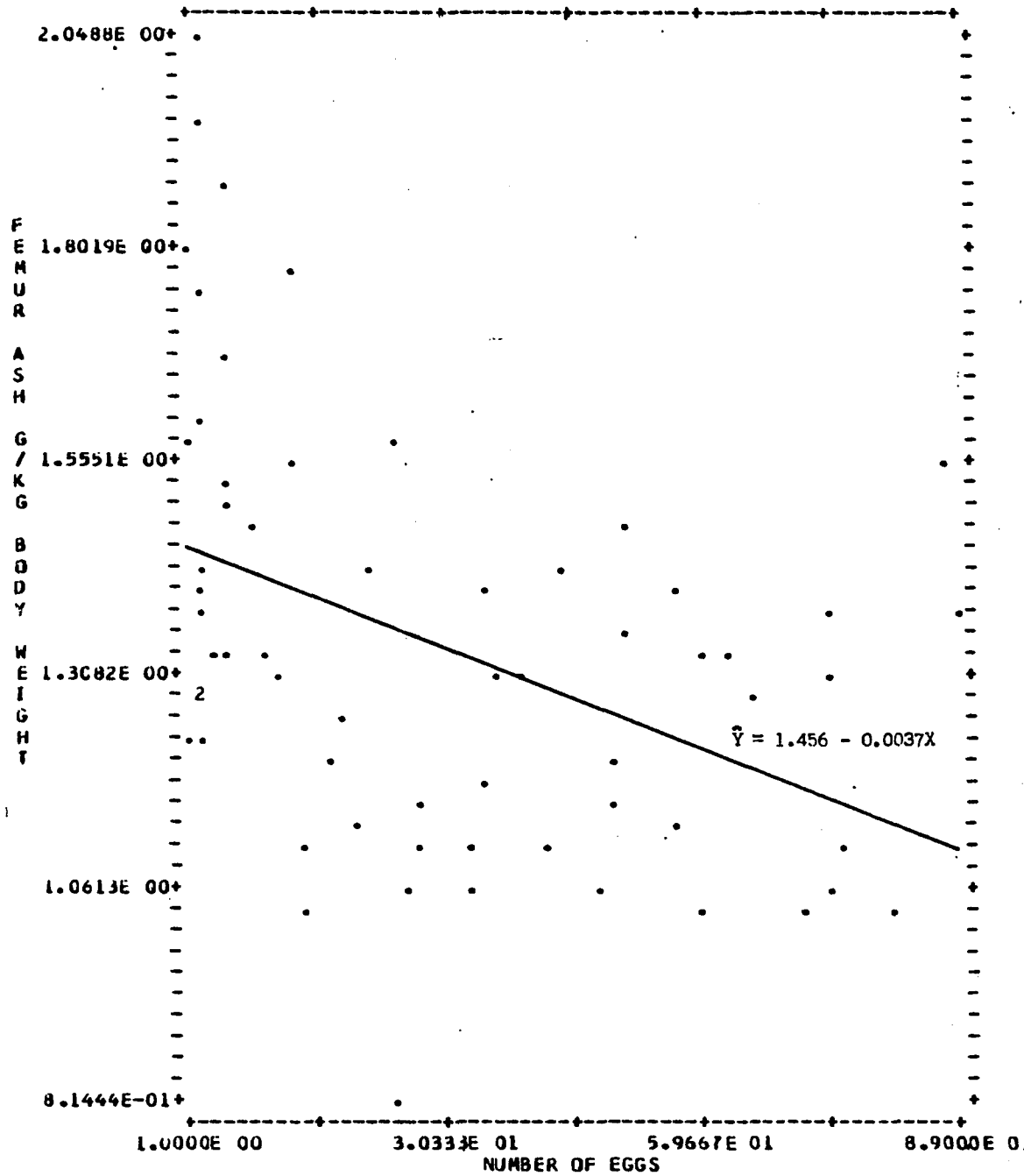

2.0488E 00+
1.8019E 00+
1.5551E 00+
1.3082E 00+
1.0613E 00+
8.1444E-01+
FEMUR ASH G/KG BODY WEIGHT
2
Ŷ = 1.456 - 0.0037X
1.0000E 00
3.0333E 01
5.9667E 01
8.9000E 01
NUMBER OF EGGS

Figure 3. Depletion of femur ash in grams per kilogram body weight which occurred during the production of the first 27 eggs. Experiment I

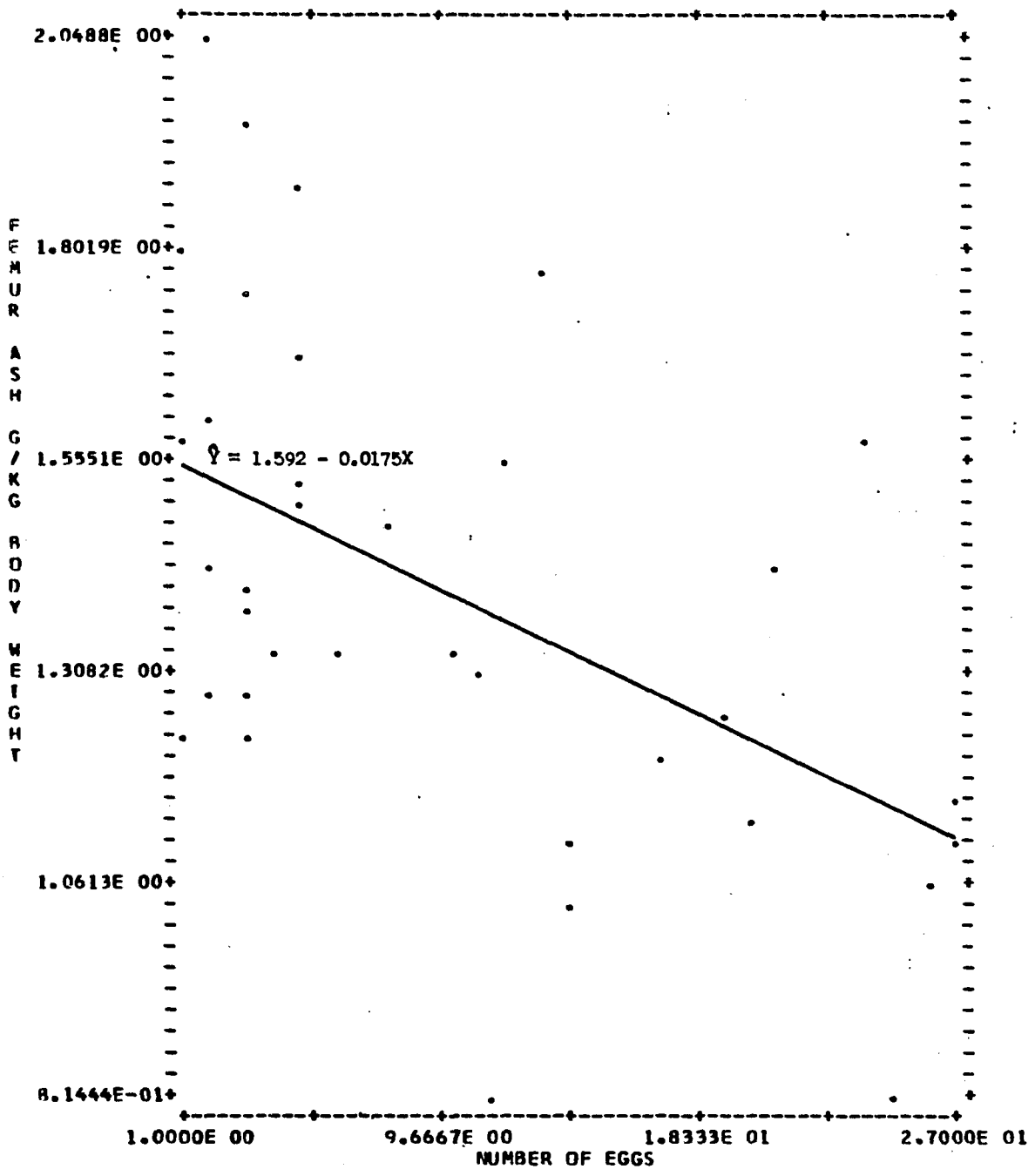

2.0488E 00
1.8019E 00
1.5551E 00
1.3082E 00
1.0613E 00
8.1444E-01
FEMUR ASH G/KG BODY WEIGHT
Ŷ = 1.592 - 0.0175X
1.0000E 00
9.6667E 00
1.8333E 01
2.7000E 01
NUMBER OF EGGS

Figure 4. Effect of rate of production on femur ash weight. Experiment I

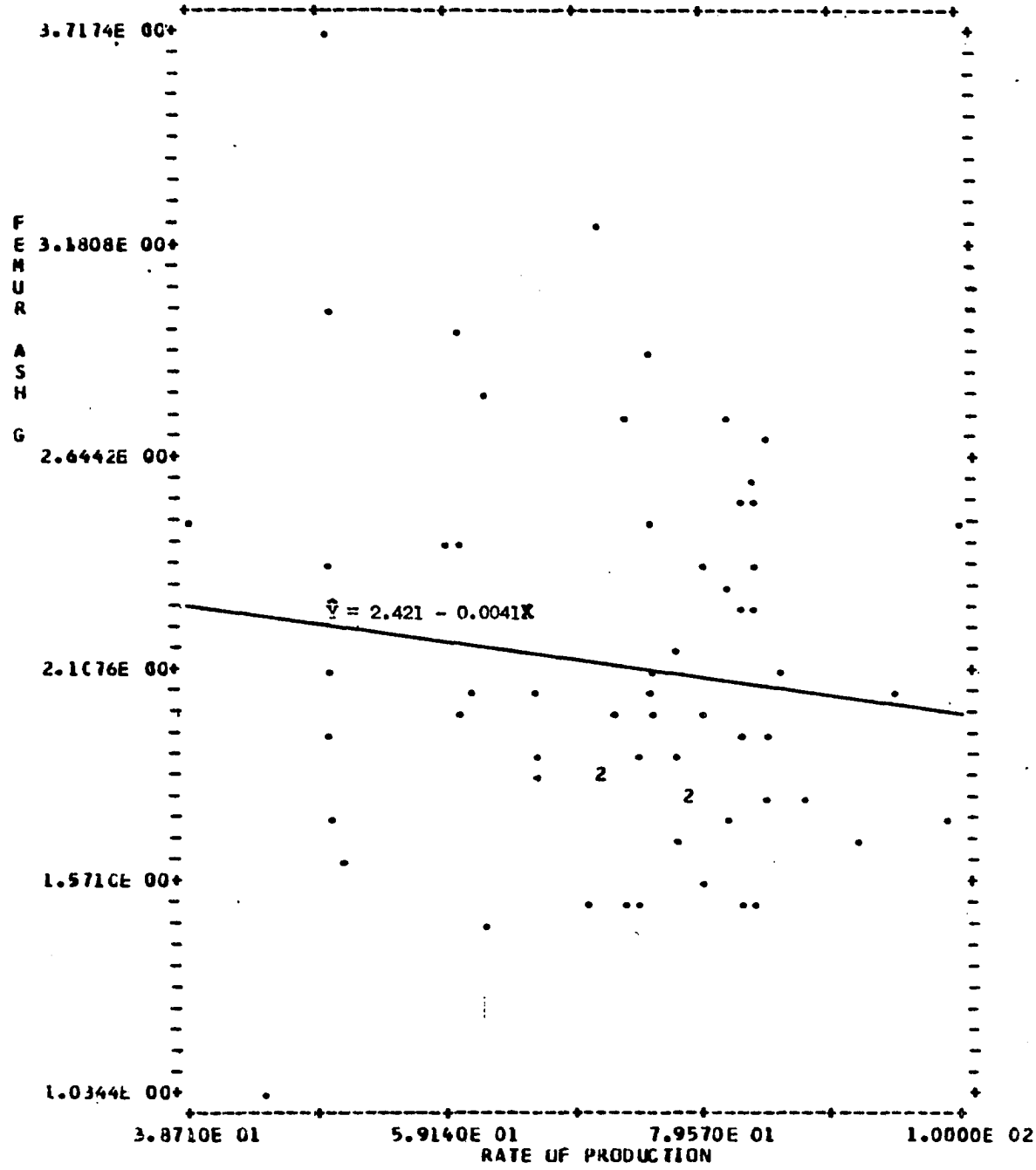
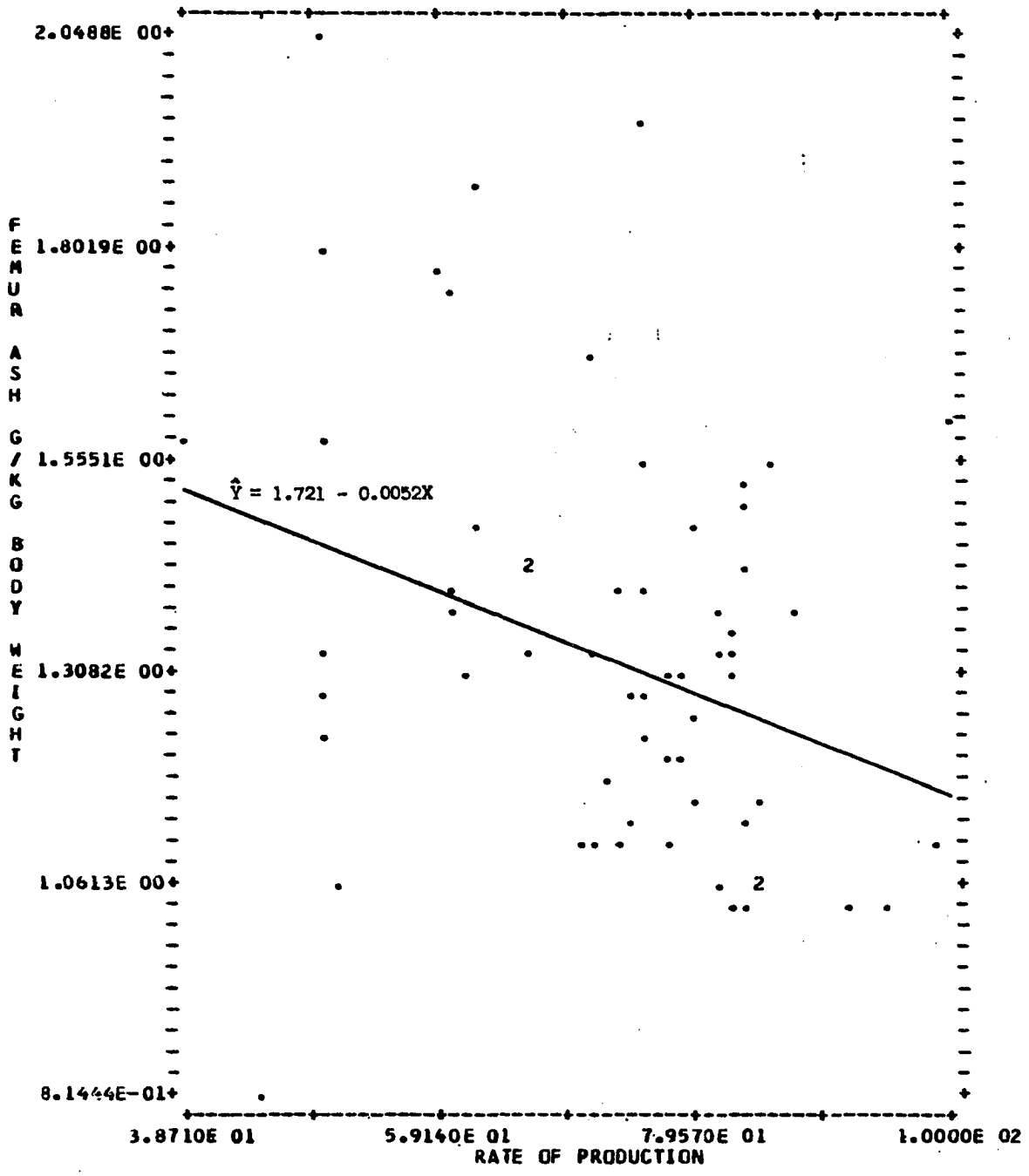

3.7174E 00
3.1808E 00
2.6442E 00
2.1076E 00
1.5710E 00
1.0344E 00
FEMUR ASH G
$\hat{Y}$ = 2.421 - 0.0041X
2
2
3.8710E 01
5.9140E 01
7.9570E 01
1.0000E 02
RATE OF PRODUCTION

Figure 5. Effect of rate of production on femur ash in grams per kilogram body weight. Experiment I


2.0488E 00
1.8019E 00
1.5551E 00
1.3082E 00
1.0613E 00
8.1444E-01
FEMUR ASH G/KG BODY WEIGHT
$\hat{Y}$ = 1.721 - 0.0052X
2
2
3.8710E 01
5.9140E 01
7.9570E 01
1.0000E 02
RATE OF PRODUCTION

the regression of $Y_2$ on number of eggs laid was highly significant ($P \leq 0.01$) indicating $Y_2$ may be a more critical measure of bone mineral status in the laying hen.

The inverse relationship between $Y_2$ and the number of eggs laid (Figure 3) indicated that depletion of femur ash began at the onset of production and progressed during the production of the first 27 eggs at an average rate of 17.5 milligrams of ash per kilogram of body weight per egg.

The effect of rate of production on $Y_1$ and $Y_2$ is illustrated in Figure 4 and Figure 5, respectively, which show an inverse relationship between the dependent variables and rate of production. The regression of $Y_1$ on rate of production was not significant, however, the regression of $Y_2$ on rate of production was significant ($P \leq 0.05$) suggesting that $Y_2$ may be a more sensitive measure of bone mineral status in the laying hen.

