Factors Influencing Bone Fragility in Chickens

By

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In the distance,
A mournful wail of a train.
It calls to all,
Come wander with me.
All hear,
And a few obey.
They are trapped.
To them the call,
Must be obeyed.
They must follow.
Thru sorrow and happiness.
Follow it to the end,
And even Further.

K. L. Hayden
(July 8, 1963)

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FACTORS INFLUENCING BONE FRAGILITY IN CHICKENS

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Bone fragility is a condition which often occurs when old hens are processed. The bone breakage associated with this condition can cause downgrading of ready-to-cook hens and bone fragments in cooked hen products can cause serious economic loss.

The purpose of the six experiments reported in this dissertation was to study factors influencing bone fragility. A modification to the Allo-Kramer Shear Press was used to determine the breaking strength of tibiae, and this along with tibia ash values were used as the criteria for measuring bone fragility.

In the first experiment a comparison was made in tibia breaking strength between caged and floor layers and roosters. It was found that the breaking strength of bones from hens and roosters maintained on the floor was significantly greater than for those birds maintained in cages. Bone ash was also significantly higher for the floor birds.

In Experiment 2, it was found that hens maintained in individual cages had significantly reduced bone strength and tibia ash values when compared to hens maintained in either pens with peanut hulls

used for litter or raised wire floors. There was no difference between floor pens with peanut hull litter and those containing raised wire floors.

The third experiment was conducted to study the influence of dienestrol diacetate and Protamone on bone strength of chickens, since it has been shown that hormones can increase blood calcium levels. It was found that feeding a level of 622 milligrams of Protamone per kilogram of diet resulted in significantly strengthening bones of both male and female chickens. The addition of 1408 milligrams of dienestrol diacetate per kilogram of diet significantly increased breaking strength of the bones of hens; however, it decreased the breaking strength of the bones of males.

It was found that increasing the level of Protamone from 622 milligrams per kilogram to 2488 milligrams resulted in a numerical, but not significant, improvement in bone breaking strength. Increasing the dietary calcium level from 3 to 6 percent resulted in increasing bone strength. The increases from dietary calcium and the addition of Protamone were found to be additive. Increasing the calcium level from 3 to 6 percent resulted in an increase in tibia ash.

In Experiment 4, three trials were conducted utilizing 12 strains of egg-production-type pullets to determine if tibia breaking strength and tibia ash varied among strains. The data indicated that there was a significant strain difference in tibia strength but that there was no apparent difference in tibia ash among strains. It was also found that caged hens had a significantly lower tibia breaking strength when compared to floor hens of the same strain.

The fifth experiment was run in order to determine the length of time necessary to develop a difference in bone characteristics

of cage and floor pullets and was found to be four weeks, with a maximum difference between groups occurring at the end of eight weeks. It was further observed that tibia ash was closely associated with breaking strength but was not as sensitive a measurement of bone fragility as was tibia breaking strength.

The effect of raising broilers in wire batteries on bone characteristics was studied in the last experiment. Bone breaking strength and tibia ash were not substantially different when battery-grown broilers were compared to broilers grown in floor pens. This would indicate that bone breakage, a characteristic of caged layers, does not occur to any large extent in eight-week-old battery-grown broilers.

INTRODUCTION

Bone fragility of hens, particularly caged layers, is a condition which often is of economic importance to the processor of ready-to-cook hens. This condition becomes a factor of importance during processing when pickers, especially batch-type, break or shatter the birds' bones during feather removal. This bone breakage causes downgrading which necessitates a lower selling value and consequently a loss of revenue to the poultryman. There is also a problem of bone fragments in cooked products. With the advent of more cage layer operations this condition has become such a problem that many processors discriminate against or even refuse to buy caged layers. Although the mineral composition of the diet is increased for caged hens as compared to those maintained on the floor the problem of bone fragility has not been solved. As the number of cage operations increases, the problem of bone fragility will become more of an economic factor and, in the event that broilers are someday grown in large numbers in wire floored pens, they too could be discriminated against by the processor. Hood et al. (1955) found that broken bones were the third most important factor in causing downgrading of eviscerated carcasses and Lloyd et al. (1970) reported 22.2 percent bone breakage from broilers grown in plastic coops.