Multiple regression of $Y_1$ on rate of production and number of eggs laid did not indicate either independent variable was significant, whereas the regression of $Y_2$ on the same independent variables indicated both independent variables contributed significantly ($P \leq 0.05$) to the observed variation in $Y_2$. Also, multiple regression of $Y_2$ on rate of production and number of eggs laid for hens that had not laid more than 30 eggs indicated that only number of eggs laid contributed significantly ($P \leq 0.01$) to the observed variation in this dependent variable.

## Experiment II

Means, standard errors and coefficients of variation for the variables measured on 8 non-layers and 57 layers during Experiment II are presented in Table 5 and Table 6 respectively. Mean terminal body weight of non-layers and layers was similar. Mean tibia weight and ash weight of the layers were 84.6% and 82.6% respectively of the non-layers and mean layer femur weight and ash weight were 83.3% and 80.0% respectively of that in non-layers. When expressed as a ratio with body weight, mean layer femur ash was only 34.5% that of the non-layer mean indicating that a substantial loss of femur ash was associated with egg production.

Expressing the bone variables on the basis of body weight explained a great deal of the observed variation as shown by the substantial reduction in the respective standard errors. Substituting metabolic body weight for body weight did not result in any further reduction of the standard errors of the bone variables.

The loss of femur ash concomitant with egg production is illustrated in Figures 6, 7, 8, 9 and 10. Figure 6 and Figure 7 show the effect of number of eggs laid on $Y_1$ (femur ash grams) and $Y_2$ (femur ash grams per kilogram body weight) respectively and illustrate that, at the onset of production, there was a depletion of femur ash which progressed with the number of eggs laid. The regressions of $Y_1$ and $Y_2$ on the number of eggs laid were highly significant ($P \leq 0.01$) and indicated a depletion rate per egg of 13 milligrams of femur ash, and 15 milligrams of femur ash per kilogram of body weight, respectively.

Table 5. Statistics of variables measured on 8 non-layers during Experiment II

| | Mean ± S.E. | Coefficient of variation |
|---|---|---|
| Terminal body wt kg | 1.47 ± 0.13 | 24.7 |
| Tibia wt g | 4.74 ± 0.33 | 19.8 |
| Tibia ash g | 2.87 ± 0.22 | 21.2 |
| Femur wt g | 4.06 ± 0.33 | 23.1 |
| Femur ash g | 2.35 ± 0.23 | 28.1 |
| Femur wt g/kg body wt | 2.96 ± 0.14 | 13.3 |
| Femur ash g/kg body wt | 1.71 ± 0.11 | 18.7 |
| Femur ash g/kg body $wt^{3/4}$ | 1.76 ± 0.12 | 19.3 |

Table 6. Statistics of variables measured on 57 layers during Experiment II

| | Mean ± S.E. | Coefficient of variation |
|---|---|---|
| Terminal body wt kg | 1.50 ± 0.04 | 18.0 |
| Age at first egg | 159 ± 1.5 | 7.2 |
| Number of eggs | 20 ± 1.7 | 62.7 |
| Rate of production % | 71.3 ± 2.6 | 27.9 |
| Tibia weight g | 4.01 ± 0.07 | 14.6 |
| Tibia ash g | 2.37 ± 0.04 | 15.8 |
| Femur weight g | 3.38 ± 0.08 | 19.5 |
| Femur ash g | 1.88 ± 0.05 | 23.6 |
| Femur g/kg body wt | 2.33 ± .04 | 18.7 |
| Femur ash g/kg body wt | 1.30 ± .04 | 22.9 |
| Femur ash g/kg body $wt^{3/4}$ | 1.72 ± .04 | 21.6 |

Figure 6. Effect of number of eggs laid on femur ash weight. Experiment II

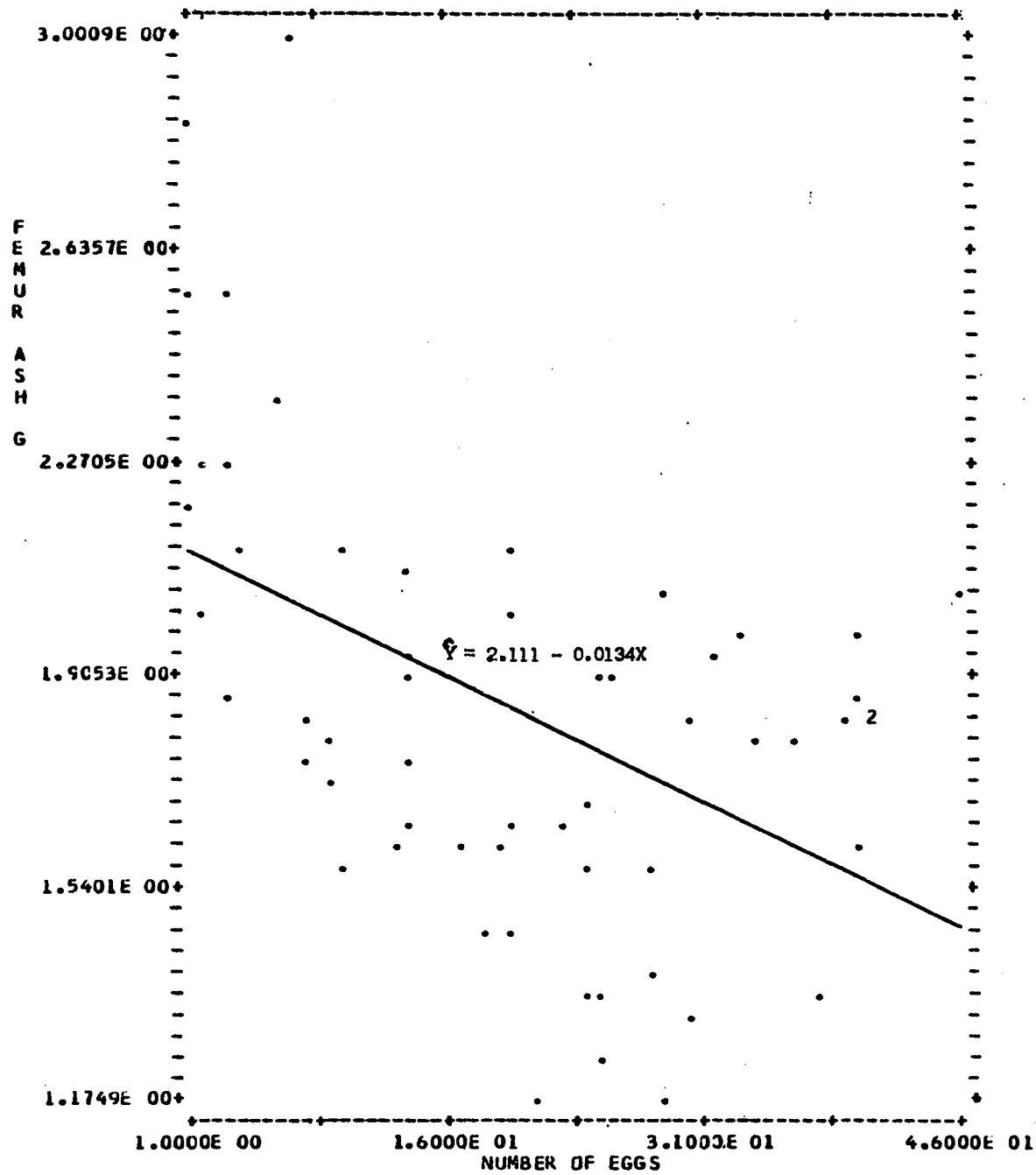

3.0009E 00
2.6357E 00
2.2705E 00
1.9053E 00
1.5401E 00
1.1749E 00
FEMUR ASH G
$\hat{Y}$ = 2.111 - 0.0134X
2
1.0000E 00
1.6000E 01
3.1000E 01
4.6000E 01
NUMBER OF EGGS

Figure 7. Effect of number of eggs laid on femur ash in grams per kilogram body weight. Experiment II

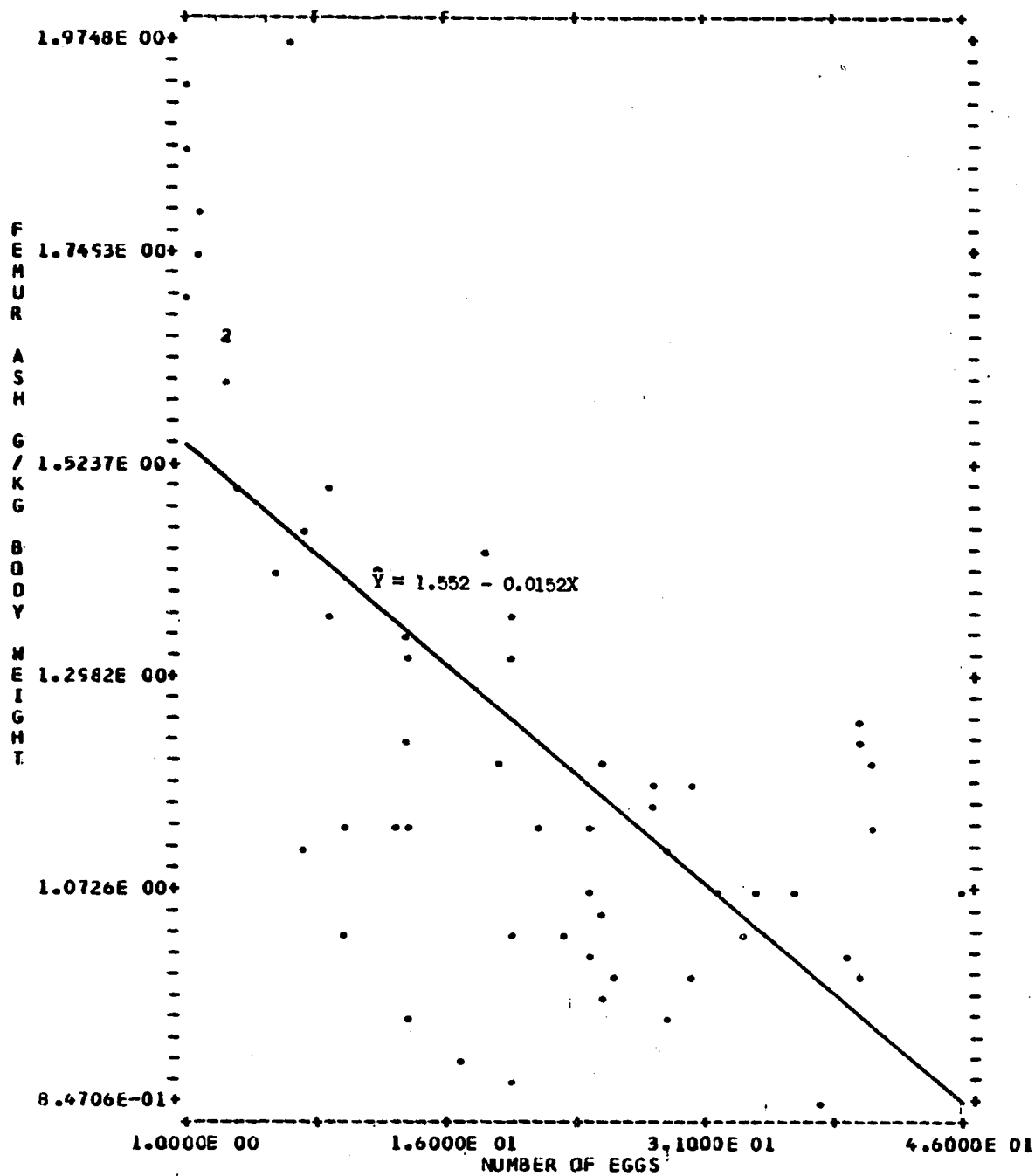

1.9748E 00+
1.7453E 00+
1.5237E 00+
1.2982E 00+
1.0726E 00+
8.4706E-01+
FEMUR ASH G/KG BODY WEIGHT
2
$\hat{Y}$ = 1.552 - 0.0152X
1.0000E 00
1.6000E 01
3.1000E 01
4.6000E 01
NUMBER OF EGGS

Figure 8. Depletion of femur ash in grams per kilogram body weight which occurred during the production of the first 30 eggs. Experiment II

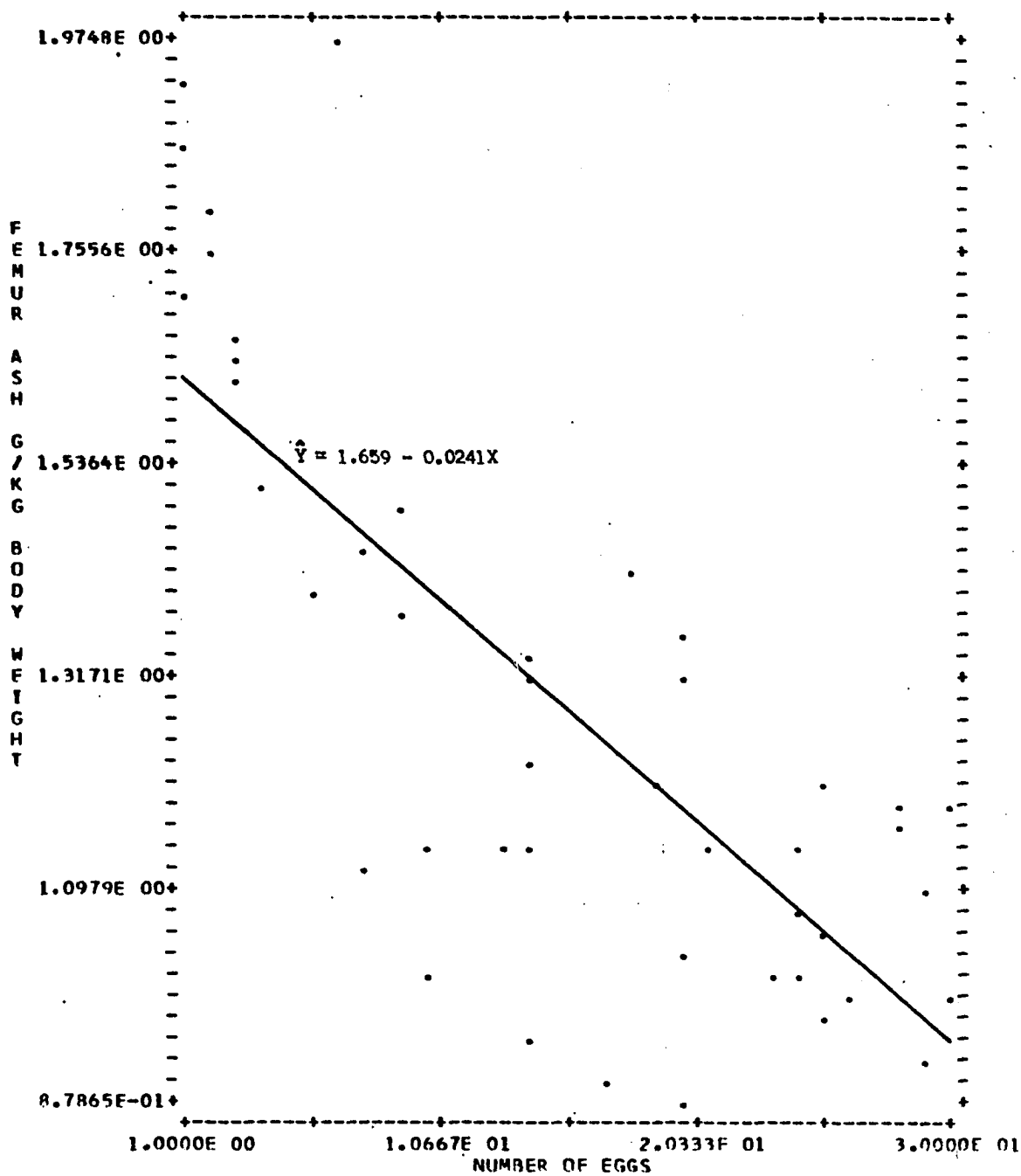

1.9748E 00+
1.7556E 00+
1.5364E 00+
1.3171E 00+
1.0979E 00+
8.7865E-01+
FEMUR ASH G/KG BODY WEIGHT
Ŷ = 1.659 - 0.0241X
1.0000E 00
1.0667E 01
2.0333E 01
3.0000E 01
NUMBER OF EGGS

Figure 9. Effect of rate of production on femur ash weight. Experiment II

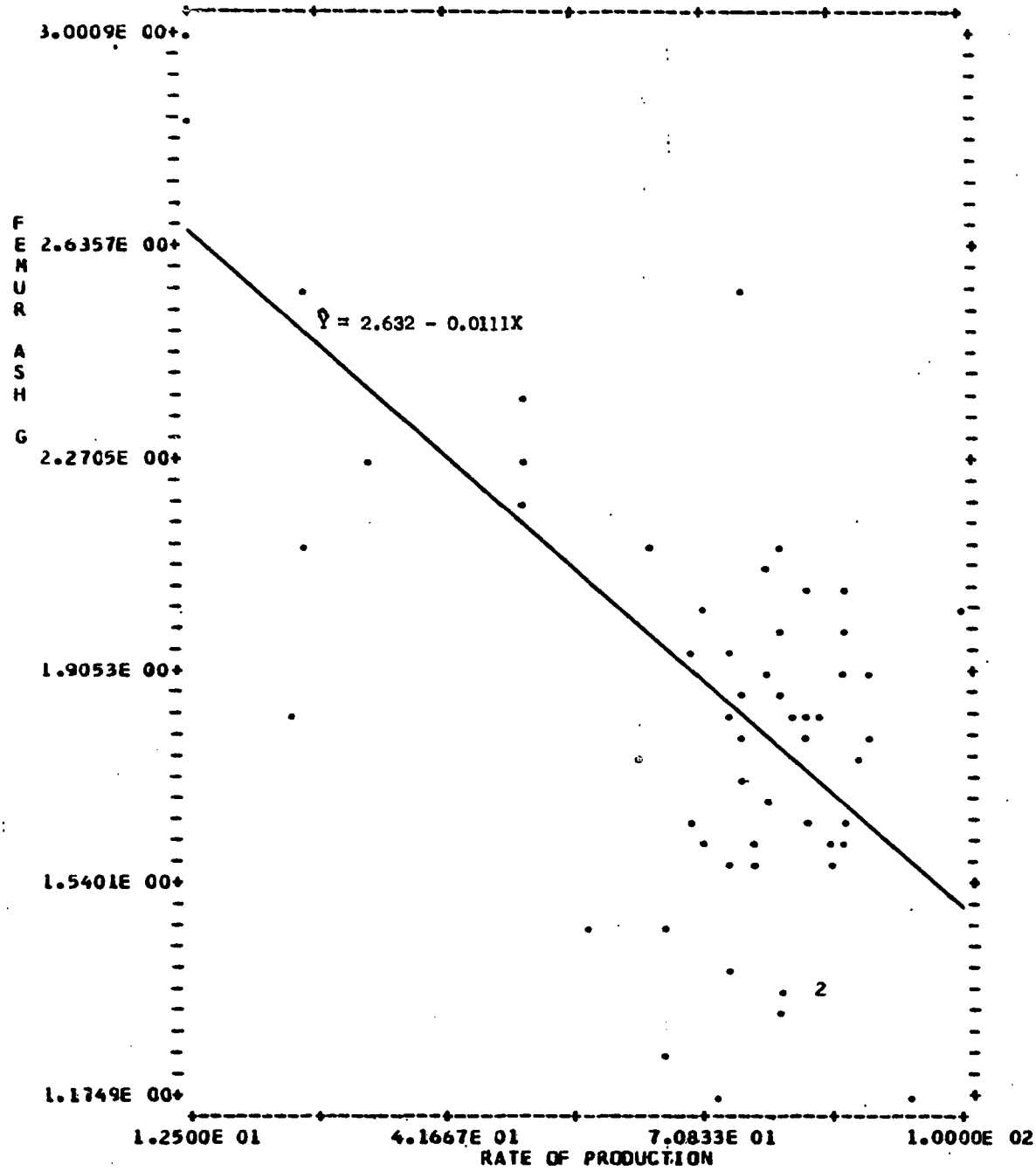
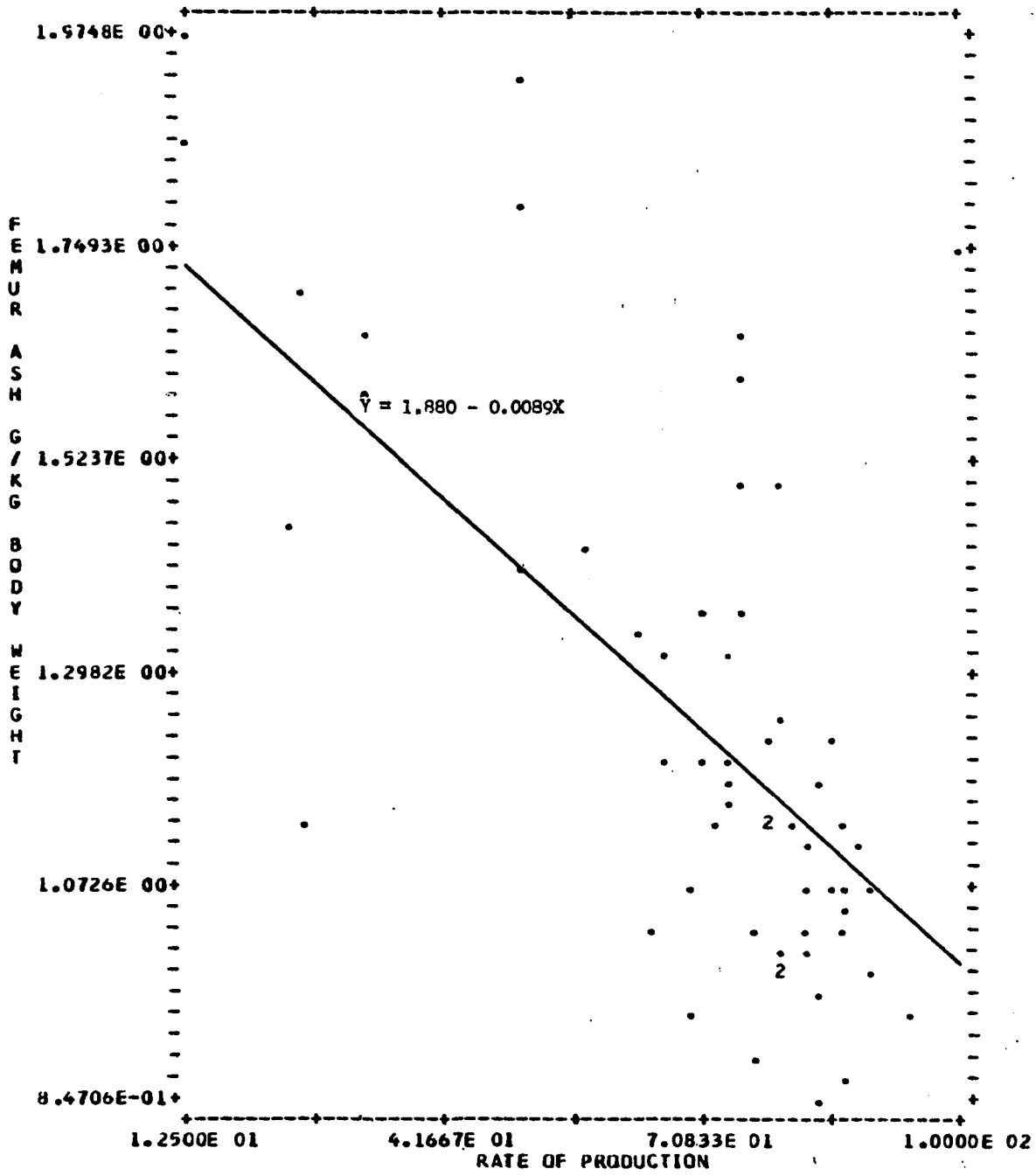

3.0009E 00
2.6357E 00
2.2705E 00
1.9053E 00
1.5401E 00
1.1749E 00
FEMUR ASH G
Ŷ = 2.632 - 0.0111X
2
1.2500E 01
4.1667E 01
7.0833E 01
1.0000E 02
RATE OF PRODUCTION

Figure 10. Effect of rate of production on femur ash in grams per kilogram body weight. Experiment II


1.9748E 00
1.7493E 00
1.5237E 00
1.2982E 00
1.0726E 00
8.4706E-01
FEMUR ASH G/KG BODY WEIGHT
Ŷ = 1.880 - 0.0089X
2
2
1.2500E 01
4.1667E 01
7.0833E 01
1.0000E 02
RATE OF PRODUCTION

The rate of depletion of femur ash which occurs during the production of the first 30 eggs is illustrated in Figure 8. On the average, approximately 24 milligrams of femur ash per kilogram of body weight per egg were lost.

Figure 9 and Figure 10 show that rate of production and $Y_1$ and $Y_2$ respectively were inversely related. Also, $Y_1$ and $Y_2$ were depleted at an average rate of 11 and 9 milligrams per percentage increase in rate of production between the limits of 20% to 100% production. The regression of $Y_1$ and $Y_2$ on rate of production indicated that rate of production contributed significantly ($P \leq 0.01$) to the observed variation in these dependent variables (Appendix, Table 18).

Multiple regression of $Y_1$ on rate of production and number of eggs laid indicated that rate of production contributed significantly ($P \leq 0.01$) to the variation in $Y_1$ whereas the contribution due to number of eggs was not significant. Multiple regression of $Y_2$ on rate of production and number of eggs laid indicated that both independent variables contributed significantly ($P \leq 0.01$) to the observed variation in $Y_2$. Also, $Y_2$ was regressed on rate of production and number of eggs laid for hens that had laid 30 eggs or less and the results indicated that both independent variables contributed significantly ($P \leq 0.01$) to the observed variation in $Y_2$.

## Experiment III

Means, standard errors and coefficients of variation for the variables measured on 20 non-layers and 98 layers during Experiment III

are presented in Table 7 and Table 8 respectively. Mean terminal body weight of the non-layers was lower than that of the layers. Mean femur weight, femur weight per kilogram body weight and femur ash weight of the layers were higher than that of the non-layers. However, femur ash per kilogram body weight was similar for both groups.

Expressing bone weight and ash weight on the basis of body weight explained a great deal of the observed variation in these variables as indicated by the reduction of the respective standard errors. Expressing femur ash in terms of metabolic body size did not result in any further reduction of the standard error.

The depletion of femur ash which occurred at the onset of production and was inversely related to number of eggs laid is illustrated in Figure 11 and Figure 12. Femur ash was lost at an average rate of 5.9 milligrams and femur ash per kilogram body weight was lost at an average rate of 3.4 milligrams per egg laid. The regressions of $Y_1$ and $Y_2$ on number of eggs laid were significant at the 1% level (Appendix, Table 19).

The loss of femur ash associated with the production of the first 29 eggs is illustrated in Figure 13 and the data indicate the rate of loss was on the average 15 milligrams per kilogram of body weight per egg.

As indicated by the very small regression coefficient in Figure 14 and a regression analysis that was non-significant, variation in rate of production did not aid in the explanation of the observed variation in $Y_1$. A small but non-significant inverse relationship between rate of production and $Y_2$ is illustrated in Figure 15.

Multiple regression of $Y_1$ and $Y_2$ on rate of production and number of eggs laid indicated that rate of production did not contribute

Table 7. Statistics of variables measured on 20 non-layers during Experiment III

| | Mean ± S.E. | Coefficient of variation |
|---|---|---|
| Terminal body wt kg | 1.26 ± .03 | 11.3 |
| Femur wt g | 3.01 ± .09 | 13.6 |
| Femur ash g | 1.61 ± .06 | 17.0 |
| Femur wt g/kg body wt | 2.40 ± .07 | 13.3 |
| Femur ash g/kg body wt | 1.29 ± .05 | 16.3 |
| Femur ash g/kg body $wt^{3/4}$ | 1.36 ± .05 | 15.8 |

Table 8. Statistics of variables measured on 98 layers during Experiment III

| | Mean ± S.E. | Coefficient of variation |
|---|---|---|
| Terminal body wt kg | 1.55 ± .02 | 10.9 |
| Age at first egg | 198 ± 3.1 | 15.2 |
| Number of eggs | 27 ± 1.8 | 65.9 |
| Rate of production % | 80.7 ± 1.49 | 18.3 |
| Femur wt g | 3.34 ± .06 | 16.4 |
| Femur ash g | 1.88 ± .04 | 20.7 |
| Femur wt g/kg body wt | 2.15 ± .03 | 12.9 |
| Femur ash g/kg body $wt_{3/4}$ | 1.21 ± .02 | 17.2 |
| Femur ash g/kg body $wt^{3/4}$ | 1.35 ± .02 | 17.5 |

Figure 11. Effect of number of eggs laid on femur ash weight. Experiment III

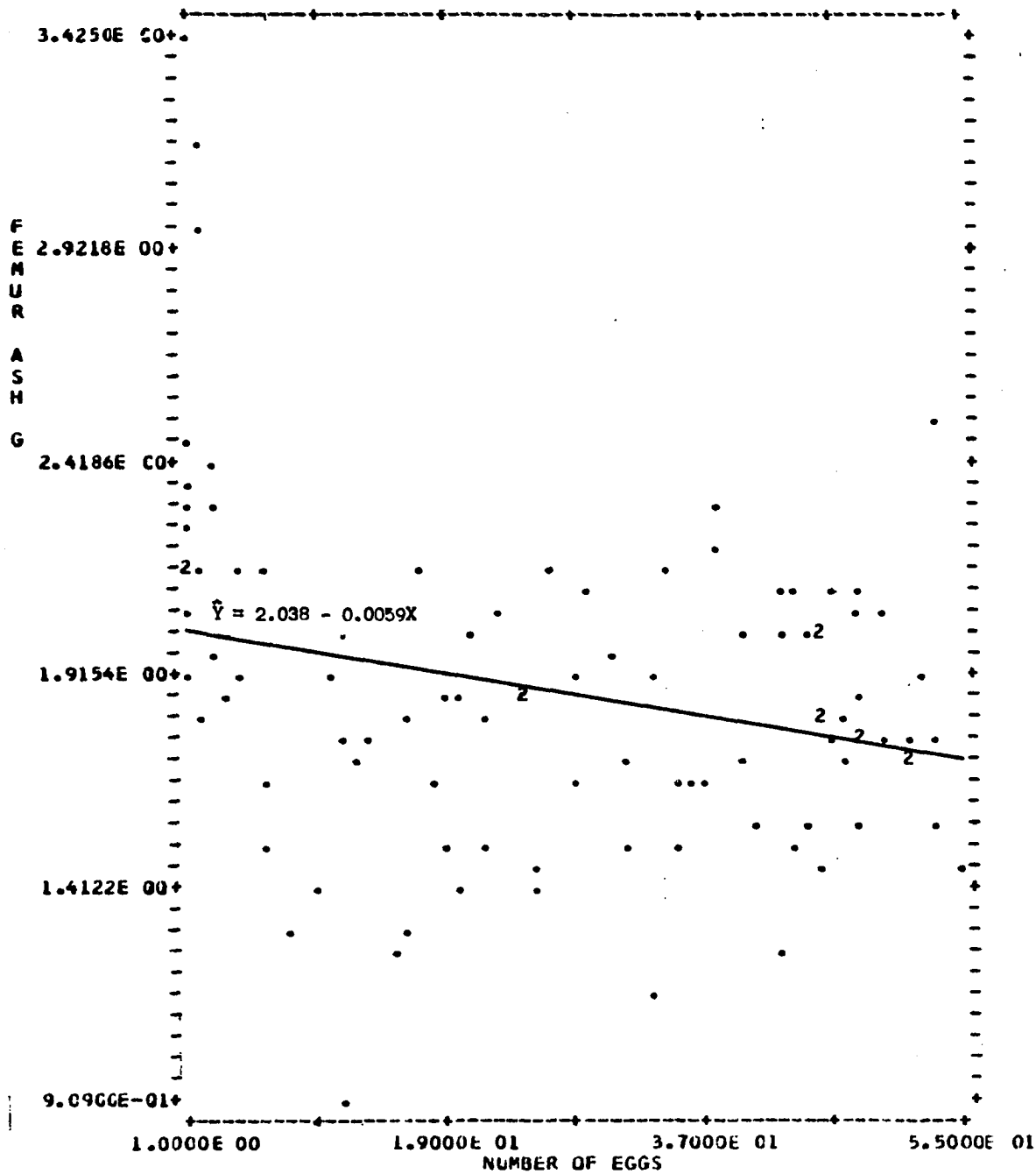

3.4250E 00
2.9218E 00
2.4186E 00
1.9154E 00
1.4122E 00
9.0900E-01
FEMUR ASH G
$\hat{Y}$ = 2.038 - 0.0059X
1.0000E 00
1.9000E 01
3.7000E 01
5.5000E 01
NUMBER OF EGGS

Figure 12. Effect of number of eggs laid on femur ash in grams per kilogram body weight. Experiment III

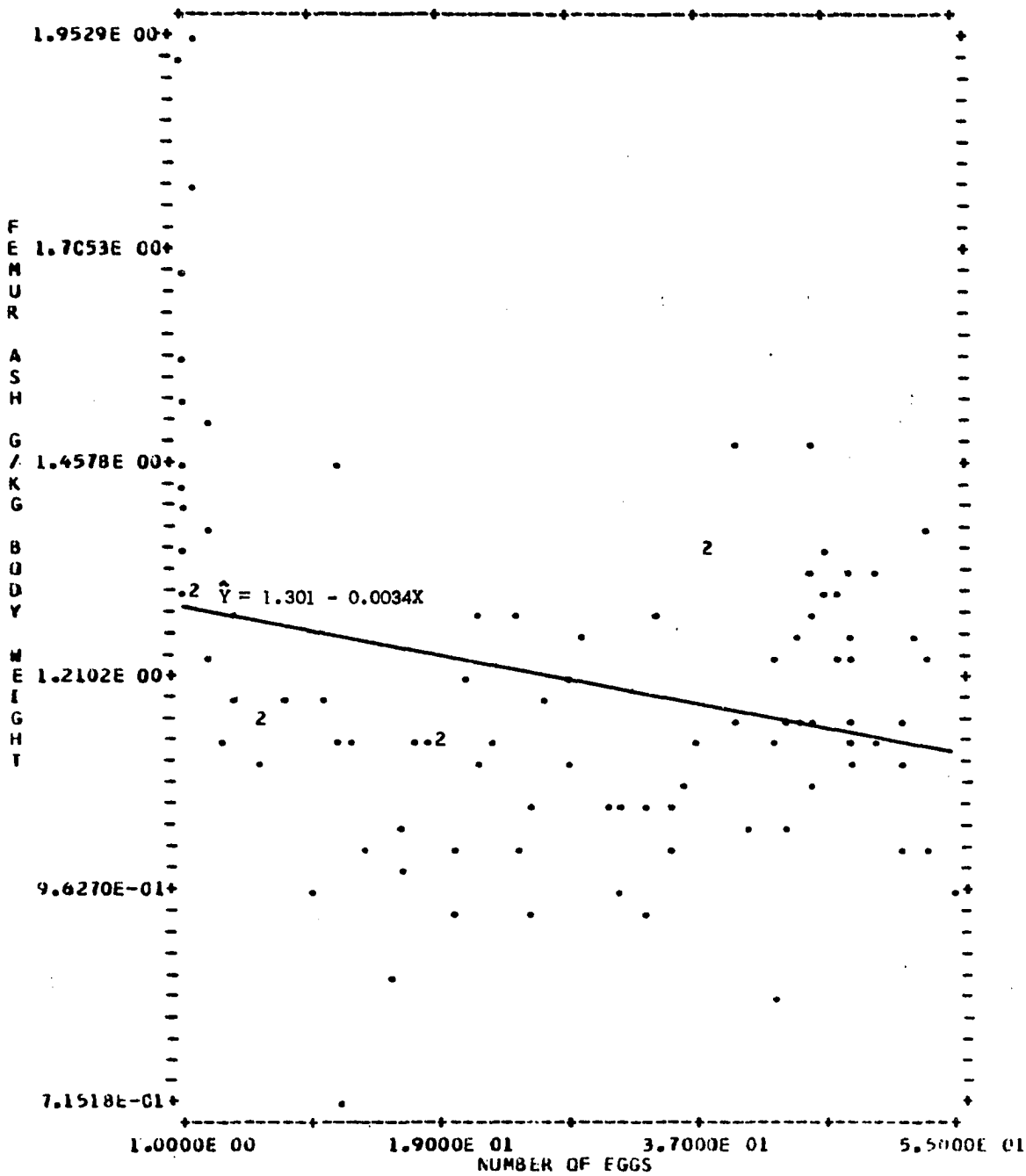

1.9529E 00
1.7053E 00
1.4578E 00
1.2102E 00
9.6270E-01
7.1518E-01
FEMUR ASH G/KG BODY WEIGHT
$\hat{Y}$ = 1.301 - 0.0034X
1.0000E 00
1.9000E 01
3.7000E 01
5.5000E 01
NUMBER OF EGGS