The fragility of bones in hens appears to be due to a condition called avian osteoporosis. This involves a reduction in bone density and mineralization which can reduce bone strength. A reduction in bone

density takes place either by osteoporosis or by osteomalacia. The most important of these two is osteoporosis or deossification. In this condition there is a quantitative loss of bone tissue. Lachman (1955) and Reifenstein (1957) described osteomalacia as a condition in which there was normal bone matrix formation without sufficient calcification. They termed osteoporosis as inadequate formation of matrix accompanied with lowered mineralization.

Osteomalacia is the counterpart of rickets occurring in adult animals and is characterized by soft flexible bones which bend rather than break when under pressure. This condition does not appear to be a factor in bone fragility.

The exact mechanism of osteoporosis is not known, but it appears to be associated with one or more of the following factors: (1) a calcium imbalance which could be due to lower estrogen levels or lower dietary calcium intake, (2) inadequate vitamin D levels, (3) deossification due to excessive parathyroid secretion, or (4) bone degeneration itself. Generally osteoporosis is associated with aging and appears to occur in all vertebrates including man. In the case of the laying hen, calcium excretion for egg shell formation greatly speeds up bone loss since the bird evidently is unable to absorb sufficient calcium from the diet, even when fortified with extra calcium, to maintain a high rate of lay without drawing additional calcium from the bones.

Bone changes usually occur first in the larger bones of the body due to their higher rate of mineral metabolism and then proceed to the smaller bones. Avian osteoporosis occurs in older birds where the calcium metabolism decreases with age and egg production.

Senile osteoporosis and Paget's disease of bone in man are quite

similar to avian osteoporosis. With the advent of better medical facilities in the United States and throughout the world, man's life-span has greatly increased. With this increased age has come problems associated with aging. Bone fragility, especially of elderly wormen, is one of these problems.

Another recent problem man has encountered is the decrease in bone density of astronauts who remain in a weightless state for relatively long periods of time. In this case and during periods of recumbency bone changes develop rather rapidly, which points to the possibility of exerc ise playing an important role in bone metabolism. It is also of interest to observe that in older people there is a tendency for a decrease in physical activity which may be a contributory factor to their bone loss problems.

Since osteoporosis appears rapidly and spontaneously in White Leghorn hens which have been bred for heavy egg production, this makes the laying hen an ideal animal for studying this phenomenon in old age and space travel.

LITERATURE REVIEW

While bone fragility or osteoporosis in aging animals and man has been observed for a great many years, it has only fairly recently become important in poultry. Most of the published data concerning bone fragility due to osteoporosis have been reported in the literature of human medicine. Unfortunately much of the work has been conducted by medical schools using only a small number of individuals for their studies. It has been, in most cases, impossible for a research group to work with large enough samples to arrive at any statistically significant conclusions. A great deal of the literature simply describes various bone disorders and then discusses the therapeutic measures which the researchers tried.

The major part of the medical research has used radiographic standards for comparisons of bone density changes, and the assumption is made that as bone density decreases so does bone strength. A comprehensive evaluation of these radiographic techniques was reported by Garn et al. (1967a).

One of the outstanding features of avian osteoporosis, as it occurs in man and other animals, is the decrease in the thickness of the cortex of the bones. The cortical bone is resorbed from within, thus enlarging the diameter of the marrow cavity. The bone tissue that is present is fully calcified; for this reason, determination of the bone ash does not always disclose the presence of osteoporosis and fails to explain the increased fragility of the bones

It has been shown (McIntosh, 1965) that in humans the largest group of osteoporotic cases occurs in postmenopausal women and in senile individuals of both sexes, rarely occurring in younger patients. Even though bone loss begins before menopause, it is apparently faster in the ovariectomized female (Garn, 1967) and this may be partially inhibited by estrogen therapy. Monroe (1951), working with 7941 individuals over 61 years of age, also found that osteoporosis was more common in women than men and that its frequency increased with advancing age. In the 70-80-year-old individuals he found 0.18 percent had serious osteoporosis problems, while 0.43 percent of the 80-90-year-old group were affected. In the group ranging in age from 90-100 years he found 1.2 percent had osteoporosis.