Figure 13. Depletion of femur ash in grams per kilogram body weight which occurred during the production of the first 29 eggs. Experiment III

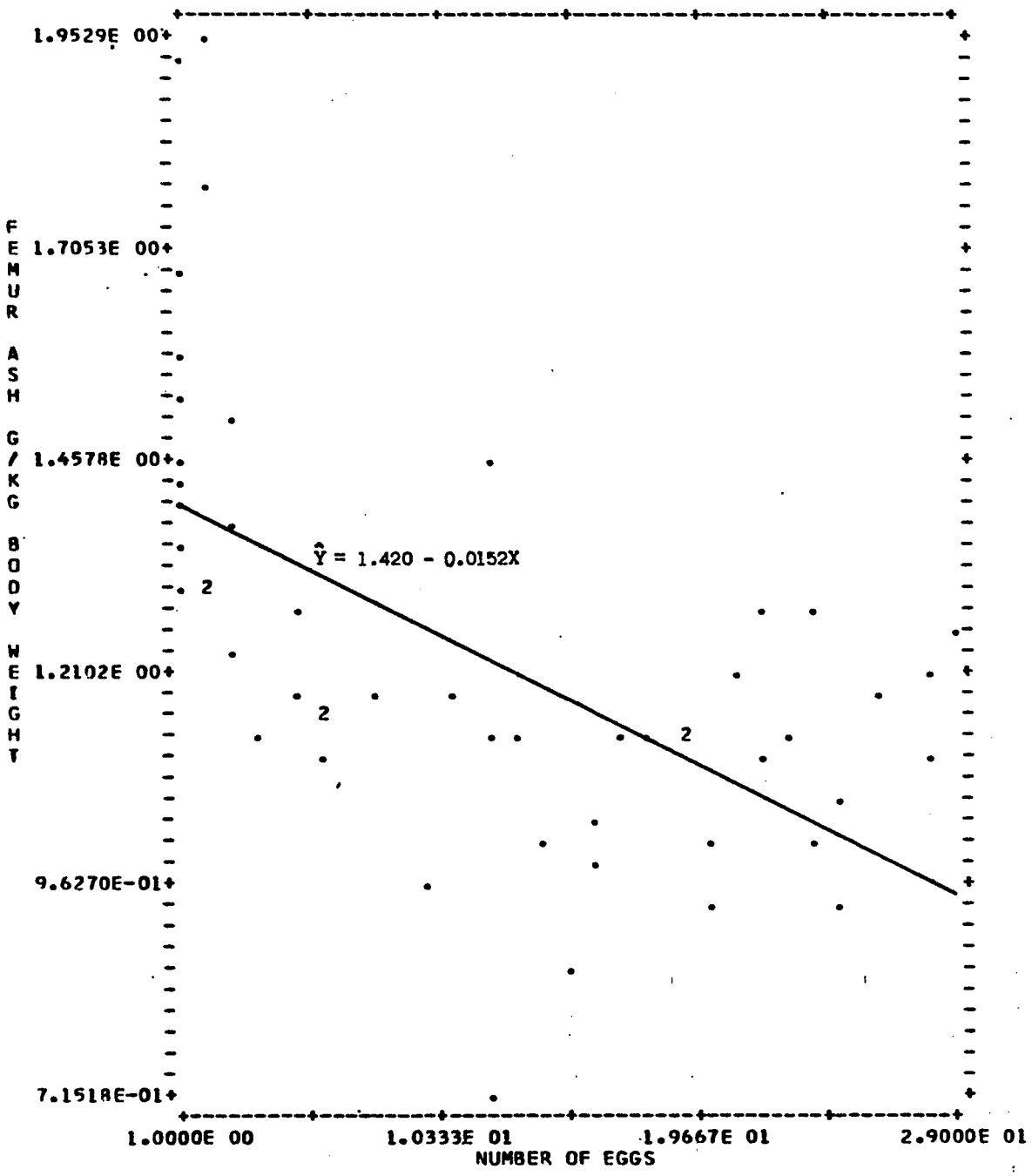

1.9529E 00
1.7053E 00
1.4578E 00
1.2102E 00
9.6270E-01
7.1518E-01
FEMUR ASH G/KG BODY WEIGHT
$\hat{Y}$ = 1.420 - 0.0152X
2
2
2
1.0000E 00
1.0333E 01
1.9667E 01
2.9000E 01
NUMBER OF EGGS

Figure 14. Effect of rate of production on femur ash weight. Experiment III

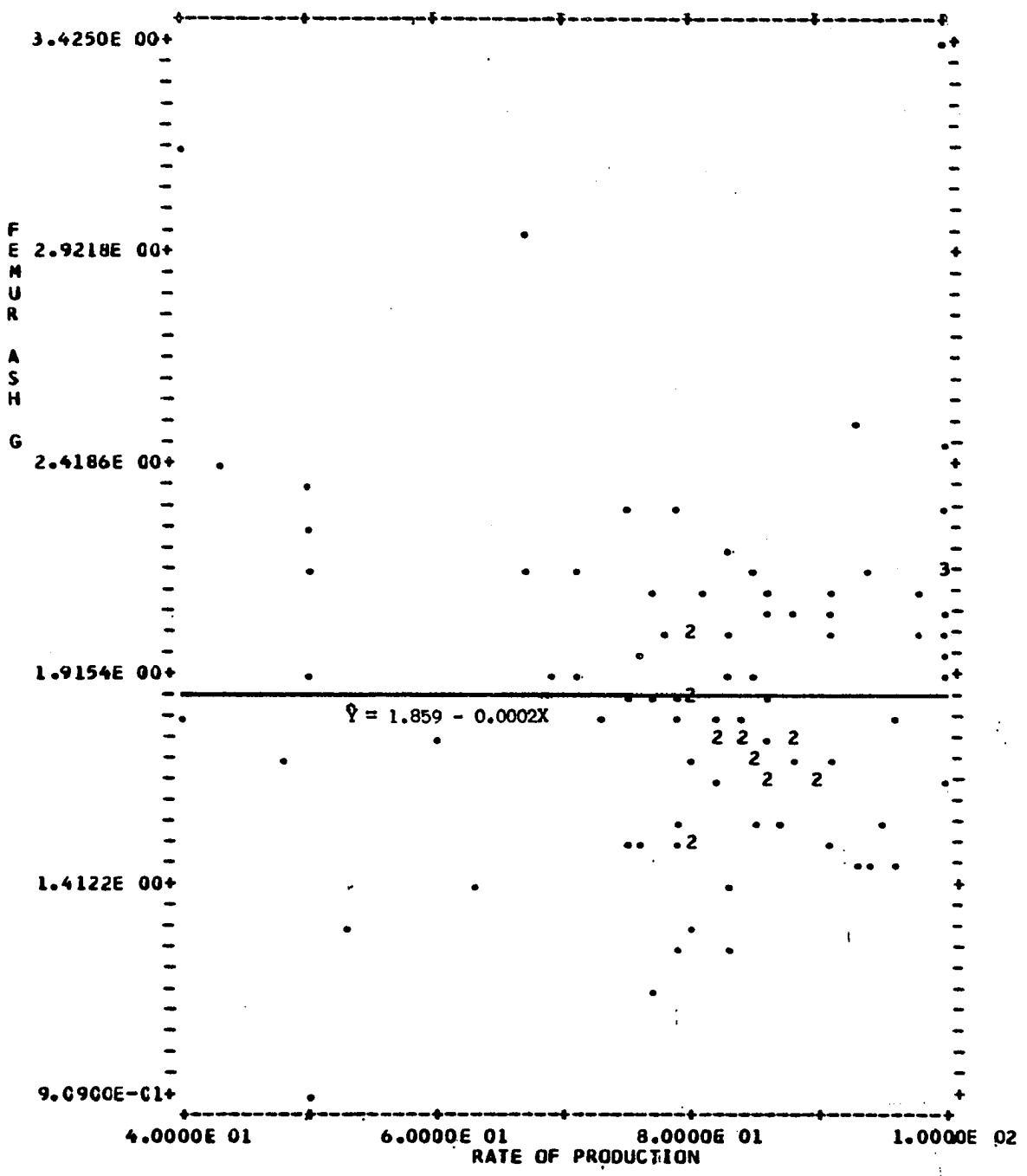
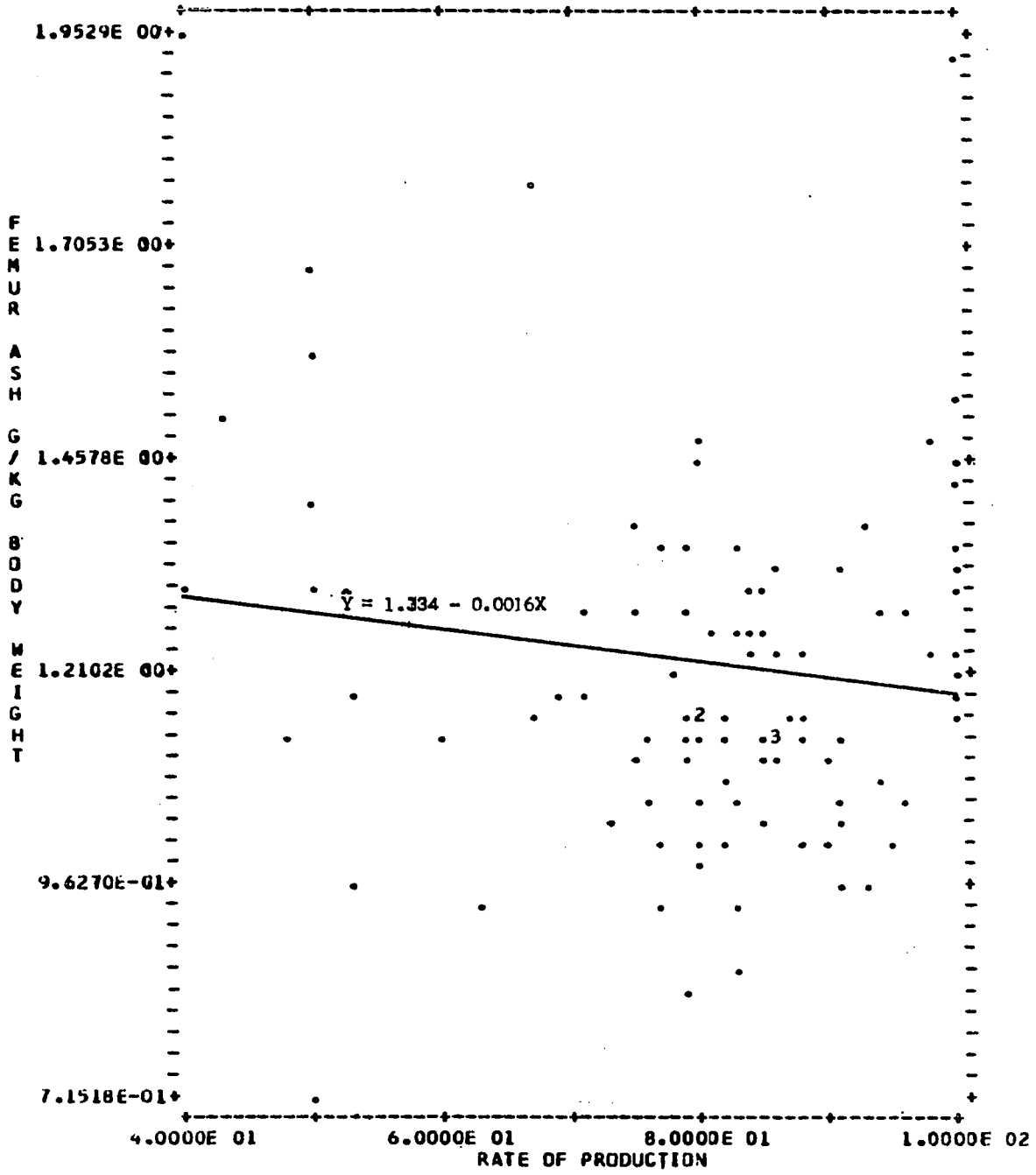

3.4250E 00+
2.9218E 00+
2.4186E 00+
1.9154E 00+
1.4122E 00+
9.0900E-01+
FEMUR ASH G
Ŷ = 1.859 - 0.0002X
4.0000E 01
6.0000E 01
8.0000E 01
1.0000E 02
RATE OF PRODUCTION

Figure 15. Effect of rate of production on femur ash in grams per kilogram body weight. Experiment III


1.9529E 00
1.7053E 00
1.4578E 00
1.2102E 00
9.6270E-01
7.1518E-01
FEMUR ASH G/KG BODY WEIGHT
Ŷ = 1.334 - 0.0016X
.2
.3
4.0000E 01
6.0000E 01
8.0000E 01
1.0000E 02
RATE OF PRODUCTION

significantly to the observed variation in $Y_1$ or $Y_2$ whereas number of eggs laid contributed significantly ($P \leq 0.01$) to the variation in both dependent variables.

## Experiment IV

Means, standard errors and coefficients of variation for variables measured on 7 males and 7 non-laying female broilers during Experiment IV are present in Table 9. As expected, the mean body weight and femur weight of the males was greater than that of the females but femur ash and femur weight in grams per kilogram body weight were similar for the two groups. Mean femur ash values, expressed on the basis of body weight or metabolic body weight, were higher for the non-laying females than for the male. Femur ash expressed as a percent of femur weight was substantially higher for the non-laying females (59.9%) compared to the males (50.8%).

Adjusting bone weight and ash weight for differences in body weight markedly reduced the respective standard errors but adjusting for metabolic body size did not result in any further reduction of the respective standard errors.

Means, standard errors and coefficients of variation for variables measured on 33 laying broiler-type females are presented in Table 10. Mean terminal body weight of the layers was similar to that of the non-laying females in Table 9. Mean layer femur weight and femur ash weight were 82.3% and 79.3% respectively of that in non-layers. That a loss of femur ash was associated with egg production is also indicated by the

Table 9. Statistics of variables measured on 7 non-laying female broilers with a developing ovary and 7 male broilers during Experiment IV[a]

| | Mean ± S.E. | Coefficient of variation |
|---|---|---|
| Non-layers with a developing ovary | | |
| Terminal body wt kg | 3.88 ± 0.14 | 9.6 |
| Femur wt g | 8.88 ± 0.32 | 9.6 |
| Femur ash g | 5.32 ± 0.21 | 10.3 |
| Femur wt g/kg body wt | 2.30 ± 0.08 | 8.9 |
| Femur ash g/kg body wt | 1.38 ± 0.05 | 9.5 |
| Femur ash g/kg body $wt^{3/4}$ | 1.93 ± 0.06 | 8.7 |
| Femur ash g/femur wt g % | 59.92 ± 0.27 | 11.8 |
| Males | | |
| Terminal body wt kg | 4.44 ± 0.15 | 8.8 |
| Femur wt g | 10.59 ± 0.29 | 7.2 |
| Femur ash g | 5.38 ± 0.14 | 6.9 |
| Femur wt g/kg body wt | 2.39 ± 0.03 | 3.1 |
| Femur ash g/kg body wt | 1.22 ± 0.03 | 7.0 |
| Femur ash g/kg body $wt^{3/4}$ | 1.76 ± 0.04 | 6.0 |
| Femur ash g/femur wt g % | 50.80 ± 0.82 | 4.2 |

[a]Range of ages of birds within each group was: 3 - 164 days; 2 - 171 days; 2 - 178 days.

Table 10. Statistics of variables measured on 33 laying broilers during Experiment IV

| | Mean ± S.E. | Coefficient of variation |
|---|---|---|
| Terminal body wt kg | 3.89 ± 0.06 | 8.1 |
| Age at first egg | 166 ± 1.3 | 4.4 |
| Number of eggs | 12 ± 1.1 | 53.1 |
| Rate of production % | 60.4 ± 3.0 | 28.2 |
| Femur wt g | 7.31 ± 0.18 | 13.8 |
| Femur ash g | 4.15 ± 0.13 | 17.5 |
| Femur wt g/kg body wt | 1.89 ± 0.05 | 14.8 |
| Femur ash g/kg body wt | 1.07 ± 0.03 | 17.5 |
| Femur ash g/kg body $wt^{3/4}$ | 1.50 ± 0.05 | 17.2 |

fact that mean femur ash expressed on the basis of body weight for layers was 77.5% that of non-layers.

A substantial reduction in the respective standard errors of femur weight and ash weight was observed when these variables were adjusted for differences in body weight. The explanation of the observed variation in bone weight and bone ash variables was not improved by expressing these variables in terms of metabolic body weight.

An observed inverse relationship between femur ash and number of eggs laid is present in Figure 16 and Figure 17 and the regression equations indicate femur ash was depleted at an average rate of 59 milligrams per egg and 18 milligrams per kilogram body weight per egg respectively. The regressions of $Y_1$ and $Y_2$ on number of eggs laid were significant at the 1% level (Appendix, Table 20).

A small but non-significant direct relationship between $Y_1$, $Y_2$ and rate of production were observed and are illustrated in Figure 18 and Figure 19 respectively.

Multiple regressions of $Y_1$ and $Y_2$ on rate of production and number of eggs laid indicated that only number of eggs laid contributed significantly to the observed variation in $Y_1$ and $Y_2$.

Figure 20 illustrates the development of medullary bone that takes place in the femur bone just prior to the onset of egg production. That the deposition of this medullary bone occurs on an organic matrix is suggested by the pillar arrangement observed. Also illustrated is a humerus bone, which contained no medullary bone, from the same hen. This suggests that either medullary bone does not develop in all bones of the

Figure 16. Effect of number of eggs laid on femur ash weight. Experiment IV

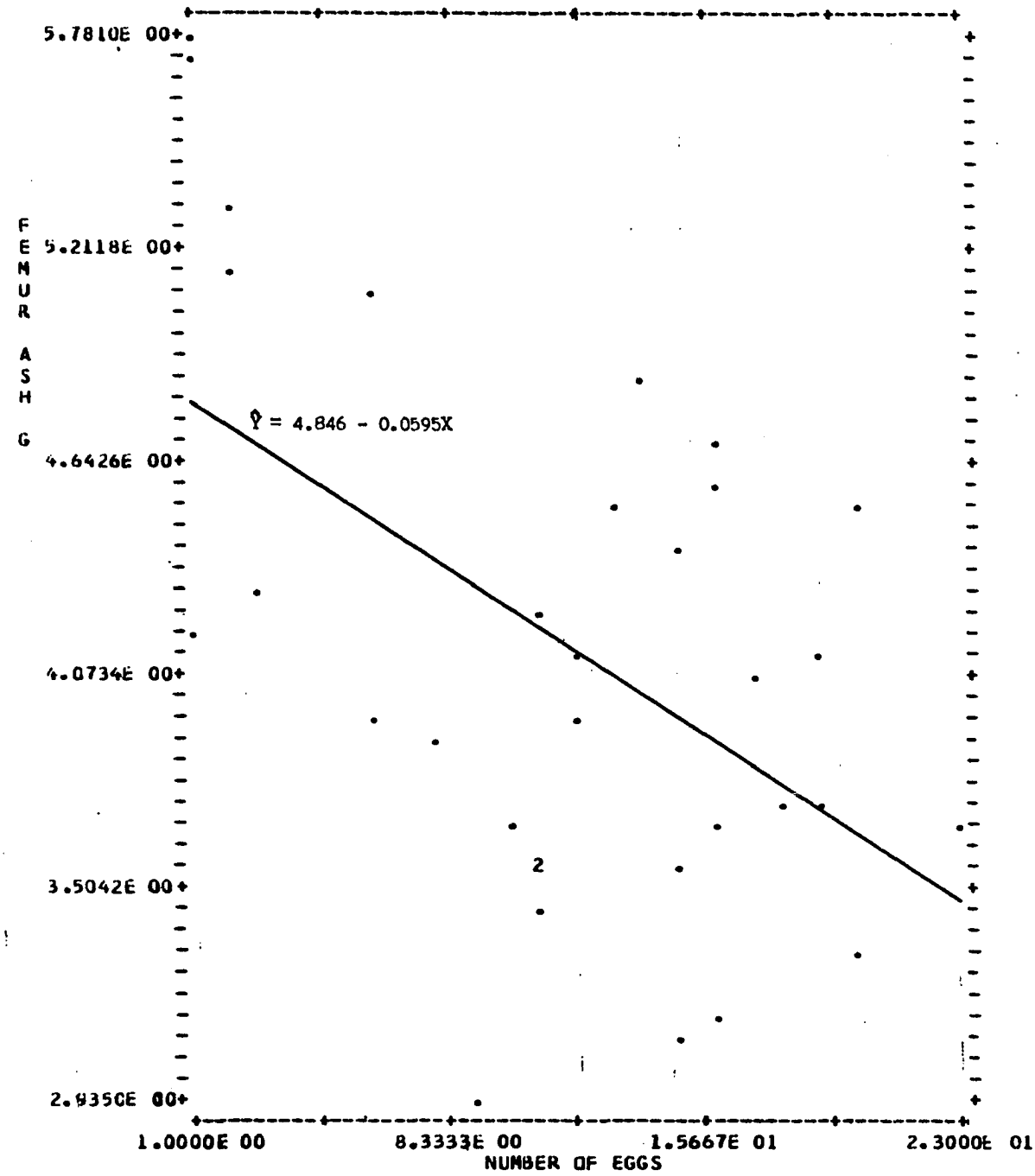

5.7810E 00
5.2118E 00
4.6426E 00
4.0734E 00
3.5042E 00
2.9350E 00
FEMUR ASH G
$\hat{Y}$ = 4.846 - 0.0595X
2
1.0000E 00
8.3333E 00
1.5667E 01
2.3000E 01
NUMBER OF EGGS

Figure 17. Depletion of femur ash in grams per kilogram body weight which occurred during the production of the first 23 eggs. Experiment IV

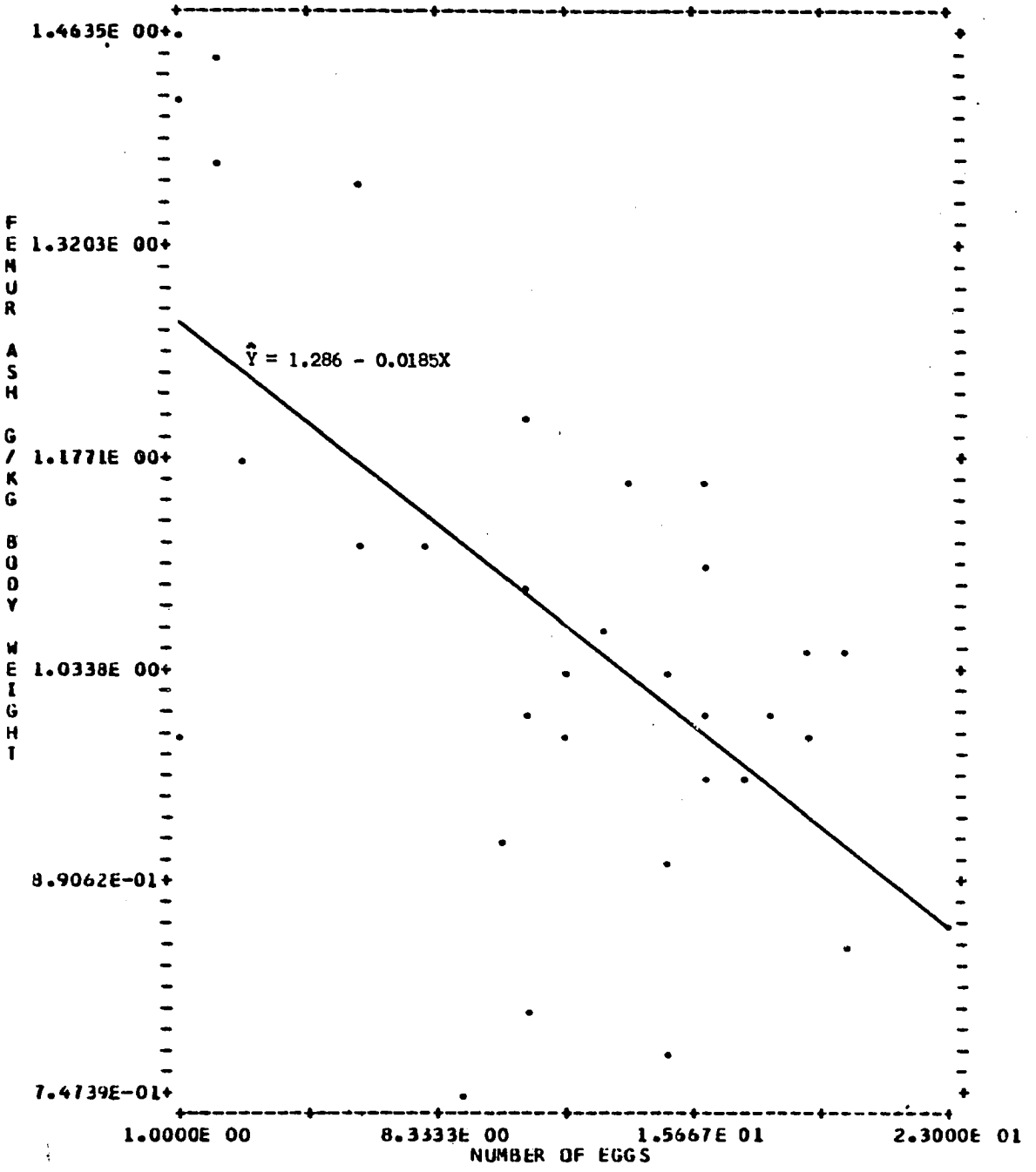

1.4635E 00
1.3203E 00
1.1771E 00
1.0338E 00
8.9062E-01
7.4739E-01
FEMUR ASH G/KG BODY WEIGHT
Ŷ = 1.286 - 0.0185X
1.0000E 00
8.3333E 00
1.5667E 01
2.3000E 01
NUMBER OF EGGS

Figure 18. Effect of rate of production on femur ash weight. Experiment IV

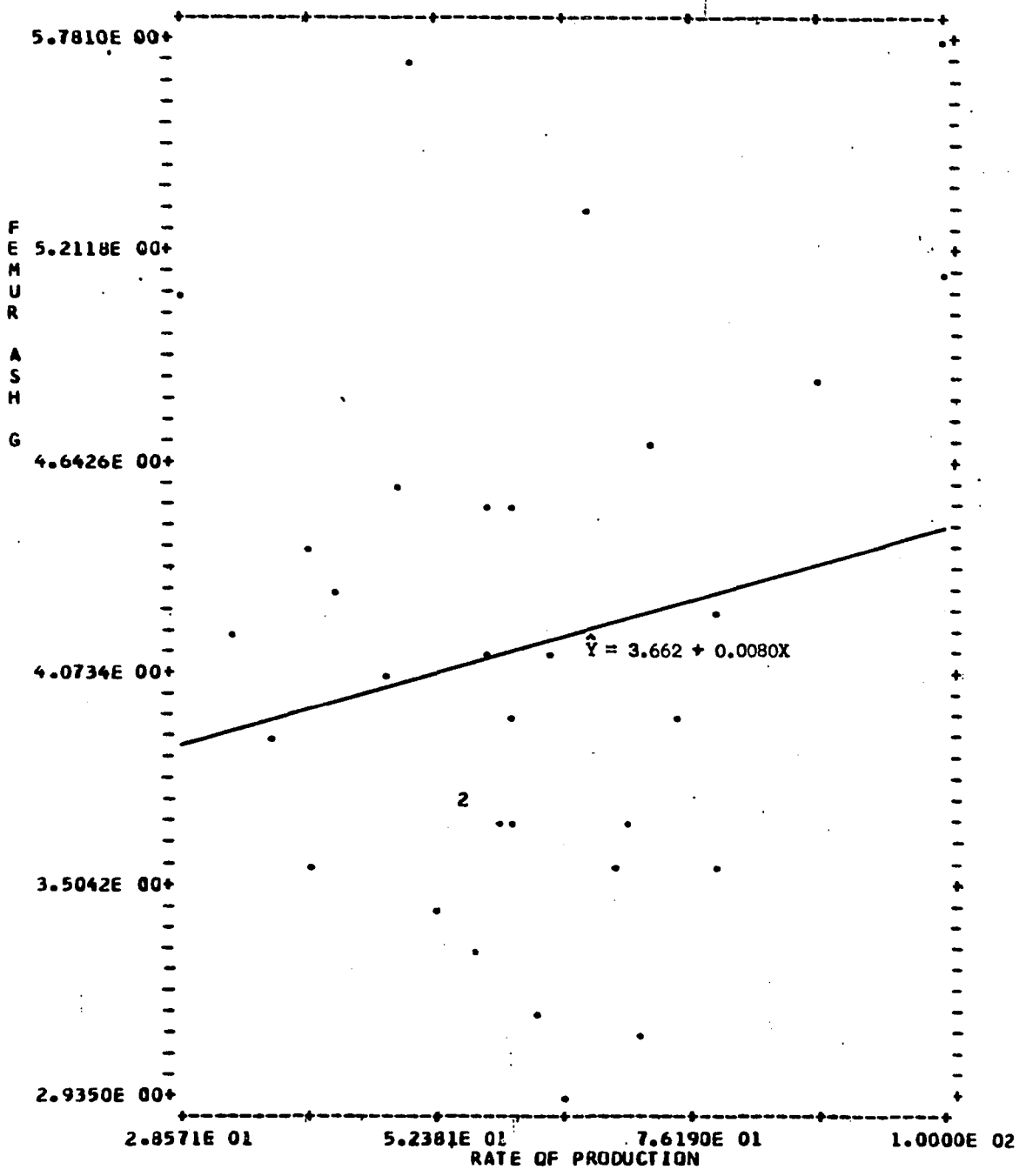
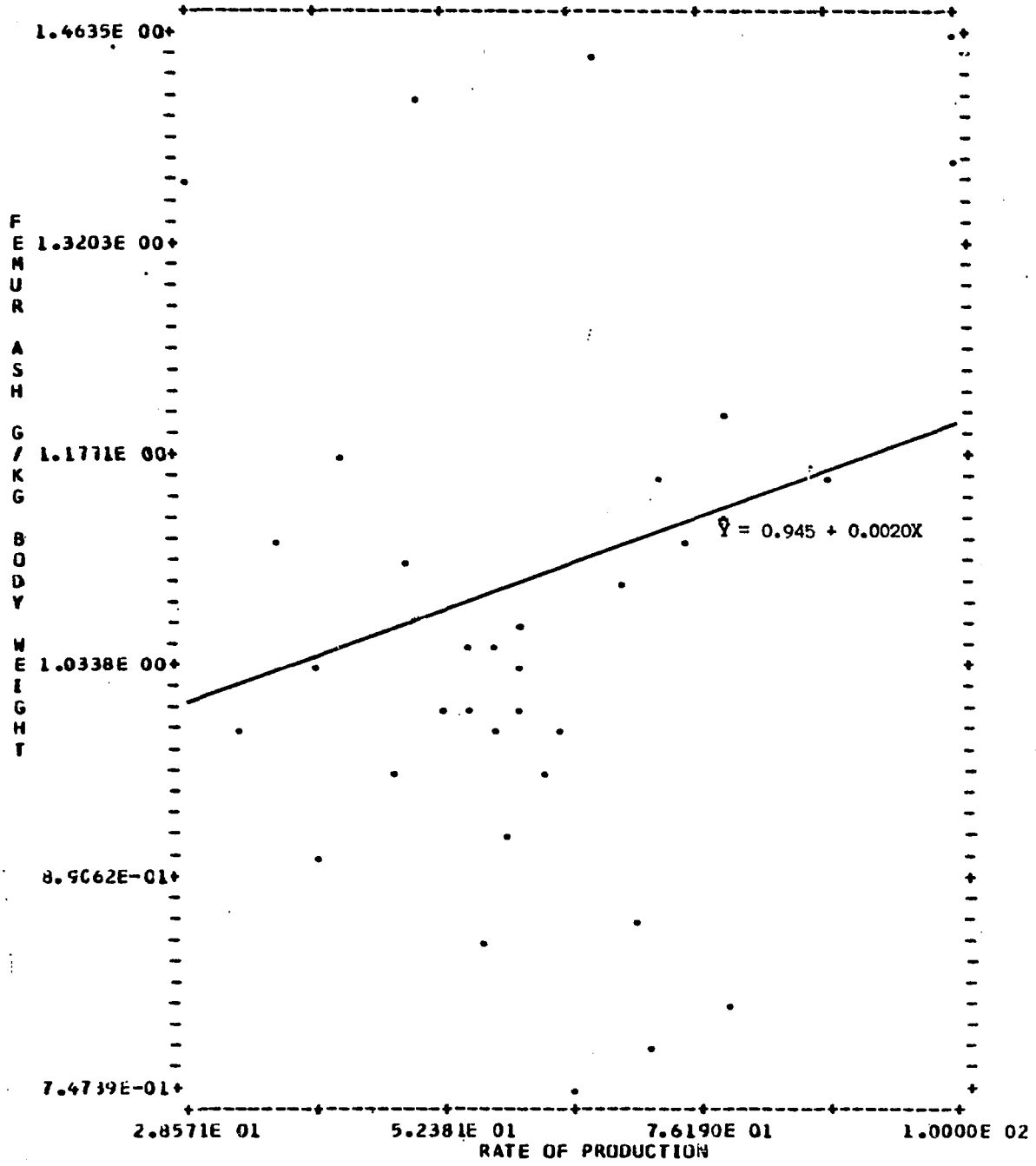

5.7810E 00+
5.2118E 00+
4.6426E 00+
4.0734E 00+
3.5042E 00+
2.9350E 00+
FEMUR ASH G
Ŷ = 3.662 + 0.0080X
2
2.8571E 01
5.2381E 01
7.6190E 01
1.0000E 02
RATE OF PRODUCTION

Figure 19. Effect of rate of production on femur ash in grams per kilogram body weight. Experiment IV


1.4635E 00
1.3203E 00
1.1771E 00
1.0338E 00
8.9062E-01
7.4739E-01
FEMUR ASH G/KG BODY WEIGHT
Ŷ = 0.945 + 0.0020X
2.8571E 01
5.2381E 01
7.6190E 01
1.0000E 02
RATE OF PRODUCTION

skeleton or the humerus was depleted of its medullary bone.