According to Smith (1967) the bulk of evidence that age-related osteoporosis is a disorder of mineral metabolism is derived from extensive studies in patients with vertebral deformities. A form of osteoporosis occurring in middle-age or elderly people has been described by Wiles (1956). This form is characterized by osteoporosis of the bodies of the vertebrae without generalized skeletal osteoporosis. All observed cases of primary osteoporosis of the vertebrae occurred in older people.

Nordin (1964) found that osteoporosis was present in 75 percent of more of the cases of fractured femurs in humans. Fractures of this nature are quite common in elderly people and are often slow to heal due to poor ossification.

Walker (1965) estimates that between 10 and 50 percent of the people in the United States over 65 years of age are severely osteoporotic and indicated that this may be due to improper calcium

intake or hormone levels. In many of the clinical cases there is a reduction of bone mass or mineral matter without a reduction in percent mineral composition of the bones. Osteoporosis differs from rickets and osteomalacia which are both associated with a reduction in percent mineral composition of the bones.

Wray et al. (1963) worked with bone from 25 patients, ranging from 62--93 years of age, who required surgical fixation of a fractured hip. They found that 10 of the patients had reduced bone ash and the demineralization which occurred in the more severe cases was as high as 78 percent.

Even though osteoporosis is most often observed in fractured pelvic bones, femurs, and the vertebrae, it does occur throughout the skeletal system. Rapid loss of tooth-bearing bone in individuals with generalized osteoporosis was reported by Groen et al. (1960). Israel (1967) also found in aging individuals the same condition in both men and women, but to a lesser extent than did Groen et al. (1960). It was brought out by Lansing (1952) that osteoporosis of the temporal bone in aged men can lead to brittleness of this bone and leave it more susceptible to fracture.

Garn et al. (1967b) studied 13,000 subjects from seven countries and found that bone loss was universal and that it was found in all human races studied. Baker and Little (1965), working with cadavers from Peru, found evidence which suggested there was a loss in skeletal density in aging Peruvians, especially women. Baker and Angel (1965) also demonstrated, while working with cadavers ranging in age upward from 40 years old, that females had lower bone densities and this lower density was entirely related to lower mineral content of the bone. They also found that there was a race difference in bone density

in that Negroes had denser bone than did Caucasians. Garn et al. (1964) found that Chinese and Japanese individuals, whether American-born or born abroad, had less compact bone per unit length than did bones of Americans of European ancestry. Garn et al. (1967b) stated that bone loss is a phenomenon that affects individuals within a given population to varying degrees. There are, however, situations that hasten adult bone loss such as dietary and hormonal factors (Smith, 1967), small physical stature (Garn and Hull, 1966), early menopause (McIntosh, 1965), and gastrectomy (Morgan et al., 1966). It was shown by Garn et al. (1967b) that feeding levels of 300-1,500 milligrams per day of calcium did not seem to affect the rate of osteoporosis development due to the above mentioned causes.

Most of the recent work on primary osteoporosis suggests that it is generally a result of increased bone resorption rather than decreased bone formation. The cause is not apparent, but it has been suggested (Nordin, 1964) that it could be due to prolonged negative calcium imbalance or degenerative changes in the bone itself. This may be associated with a hormonal change. The fact that it is most prevalent in women who have undergone menopause indicates that a decrease in estrogen production could play an important role. Spencer et al. (1964) also suggested that patients with osteoporosis have a decreased ability to absorb added calcium from the intestine as compared to young persons or to patients of comparable age without osteoporosis.

Since bone is an active tissue and is continually undergoing growth and destruction by the osteoblasts and osteoclasts, it could be postulated that exercise or bone stress could influence its density just as exercise influences other tissue formation such as

muscle mass. Evidence was presented by Geiser and Trueta (1958), working with rabbits, that there is an increase in bone density of long bones subjected to physical stress. These same bones decreased in density when the stress was removed. Bassett (1965) found that bones under stress increased in strength. It has been well documented (Mack, 1969; Mack et al., 1967) that the astronauts on the Gemini IV, Gemini V, and Gemini VII space missions had a reduction in bone mass which was associated with lack of exercise. The same bone density losses were also reported from recumbent volunteers on the ground.

Bone fragility is not limited to man and chickens, but has been observed in most higher animals. It was shown by Gardner (1943) that the breaking strength of the femurs of 241 mice decreased as the mice advanced in age. He also noted that the breaking strength of the femurs of both male and female mice was greatly increased by injecting estrogens. Roentgen-ray photographs of the bones were made and they found that the bones with the greater breaking strengths also had higher densities.