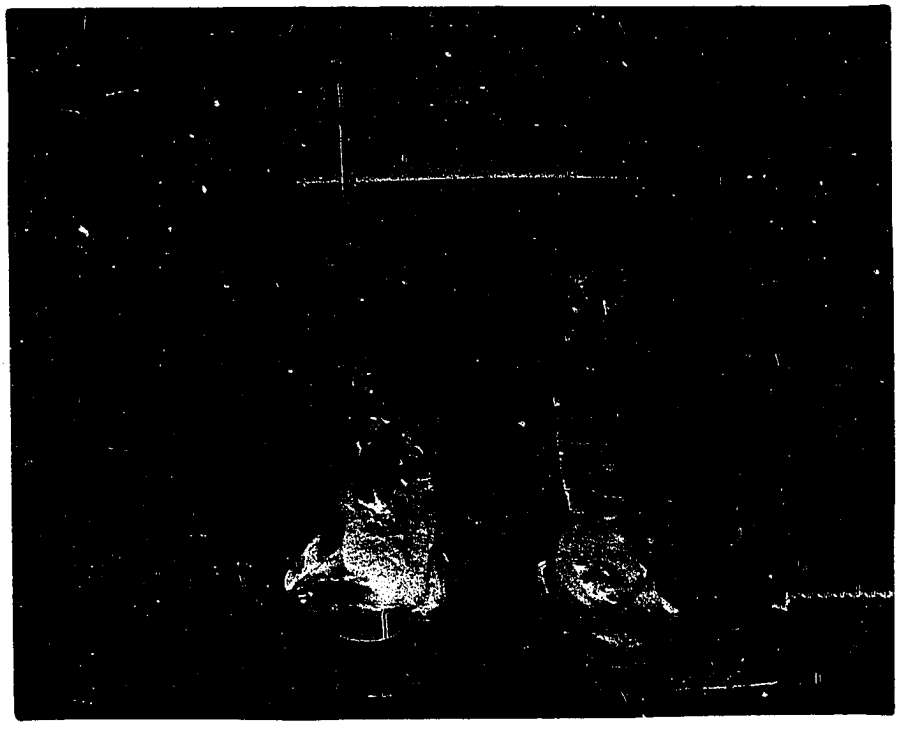


Figure 20. The development of medullary bone in the femur bone and lack of medullary bone in the humerus bone from the same hen

## Experiment V

Means and standard errors for body weight and fat-free dry total skeletal weights for 18 D-Line and 11 E-Line laying hens are presented in Table 11. As planned, the mean terminal body weight and mean fat-free dry total skeletal weight and ash weight were significantly different. Variation in skeletal weight was considerably greater than skeletal ash weight within each line as indicated by the magnitude of the respective

standard errors. Expressing the fat-free dry total skeletal weight and total skeletal ash weight on the basis of terminal body weight explained a great deal of the observed variation in these variables within each line as indicated by the reduction of the respective standard errors. After adjusting for differences due to body weight, mean total skeletal ash of the D-Line hens was approximately 3.6 grams heavier than that of the E-Line hens whereas unadjusted it was approximately 34.1 grams heavier.

Table 11. Body weights and fat-free-dry total skeletal weights for 43-week-old D- and E-Line laying hens

| Variable | D-Line Mean[a] ± S.E. | E-Line Mean[b] ± S.E. | "t" Statistic[c] |
|---|---|---|---|
| Terminal body wt kg | 1.96 ± 0.06 | 1.45 ± 0.06 | 8.50 |
| Total skeletal wt g | 88.40 ± 2.85 | 54.35 ± 2.47 | 8.24 |
| Total skeletal ash wt g | 51.58 ± 1.51 | 32.76 ± 1.42 | 8.44 |
| $\frac{\text{Total skeletal ash wt g}}{\text{Terminal body wt kg}}$ | 26.41 ± 0.45 | 22.78 ± 0.97 | 3.85 |
| $\frac{\text{Total skeletal ash wt g}}{\text{Terminal body wt kg}^{3/4}}$ | 31.18 ± 0.52 | 24.90 ± 0.97 | 6.01 |

[a]Mean of 18 laying hens.

[b]Mean of 11 laying hens.

[c]$t_{0.01,27} = 2.77$.

Means and standard errors of individual fat-free dry bone weights, bone weight as a percent of the total skeletal weight, individual bone ash weight, bone ash as a percent of the total skeletal ash, ash as a percent

of the bone, bone ash per kilogram body weight and bone ash on a metabolic body weight basis of 43-week-old D- and E-Line laying hens are presented in Table 12 and Table 13 respectively.

Corresponding individual bone weight and bone ash weight differed considerably between Lines (Figure 21 and Figure 22 respectively). However, when expressed as a percent of the total skeletal weight and total skeletal ash weight respectively these differences disappeared (Table 12 and Table 13). The data show that bone weights, within Lines, varied considerably more than bone ash weights as indicated by the smaller standard errors for bone ash weights. Also, expressing bone ash on the basis of body weight reduced the respective standard errors within each Line. The result of expressing femur ash weight and total skeletal ash weight on the basis of body weight is illustrated in Figure 23. The interspersion of observations, compared to Figure 22 in which, except for one point, the observations within each Line fell into separate groups, indicates a reduction in the relative differences between Lines and hence suggests that bone variables expressed on the basis of body weight would be a more critical measure of bone mineral status in the laying hen.

Individual bones within the skeleton differed considerably when expressed as bone ash as a percent of the bone weight (Table 12 and Table 13). The tarsus bones of D- and E-Line birds were the lowest in mean percent ash (52.2% and 53.9% respectively) whereas the humerus bones were the highest (61.6% and 64.4% respectively).

The procedure adopted to determine which bone(s) would serve best as an indicator of skeletal mineralization, was to calculate the

Table 12. Individual bone variable means[a] and standard errors for 43-week-old D-Line laying hens

| | Bone (g) | Percent of total skeleton weight | Ash (g) | Percent of total skeletal ash | Ash (%) | Ash g/kg body wt | Ash g/kg (body wt)$^{3/4}$ |
|---|---|---|---|---|---|---|---|
| Femur[b] | 5.41±.18 | 6.12±.06 | 3.09±.10 | 5.98±.06 | 57.1±0.53 | 1.58±.036 | 1.87±.042 |
| Tibia[b] | 6.60±.20 | 7.48±.08 | 3.94±.11 | 7.65±.07 | 59.8±0.38 | 2.02±.033 | 2.38±.037 |
| Humerus[b] | 3.94±.14 | 4.46±.08 | 2.42±.08 | 4.70±.08 | 61.6±0.48 | 1.24±.027 | 1.46±.032 |
| Ulna[b] | 2.07±.07 | 2.35±.05 | 1.25±.04 | 2.43±.05 | 60.4±0.59 | 0.64±.016 | 0.76±.018 |
| Radius[b] | 0.79±.02 | 0.89±.02 | 0.48±.02 | 0.93±.08 | 60.9±0.66 | 0.25±.005 | 0.29±.006 |
| Scapula[b] | 1.00±.03 | 1.14±.01 | 0.61±.02 | 1.19±.01 | 61.2±0.40 | 0.31±.005 | 0.37±.006 |
| Corocoid[b] | 1.88±.07 | 2.13±.02 | 1.14±.04 | 2.20±.02 | 60.3±0.45 | 0.58±.012 | 0.69±.015 |
| Clavicle[b] | 1.01±.05 | 1.13±.03 | 0.61±.03 | 1.18±.04 | 60.9±0.54 | 0.31±.011 | 0.37±.014 |
| Sternum[b] | 6.93±.29 | 7.84±.17 | 4.07±.16 | 7.90±.18 | 58.9±0.68 | 2.08±.056 | 2.46±.068 |
| Ribs | 3.69±.18 | 4.16±.12 | 2.09±.10 | 4.03±.13 | 56.7±1.13 | 1.07±.047 | 1.26±.055 |
| Pygostyle | 0.22±.01 | 0.25±.01 | 0.12±.01 | 0.24±.01 | 56.2±1.70 | 0.06±.002 | 0.07±.003 |
| Thor vert | 1.88±.10 | 2.13±.08 | 1.04±.05 | 2.02±.08 | 55.9±1.03 | 0.54±.023 | 0.63±.027 |
| Lumb vert | 4.10±.22 | 4.60±.01 | 2.32±.10 | 4.47±.11 | 57.1±0.97 | 1.18±.028 | 1.39±.038 |
| Neck | 7.49±.22 | 8.52±.20 | 4.34±.13 | 8.46±.22 | 58.1±1.06 | 2.24±.075 | 2.64±.080 |
| Metatarsus[b] | 6.18±.19 | 7.01±.08 | 3.64±.10 | 7.08±.09 | 59.1±0.50 | 1.87±.024 | 2.20±.028 |
| Tarsus | 3.55±.11 | 4.05±.11 | 1.85±.06 | 3.61±.10 | 52.2±0.98 | 0.95±.032 | 1.13±.035 |
| Metacarpus and carpus | 2.75±.69 | 3.10±.14 | 1.54±.09 | 2.97±.13 | 56.2±1.08 | 0.78±.034 | 0.93±.042 |
| Ilium, Ischium, Pubis | 7.17±.33 | 8.07±.14 | 4.11±.17 | 7.93±.12 | 57.6±0.81 | 2.10±.052 | 2.48±.066 |

[a]Average of 18 hens.

[b]Single bone.

Table 13. Individual bone variable means[a] and standard errors for 43-week-old E-Line laying hens

| | Bone (g) | Percent of total skeleton weight | Ash (g) | Percent of total skeletal ash | Ash (%) | Ash g/kg body wt | Ash g/kg (body wt)$^{3/4}$ |
|---|---|---|---|---|---|---|---|
| Femur[b] | 3.38±.17 | 6.22±.10 | 2.02±.11 | 6.14±.12 | 59.6±0.86 | 1.41±.086 | 1.54±.088 |
| Tibia[b] | 3.95±.18 | 7.29±.11 | 2.46±.11 | 7.51±.09 | 62.2±0.58 | 1.71±.082 | 1.87±.081 |
| Humerus[b] | 2.27±.12 | 4.18±.09 | 1.46±.07 | 4.46±.10 | 64.4±0.64 | 1.01±.041 | 1.11±.042 |
| Ulna[b] | 1.33±.08 | 2.44±.07 | 0.83±.05 | 2.53±.07 | 62.7±0.67 | 0.58±.031 | 0.63±.032 |
| Radius[b] | 0.51±.02 | 0.93±.02 | 0.32±.02 | 0.97±.02 | 62.7±0.40 | 0.22±.008 | 0.24±.008 |
| Scapula[b] | 0.63±.03 | 1.16±.02 | 0.39±.02 | 1.20±.02 | 62.7±0.58 | 0.27±.012 | 0.30±.012 |
| Corocoid[b] | 1.04±.05 | 1.91±.02 | 0.65±.03 | 1.97±.03 | 62.3±0.59 | 0.45±.022 | 0.49±.022 |
| Clavicle[b] | 0.58±.08 | 1.07±.03 | 0.36±.02 | 1.12±.03 | 62.6±0.48 | 0.25±.013 | 0.28±.013 |
| Sternum[b] | 3.68±.21 | 6.73±.12 | 2.21±.12 | 6.73±.10 | 60.4±0.73 | 1.53±.070 | 1.68±.075 |
| Ribs | 2.38±.14 | 4.37±.08 | 1.35±.08 | 4.10±.10 | 56.6±1.00 | 0.94±.054 | 1.02±.057 |
| Pygostyle | 0.14±.01 | 0.26±.01 | 0.08±.01 | 0.24±.01 | 55.2±1.65 | 0.05±.003 | 0.06±.004 |
| Thor vert | 1.56±.11 | 2.85±.11 | 0.93±.06 | 2.81±.10 | 59.8±0.97 | 0.64±.042 | 0.70±.044 |
| Lumb vert | 2.51±.14 | 4.60±.13 | 1.51±.08 | 4.60±.13 | 60.4±1.24 | 1.05±.059 | 1.15±.061 |
| Neck | 4.44±.18 | 8.20±.14 | 2.63±.09 | 8.09±.19 | 59.4±0.92 | 1.83±.051 | 2.00±.049 |
| Metatarsus[b] | 3.57±.13 | 6.61±.12 | 2.20±.07 | 6.77±.14 | 61.8±0.72 | 1.53±.047 | 1.67±.042 |
| Tarsus | 2.10±.09 | 3.90±.15 | 1.13±.03 | 3.48±.14 | 53.9±0.84 | 0.78±.025 | 0.86±.022 |
| Metacarpus and carpus | 1.99±.14 | 3.66±.19 | 1.10±.07 | 3.37±.18 | 55.7±1.18 | 0.76±.033 | 0.83±.038 |
| Ilium, Ischium, Pubis | 4.63±.27 | 8.48±.19 | 2.76±.17 | 8.38±.24 | 59.7±1.05 | 1.93±.131 | 2.10±.135 |

[a]Mean of 11 hens.

[b]Single bone.

Figure 21. The relationship between femur bone weight and total skeletal weight of D-Line and E-Line laying hens. Experiment V

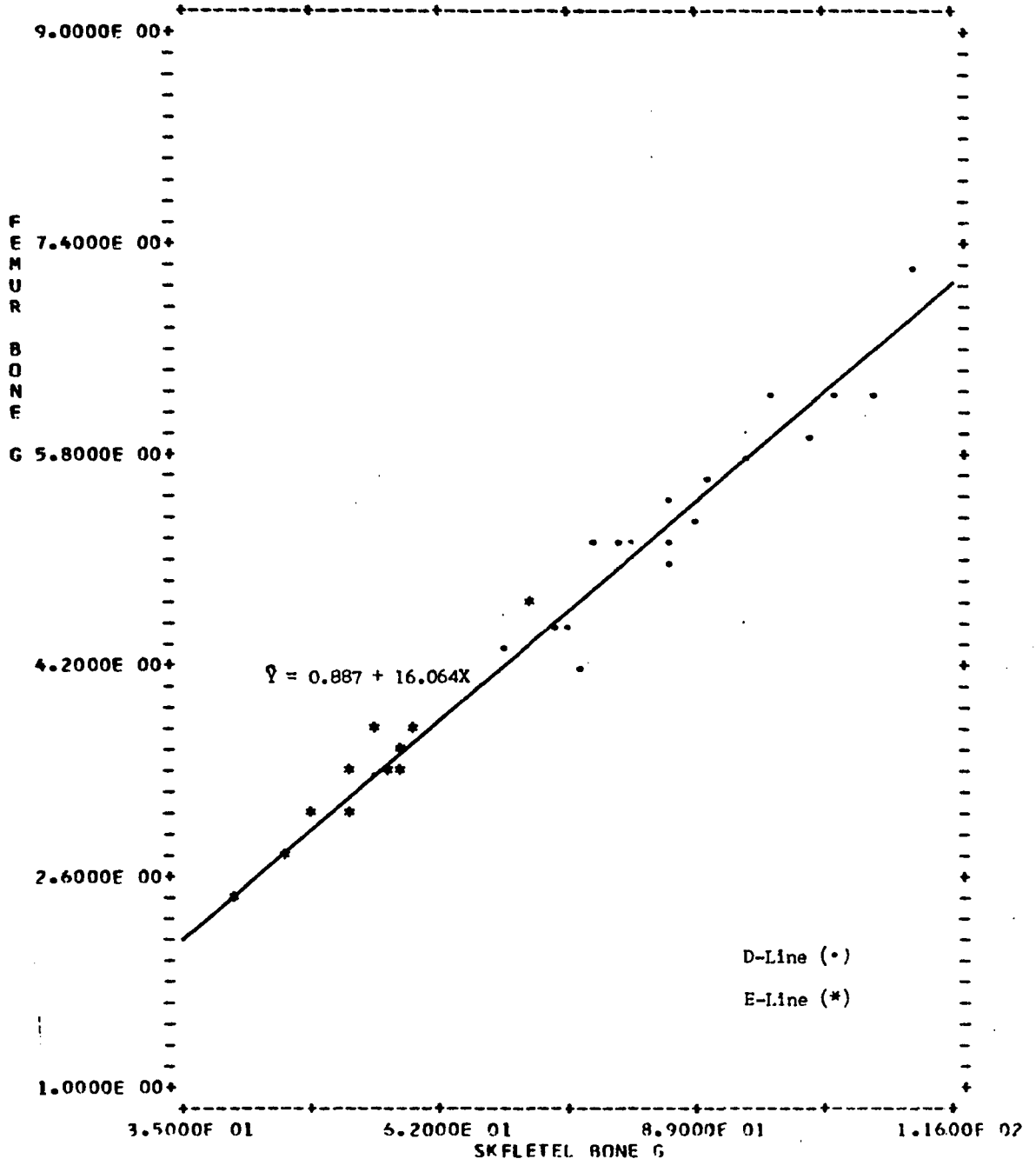

9.0000E 00
7.4000E 00
5.8000E 00
4.2000E 00
2.6000E 00
1.0000E 00
FEMUR BONE G
Ŷ = 0.887 + 16.064X
D-Line (•)
E-Line (*)
3.5000E 01
5.2000E 01
8.9000E 01
1.1600E 02
SKELETEL BONE G

Figure 22. The relationship between femur ash weight and total skeletal ash weight of D-Line and E-Line laying hens. Experiment V

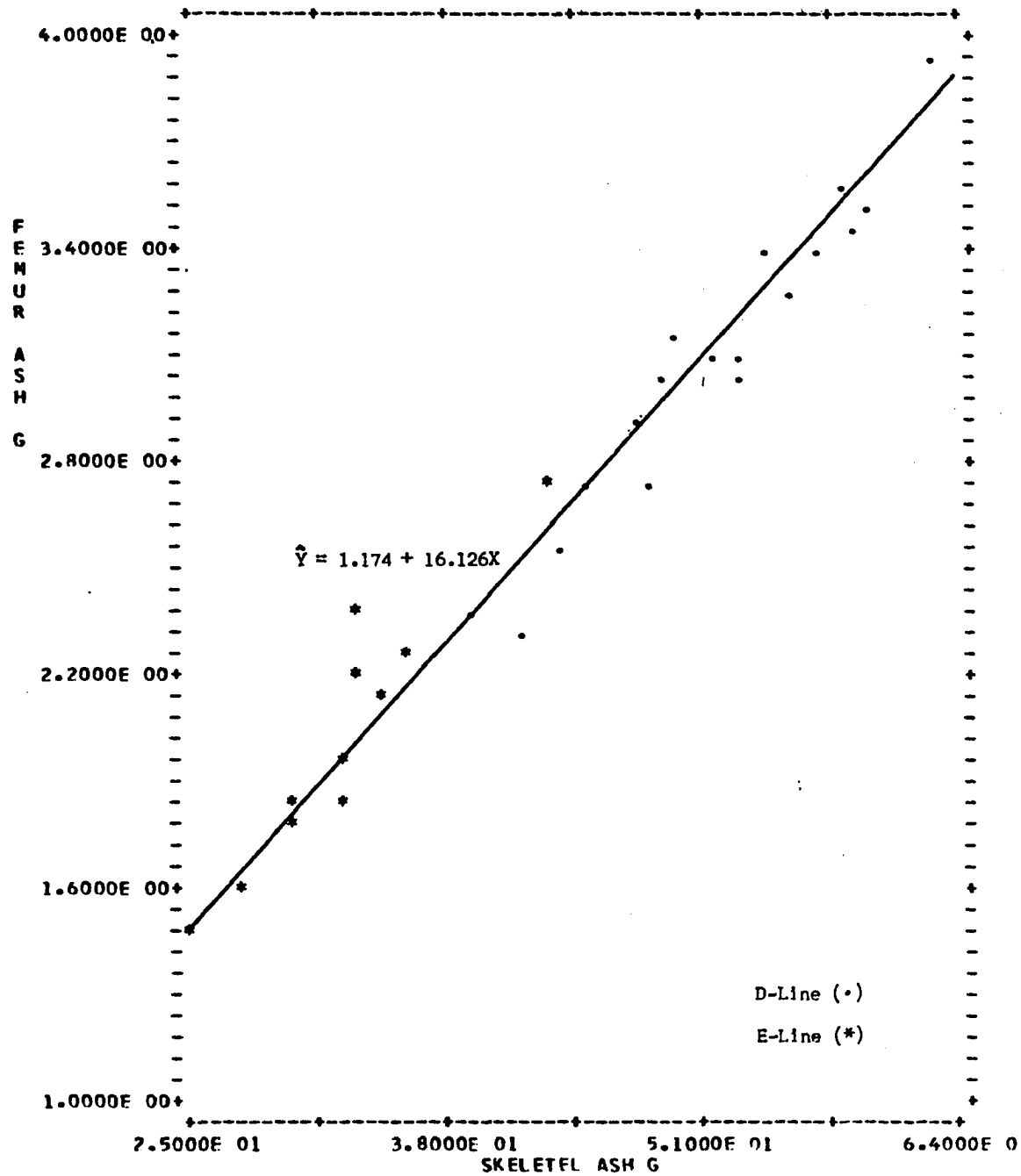
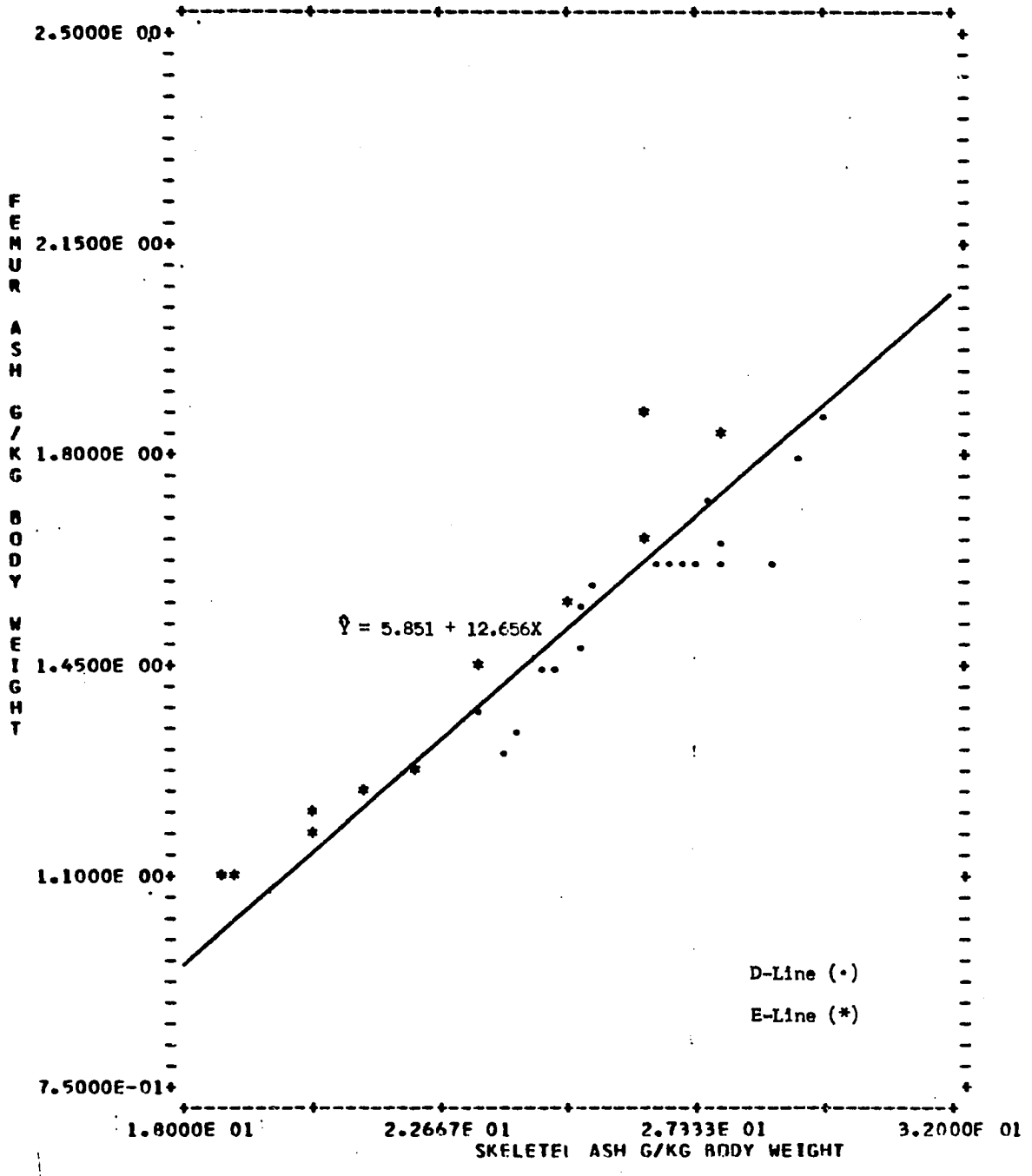

4.0000E 00
3.4000E 00
2.8000E 00
2.2000E 00
1.6000E 00
1.0000E 00
FEMUR ASH G
$\hat{Y}$ = 1.174 + 16.126X
D-Line (•)
E-Line (*)
2.5000E 01
3.8000E 01
5.1000E 01
6.4000E 01
SKELETEL ASH G

Figure 23. The relationship between femur ash weight in grams per kilogram body weight and total skeletal ash weight in grams per kilogram body weight. Experiment V


2.5000E 00
2.1500E 00
1.8000E 00
1.4500E 00
1.1000E 00
7.5000E-01
FEMUR ASH G/KG BODY WEIGHT
Ŷ = 5.851 + 12.656X
D-Line (•)
E-Line (*)
1.8000E 01
2.2667E 01
2.7333E 01
3.2000E 01
SKELETEI ASH G/KG BODY WEIGHT

regressions of total skeletal weight, total skeletal ash and total skeletal ash per kilogram body weight on each of the 19 groups of bones which had been weighed separately. Correlation coefficients for these regressions are presented in Table 14. The data show all bone weights were highly significantly ($P \leq 0.01$) correlated to total skeletal weight for both Lines. All bone ash weights of the D-Line birds were highly significantly ($P \leq 0.01$) correlated to total skeletal ash weight. Only tarsus ash weight of the E-Line birds was not highly significantly correlated to total skeletal ash weight.

When a great deal of the observed variation in total skeletal ash and bone ash weights was accounted for by expressing these variables on the basis of body weight, individual bone group correlation coefficients decreased and consequently some correlations which were previously highly significant became significant.

In both Lines studied, regardless of the measure of the independent variable, the three bones which would be the most practical to sample, showed the highest simple correlation coefficients and which were not significantly different from each other were the femur, tibia and corocoid.

In an attempt to obtain a higher correlation, total skeletal variables were regressed on two bones weighed individually. Accordingly, all possible combinations taken two at a time of the three bones showing the highest simple correlations were tested and the results are shown in Table 15. For the D-Line hens, the highest multiple correlation coefficient for skeletal weight resulted when the femur and corocoid bones

Table 14. Simple correlation coefficients for the regressions of total skeletal weight, total skeletal ash and total skeletal ash per kilogram body weight on individual bone weight, bone ash weight and bone ash per kilogram body weight respectively for D- and E-Line laying hens

| Regression[a] | D-Line | | | E-Line | | |
|---|---|---|---|---|---|---|
| | $Y_1$ on $X_1$ | $Y_2$ on $X_2$ | $Y_3$ on $X_3$ | $Y_1$ on $X_1$ | $Y_2$ on $X_2$ | $Y_3$ on $X_3$ |
| Femur | .972** | .982** | .965** | .977** | .973** | .988** |
| Tibia | .971** | .977** | .926** | .973** | .981** | .983** |
| Corocoid | .976** | .980** | .942** | .988** | .987** | .982** |
| Humerus | .933** | .920** | .789** | .956** | .940** | .939** |
| Ulna | .367** | .888** | .777** | .935** | .942** | .919** |
| Radius | .922** | .890** | .741** | .960** | .950** | .940** |
| Scapula | .971** | .962** | .873** | .977** | .972** | .964** |
| Clavicle | .930** | .928** | .722** | .886** | .868** | .902** |
| Metatarsus | .966** | .957** | .846** | .958** | .938** | .936** |
| Tarsus | .789** | .793** | .782** | .744** | .650* | .677* |
| Sternum | .917** | .898** | .721** | .990** | .990** | .971** |
| Ilium, Ischium, Pubis | .983** | .979** | .898** | .968** | .954** | .981** |
| Neck | .837** | .758** | .792** | .965** | .921** | .914** |
| Ribs | .894** | .893** | .896** | .972** | .957** | .960** |
| Metacarpus and carpus | .848** | .811** | .688** | .811** | .726** | .639* |
| Pygostyle | .899** | .865** | .782** | .937** | .868** | .718** |
| Thoracic vertebrae | .825** | .767** | .643** | .932** | .937** | .946** |
| Lumbar vertebrae | .946** | .934** | .579** | .936** | .925** | .928** |
| Sacral vertebrae | | | | .875** | .880** | .853** |

[a] $Y_1$ - total skeletal wt g; $Y_2$ - total skeletal ash g; $Y_3$ - total skeletal ash g/kg body wt; $X_1$ - bone wt g; $X_2$ - bone ash g; $X_3$ - bone ash g/kg body wt.

*Indicates significance at $P \leq 0.05$.

**Indicates significance at $P \leq 0.01$.

Table 15. Correlation coefficients for the multiple regression of the dependent variables on combinations of two separate independent variable weights for D- and E-Line laying hens

| Dependent variable Independent variables | D-Line | | | E-Line | | |
|---|---|---|---|---|---|---|
| | $Y_1$[a] | $Y_2$[b] | $Y_3$[c] | $Y_1$ | $Y_2$ | $Y_3$ |
| Femur and tibia | .964 | .978 | .944 | .965 | .972 | .988 |
| Femur and corocoid | .971 | .980 | .954 | .982 | .977 | .979 |
| Tibia and corocoid | .968 | .973 | .917 | .988 | .986 | .985 |

[a]$Y_1$ - total skeletal wt g.

[b]$Y_2$ - total skeletal ash wt g.

[c]$Y_3$ - total skeletal ash g/kg body wt.

were used whereas tibia and corocoid bones yielded the highest multiple correlation coefficient for the E-Line hens. The same combinations of bone ash weights yielded the highest multiple correlation coefficients with respective skeletal ash weights for each Line. When the dependent and independent variables were expressed as a ratio to body weight, the femur and corocoid combination yielded the highest multiple correlation coefficient for the D-Line hens whereas femur and tibia yielded the highest for the E-Line hens. The multiple correlation coefficients within and between Lines for these groups of bones were not significantly different from each other and thus suggest if a combination was desirable the femur and tibia would serve as good as any other pair.

Femur ash in grams per kilogram body weight was also regressed on the ash weight per kilogram of both femurs from the skeleton weighed individually to determine if any improvement in the correlation coefficient

would result and if the extra time required to prepare the second sample was justifiable. The correlation coefficients using one and both femur(s) were 0.964 and 0.949 respectively, thus indicating a single femur was adequate.

For prediction purposes, the D- and E-Line data were combined and prediction equations were derived by regression of total skeletal weight on the weight of a single femur bone ($\hat{Y}_1 = 0.887 + 16.06X_1$; $r = 0.985$); total skeletal ash weight on the ash weight of a single femur bone ($\hat{Y}_2 = 1.174 + 16.13X_2$; $r = 0.980$); and total skeletal ash in grams per kilogram body weight on a single femur ash weight in grams per kilogram body weight ($\hat{Y}_3 = 5.581 + 12.66X_3$; $r = 0.938$). These equations were tested on the skeletons from eight A-Line birds that were sacrificed at the same age but had produced a different number of eggs. The results are presented in Table 16. In general, the predicted values agreed quite well with the observed. The agreement improved, however, as the number of eggs laid by a hen increased, which is understandable considering that the predicted equations were derived from data obtained on hens that had been in production for at least 15 weeks. Also, expressing femur ash and total skeletal ash on the basis of body weight resulted in improved agreement between the observed and predicted values.

Table 16. Observed skeletal weight, skeletal ash weight and skeletal ash grams per kilogram body weight of eight A-Line hens and corresponding predicted values calculated from equations derived by the combination of the D-Line and E-Line data

| Hen | Terminal age days | Number of eggs laid | Rate[a] of production % | Skeletal wt g | | Skeletal ash g | | Skeletal ash g/kg body wt | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Observed | Predicted[b] | Observed | Predicted[c] | Observed | Predicted[d] |
| 1 | 252 | 1 | 50.0 | 61.69 | 66.44 | 34.47 | 36.60 | 19.98 | 21.97 |
| 2 | 252 | 3 | 60.0 | 73.60 | 71.39 | 40.11 | 39.77 | 22.95 | 23.18 |
| 3 | 252 | 33 | 70.2 | 50.21 | 46.40 | 27.11 | 24.87 | 19.90 | 19.50 |
| 4 | 252 | 40 | 69.0 | 68.47 | 71.49 | 37.59 | 40.52 | 21.22 | 23.28 |
| 5 | 252 | 42 | 71.2 | 56.24 | 55.73 | 30.25 | 31.00 | 18.01 | 19.79 |
| 6 | 252 | 56 | 73.7 | 77.35 | 78.01 | 44.28 | 45.74 | 22.68 | 23.76 |
| 7 | 252 | 63 | 81.8 | 78.16 | 74.65 | 45.87 | 44.58 | 22.45 | 22.52 |
| 8 | 252 | 72 | 92.3 | 49.44 | 49.36 | 27.47 | 27.93 | 17.29 | 17.07 |

[a]Rate of production = number of eggs laid/number of days in production.

[b]$\hat{Y} = 0.887 + 16.06X.$

[c]$\hat{Y} = 1.174 + 16.13X.$

[d]$\hat{Y} = 5.581 + 12.66X.$

# DISCUSSION

The initial problem confronted was to study factors affecting eggshell quality. In view of the reports of Driggers and Comar (1949) and Jowsey et al. (1956) that 25% to 65% of the calcium in the eggshell was derived from the skeleton of the producing hen it seemed logical that factors affecting the development and maintenance of the hen's skeleton (secondary bone) would have been studied in detail. To my knowledge there are no reports in the literature which illustrate the development of and factors affecting the maintenance of the laying hen's skeleton. As a result the experiments reported herein were designed to answer three basic questions: (1) which bone(s) would serve best as an indicator(s) of the status of mineralization of the skeleton of the laying hen; (2) in what units should the criteria for evaluation of mineral status be measured and (3) what factors affect the development and maintenance of secondary bone in the laying hen.

The most fundamentally important question to which an answer was sought was which bone(s) would serve best as an indicator(s) of the status of mineralization of the skeleton of the laying hen. Experiment V was conducted to attempt to answer this question. The two widely divergent body weight Lines were used for the purpose of covering the range of body weights of hens that would be used in commercial egg production operations. The results of Experiment V indicate that a single femur bone would be as good as any bone group sample for the prediction of skeletal weight. This is in contrast to the findings of Morris et al. (1966) who reported that a combination of the weights of tibia and corocoid bones weighed separately was the best predictor of skeletal

weight. A possible explanation for the discrepancy between the studies of Morris and those reported here might be that the 60 birds used by Morris et al. (1966) were from three different breeds and/or crossbreeds and from nine different dietary and/or environmental treatments, many of which would have resulted in a severe depletion of skeletons. The only variable in Experiment V was body weight of the two Lines. Also, Morris et al. (1966) did not attempt to standardize age at sacrifice, nor was number of eggs laid, rate of production or body weight reported for all birds used in their analysis. They did report, however, that some of the birds used had not laid an egg.

In the experiment reported here, a great deal of the observed variation in bone and skeletal weight within a Line and between the Lines was explained by adjusting for differences in body weight. This indicates a more sensitive measure for comparison of any treatment effects on skeletal development and maintenance would be a ratio of the skeletal variable to body weight.

Investigators have used percent ash of the tarsus bones as their criteria when evaluating the mineral status of a skeleton. Also, investigations for a biological vitamin D assay have been developed using percent ash of the tarsus bones (Baird and MacMillan, 1942), (Evans and Carver, 1944) and (Campbell et al., 1945a, b). The results from Experiment V indicate that there is a marked difference between bone groups within the skeleton with respect to percent ash. The tarsus were the lowest in bone ash percent, hence could not be expected to reflect the mineralization of all the other bone groups. Also, as reported by Morris et al. (1966), tarsus weight showed the smallest

correlation coefficient with total skeletal weight which again questions the use of the tarsus as an indicator of the status of the remainder of the skeleton.

In view of the large differences between bones within the skeleton in percent ash, bone weight would not be a reliable indication of the mineral content of the skeleton and, if one is interested in the mineral status, bone ash must be measured.

Variation among single femur ash weights explained as large or a larger proportion of the variation in total skeletal ash than any other individual bone ash weight, both femur ash weights taken separately or any combination of two of the three bone ash weights showing the highest simple correlation coefficients. This indicates that femur ash weight would serve as the best indicator of the status of skeletal mineralization of the laying hen. As was the case with bone weight, the variation in ash weight within and between Lines was markedly reduced by adjusting for variation in body weights.

The results of Experiment V thus indicate that femur ash would serve as the best indicator of the mineral status of the skeleton of the laying hen and that it should be expressed in grams of femur ash per kilogram of body weight. The observation that the variation in mineral content of the femur explained a larger proportion of the variation in total skeletal mineral content than the other bones suggests that femur mineral was more labile. This is in agreement with the reports of Hurwitz (1965) and Hurwitz and Bar (1966). The agreement between the observed and predicted skeletal weight and skeletal ash weight improved as the number

of eggs laid by the hens were used to test the prediction equation approached that of the hens used in the calculation of the prediction equations. This indicates a skeletal-status prediction equation for all periods in the production cycle must be derived from data collected from all periods in the production cycle in order to be very accurate. It also suggests that the status of the skeleton changes as the hen progresses through her production periods. Also, although the results are not reported herein, the prediction equation of Morris et al. (1966) was tested with the total skeletons observed in this study and the agreement between the observed and predicted values were in general not very good.

Variability is typical of experimental data and any method which can be used to reduce the inherent variability in the experimental material will increase the accuracy of experiments. Skeletal, bone and bone ash weights were highly variable in all experiments. Expressing these variables on the basis of body weight markedly reduced this variability hence bone weight or ash weight per kilogram live weight would be a more sensitive measure of the mineral status of skeleton of the laying hen than would these same values unadjusted for body weight.

Femur ash weight would not be a good criteria for evaluation of the mineral status of the laying hen because 1.5 and 2.0 kilogram hens conceivably could have the same femur ash weight of, for example, 3.0 grams and, looking only at the ash weight, tells us nothing of the relative mineral status of the femurs of these hens. Expressing femur ash in grams per kilogram body weight yields values of 2 and 1.5 respectively. If the value of 2 grams of femur ash per kilogram of body weight

represented a non-depleted femur mineral content, then 1.5 grams of femur ash per kilogram of body weight indicates that the femur of that hen had been depleted of 25% of its mineral.

Femur bone weight would be an even less reliable criterion than femur ash weight for evaluation of the mineral status for the same reason as above and also because of the difference in percent ash which could occur between a non-depleted and a depleted femur.

There were no dietary treatment differences imposed within an experiment after the birds were placed into individual wire laying cages for the collection of production records. Hence, the experiments can be looked upon as uniformity trials and an estimate of the variability can be obtained which will aid in the design of future experiments conducted to study factors affecting the development and maintenance of secondary bone.

A depletion of femur ash was observed to be concomitant with the onset of egg production and progressed rapidly during the production of approximately the first 30 eggs. This is indicated by depletion rates during this period of 17.1, 19.4, 15.6, and 18.1 milligrams of femur ash per kilogram of body weight per egg for Experiments I, II, III and IV respectively. This observation is in contrast to that of Hurwitz and Griminger (1960) who reported a negative calcium balance occurred during the production of only the first few eggs. If femur ash was the criterion used to assess the depletion, rates of 34.2, 21.4, 24.8 and 57.9 milligrams femur ash per egg were observed and the differences between experiments appear very large. In actual fact, differences in depletion

rates among experiments were small when adjustments for differences in body weight were made. When adjustment was made for differences in body weight, some adjustment was automatically made for differences between hens in feed consumption (hence mineral consumption) and size of egg produced (hence mineral excreted), which are both related to body size.

Rates of depletion of femur ash determined for the duration of the respective experiments cannot be compared because the regression coefficient within each experiment was calculated using a different number of hens which were in production for different lengths of time, hence had laid different numbers of eggs. Also, the differences between the rate of depletion during the production of the first 30 eggs and that of the duration of the respective experiments suggests that a simple linear relationship was not the best fit for the ranges in number of eggs laid during the corresponding experiments.

The variation in rate of production highly significantly affected the variation in femur ash (in grams per kilogram body weight) in Experiment II only. The explanation for this observation is not readily apparant at this time.

Many questions about the factors affecting development and maintenance of secondary bone remain unanswered and some of the answers may be obtained from data collected during the experiments reported herein. However, before these answers can be obtained further calculations will need be made by computer and hence funds acquired to pay for computer time.

## SUMMARY

An experiment using two genetic Lines of S. C. W. Leghorn hens which had been developed by selection for large and small body weights was conducted to study total skeletons for the selection of a sample bone(s) which would be most representative of the mineral status of the total skeleton. Three experiments using S. C. W. Leghorn egg production type chickens and one experiment using broiler type chickens were conducted to study factors that affect the development and maintenance of secondary bone in the domestic laying hen.

Physiological factors measured on individual birds were 140-day body weight, terminal body weight, terminal age, age at first egg, number of eggs laid, rate of egg production and fat-extracted dry bone and ash weight of individual bones.

Individual bones within the skeleton differed markedly with respect to percent ash. The tarsus bones were the lowest in bone ash percent (approximately 53%), while the humerus bones were the highest in bone ash percent (approximately 63%). In view of this large difference between individual bones within the skeleton in percent ash, individual bone weight or percent ash would not be a reliable indication of the mineral content of the skeleton of the laying hen.

A single femur bone and ash weight predicted total skeletal weight and total skeletal ash weight respectively as well as both femur bones, any other individual bone group or any combination of two bone groups weighed separately which showed the highest simple correlation coefficients with the total skeletal variables.

A skeletal-status prediction equation must be calculated from data collected from all periods in the production cycle of the laying hen. Also, dietary and environmental treatments used during the collection of the data involved in the calculation of this equation must be defined.

A depletion of femur ash was observed to be concomitant with the onset of egg production and progressed rapidly during the production of approximately the first 30 eggs. Rates of depletion during this period were 34.2, 21.4, 24.8 and 57.9 milligrams femur ash per egg in Experiments I, II, III and IV respectively. When an adjustment was made for differences in body weight, however, the depletion rates were 17.1, 19.4, 15.6 and 18.1 milligrams of femur ash per kilogram of body weight per egg were observed in Experiments I, II, III and IV respectively. This emphasizes that the criterion for the evaluation of the mineral status of the skeleton of the laying hen should be femur ash weight in grams per kilogram body weight.

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ACKNOWLEDGEMENTS

The gratitude which this author holds for Dr. S. L. Balloun, Chairman of the committee during these studies, is beyond the comprehension of anyone who might read this page.

The author wishes to express his sincere gratitude to Dr. D. F. Cox for his service on this committee, for his consultation on statistical analysis of the data and for his genuine concern for the student.

Thanks are extended to Dr. D. R. Griffith, Dr. R. C. Ewan and Dr. W. W. Marion for their courses, inspirations, friendship and service on this committee.

Thanks also are extended to Mrs. Kaminski (Jeannette) for her typing of this thesis.

Finally, to my wonderful wife Darlene and our sons James and Donald who have suffered a great deal of unavoidable neglect during this study, my sincere apology.

# APPENDIX

Table 17. Analysis of variance for data of Experiment I

| Dependent variable | Independent variable | d.f. | m.s. | F |
|---|---|---|---|---|
| $Y_1$[a] | | 61 | 0.242 | |
| | $X_1$[b] after $\bar{X}$ | 1 | 0.189 | 0.78 |
| Residual | | 60 | 0.243 | |
| $Y_1$ | | 61 | 0.242 | |
| | $X_2$[c] after $\bar{X}$ | 1 | 0.874 | 3.78 |
| Residual | | 60 | 0.231 | |
| $Y_1$ | | 61 | 0.242 | |
| | $X_1$ after $\bar{X}$ | 1 | 0.189 | 0.78 |
| | $X_2$ after $\bar{X}$, $X_1$ | 1 | 0.685 | 2.91 |
| Residual | | 59 | 0.235 | |
| $Y_2$[d] | | 61 | 0.061 | |
| | $X_1$ after $\bar{X}$ | 1 | 0.304 | 5.32* |
| Residual | | 60 | 0.057 | |
| $Y_2$ | | 61 | 0.061 | |
| | $X_2$ after $\bar{X}$ | 1 | 0.609 | 11.72** |
| Residual | | 60 | 0.052 | |
| $Y_2$ | | 61 | 0.061 | |
| | $X_1$ after $\bar{X}$ | 1 | 0.304 | 5.32* |
| | $X_2$ after $\bar{X}$, $X_1$ | 1 | 0.342 | 6.55* |
| Residual | | 59 | 0.052 | |

[a] $Y_1$ - femur ash g.

[b] $X_1$ - rate of production.

[c] $X_2$ - number of eggs laid.

[d] $Y_2$ - femur ash g/kg body wt.

*Indicates significance at $P \leq 0.05$.

**Indicates significance at $P \leq 0.01$.

Table 18. Analysis of variance for data of Experiment II

| Dependent variable | Independent variable | d.f. | m.s. | F |
|---|---|---|---|---|
| $Y_1$[a] | | 56 | 0.142 | |
| | $X_1$[b] after $\bar{X}$ | 1 | 2.733 | 28.62** |
| Residual | | 55 | 0.095 | |
| $Y_1$ | | 56 | 0.142 | |
| | $X_2$[c] after $\bar{X}$ | 1 | 1.619 | 13.99** |
| Residual | | 55 | 0.116 | |
| $Y_1$ | | 56 | 0.142 | |
| | $X_1$ after $\bar{X}$ | 1 | 2.733 | 28.62** |
| | $X_2$ after $\bar{X}$, $X_1$ | 1 | 0.217 | 2.32 |
| Residual | | 54 | 0.093 | |
| $Y_2$[d] | | 56 | 0.079 | |
| | $X_1$ after $\bar{X}$ | 1 | 1.760 | 36.35** |
| Residual | | 55 | 0.048 | |
| $Y_2$ | | 56 | 0.079 | |
| | $X_2$ after $\bar{X}$ | 1 | 2.087 | 49.15** |
| Residual | | 55 | 0.042 | |
| $Y_2$ | | 56 | 0.079 | |
| | $X_1$ after $\bar{X}$ | 1 | 1.760 | 36.35** |
| | $X_2$ after $\bar{X}$, $X_1$ | 1 | 0.763 | 21.69** |
| Residual | | 54 | 0.035 | |

[a] $Y_1$ - femur ash g.

[b] $X_1$ - rate of production.

[c] $X_2$ - number of eggs laid.

[d] $Y_2$ - femur ash g/kg body wt.

**Indicates significance at $P \leq 0.01$.

Table 19. Analysis of variance for data of Experiment III

| Dependent variable | Independent variable | d.f. | m.s. | F |
|---|---|---|---|---|
| $Y_1$[a] | | 97 | 0.150 | |
| | $X_1$[b] after $\bar{X}$ | 1 | 0.001 | 0.01 |
| Residual | | 96 | 0.015 | |
| $Y_1$ | | 97 | 0.150 | |
| | $X_2$[c] after $\bar{X}$ | 1 | 1.093 | 7.77** |
| Residual | | 96 | 0.141 | |
| $Y_1$ | | 97 | 0.150 | |
| | $X_1$ after $\bar{X}$ | 1 | 0.001 | 0.01 |
| | $X_2$ after $\bar{X}$, $X_1$ | 1 | 1.369 | 9.83** |
| Residual | | 95 | 0.139 | |
| $Y_2$[d] | | 97 | 0.043 | |
| | $X_1$ after $\bar{X}$ | 1 | 0.051 | 1.17 |
| Residual | | 96 | 0.043 | |
| $Y_2$ | | 97 | 0.043 | |
| | $X_2$ after $\bar{X}$ | 1 | 0.358 | 8.97** |
| Residual | | 96 | 0.040 | |
| $Y_2$ | | 97 | 0.043 | |
| | $X_1$ after $\bar{X}$ | 1 | 0.051 | 1.17 |
| | $X_2$ after $\bar{X}$, $X_1$ | 1 | 0.309 | 7.65** |
| Residual | | 95 | 0.040 | |

[a] $Y_1$ - femur ash g.

[b] $X_1$ - rate of production.

[c] $X_2$ - number of eggs laid.

[d] $Y_2$ - femur ash g/kg body wt.

**Indicates significance at $P \leq 0.01$.

Table 20. Analysis of variance for data of Experiment IV

| Dependent variable | Independent variable | d.f. | m.s. | F |
|---|---|---|---|---|
| $Y_1$[a] | | 32 | 0.525 | |
| | $X_1$[b] after $\bar{X}$ | 1 | 0.592 | 1.13 |
| Residual | | 31 | 0.523 | |
| $Y_1$ | | 32 | 0.525 | |
| | $X_2$[c] after $\bar{X}$ | 1 | 4.427 | 11.09** |
| Residual | | 31 | 0.399 | |
| $Y_1$ | | 32 | 0.525 | |
| | $X_1$ after $\bar{X}$ | 1 | 0.592 | 1.13 |
| | $X_2$ after $\bar{X}$, $X_1$ | 1 | 4.159 | 10.35** |
| Residual | | 30 | 0.402 | |
| $Y_2$[d] | | 32 | 0.035 | |
| | $X_1$ after $\bar{X}$ | 1 | 0.038 | 1.10 |
| Residual | | 31 | 0.035 | |
| $Y_2$ | | 32 | 0.035 | |
| | $X_2$ after $\bar{X}$ | 1 | 0.427 | 19.24** |
| Residual | | 31 | 0.022 | |
| $Y_2$ | | 32 | 0.035 | |
| | $X_1$ after $\bar{X}$ | 1 | 0.038 | 1.10 |
| | $X_2$ after $\bar{X}$, $X_1$ | 1 | 0.407 | 18.19** |
| Residual | | 30 | 0.022 | |

[a] $Y_1$ - femur ash g.

[b] $X_1$ - rate of production.

[c] $X_2$ - number of eggs laid.

[d] $Y_2$ - femur ash g/kg body wt.

**Indicates significance at $P \leq 0.01$.

Table 21. Analysis of variance for hens from Experiments I, II and III that had laid between one and thirty eggs

| Experiment | Dependent variable | Independent variable | d.f. | m.s. | F |
|---|---|---|---|---|---|
| I | $Y_2$[a] | | 34 | 0.077 | |
| | | $X_1$[b] after $\bar{X}$ | 1 | 0.077 | 1.00 |
| | | $X_2$[c] after $\bar{X}$, $X_1$ | 1 | 0.731 | 12.75** |
| | Residual | | 32 | 0.057 | |
| II | $Y_2$ | | 44 | 0.088 | |
| | | $X_1$ after $\bar{X}$ | 1 | 1.435 | 25.16** |
| | | $X_2$ after $\bar{X}$, $X_1$ | 1 | 1.083 | 33.23** |
| | Residual | | 42 | 0.026 | |
| III | $Y_2$ | | 50 | 0.062 | |
| | | $X_1$ after $\bar{X}$ | 1 | 0.026 | 0.42 |
| | | $X_2$ after $\bar{X}$, $X_1$ | 1 | 0.996 | 22.80** |
| | Residual | | 48 | 0.044 | |

[a]$Y_2$ - femur ash g/kg body wt.

[b]$X_1$ - rate of production.

[c]$X_2$ - number of eggs laid.

**Indicates significance at $P \leq 0.01$.