It was found (McCay et al., 1935) that rats whose life span had been extended to nearly four years had bones so fragile that they were crushed by the scalpel in the process of dissecting away the muscle for measuring bone growth. The long bones were little more than thin empty shells.

It is well documented (Mitchell, 1962; Forbes et al., 1922; Forbes et al., 1935) that cows, particularly high producing dairy cows, go into a negative phosphorus and calcium balance during lactation. A prolonged period of milk secretion can produce temporary osteoporosis in cattle which cannot be overcome by supplemental calcium in the diet as long as the cow is giving milk.

In the adult animal the bones contain approximately 25 percent water, 30 percent organic matter, and 45 percent ash. In the ash portion calcium constitutes about 37 percent and phosphorus about 18.5 percent (Swenson, 1970). The bone salts are deposited within the bone matrix and are composed primarily of calcium, magnesium, sodium, potassium, phosphorus, carbon dioxide, citric acid, chlorine, and fluorine. Other minerals such as zinc (Kienholz et al., 1964) have also been found in bone in very small quantities.

Fluoride has been shown to increase the calcium content of rat bones when present in the water at the rate of 20 parts per million (Saville, 1967). The force required to break the femur was a linear function of body weight; as the weight of the rat increased so did the breaking strength of its bones. Fluoride was used therapeutically to treat osteoporosis by Rich and Ensinck (1961). Leone et al. (1955) suggested the possible use of fluoride to combat osteoporosis that commonly occurs in aging women. Rich and Ivanovich (1965) found that in a 65-year-old man who had primary osteoporosis, 40-50 milligrams of fluoride given daily during a 122-week treatment period increased calcium retention when bone density was determined by radiographic measurements.

As the hen lays, she depletes her skeletal system of calcium by deposition of calcium in the shell. Common (1938) showed that up to 24 percent of the total body calcium of laying hens fed a low-calcium diet can be withdrawn from the skeleton for shell formation. He also found that changes in the composition of the inorganic material of the skeleton could be accomplished by varying the calcium carbonate level in the diet. The whole-body calcium content of the pullets was raised considerably by feeding a high-calcium diet prior to the

onset of lay. This added calcium was stored in the bones. Cox and Balloun (1970, 1971) reported that minerals began to decrease in the femur of laying hens at the onset of egg production. Hurwitz and Bar (1969) demonstrated that the laying hen utilizes her skeletal system for a calcium reserve which is called up during egg shell formation. It was earlier shown by Hurwitz and Bar (1966) that hens on dietary calcium depletion studies had a progressive decrease in blood and egg shell calcium along with a marked depletion of femur calcium. Taylor and Moore (1954, 1956) reported a reduction in percent shell immediately after pullets were placed on a low-calcium diet; the reduction became progressively greater with each egg laid. Skeletal loss of calcium accompanied egg production on low-calcium diets, indicating that bone calcium was used for shell formation. They also found that the percent bone ash of pullets dropped on a low-calcium diet. Even under normal feeding conditions a considerable portion of the calcium deposited in the shell comes from the skeleton (Wasserman, 1958) and a large calcium turnover occurs in various bones of the laying hen (Hurwitz, 1965).

Urist and Deutsch (1960a) found that osteoporosis developed in White Leghorn chickens during the first year of production and became severe during the molt. It was found that, under normal conditions, immature and young pullets, cockerels, and nonmolting roosters do not have osteoporosis. In the case of hens with osteoporosis they found that loss of bone density can produce bones so fragile that fractures may be caused by minor trauma such as excitement or handling of the birds.

It is a well known and accepted fact that vitamin D plays an important role in proper bone formation in growing animals. Itoh

and Hatano (1964) reported that vitamin D has specific effects on the calcium metabolism of individual bones in growing chicks. They found that the most active metabolism of calcium was in the wing bones, but that the femur and tibia furnished a good index for calcium metabolism study. Lachat and Halvorson (1936) found that female White Leghorn chicks had more efficient utilization of vitamin D and proved more satisfactory as a research animal than males. Growth and calcification were also more seriously affected by deprivation of vitamin D in the females.

Percent bone ash would be expected to increase with elevated blood calcium levels. Administration of estrogens has been reported to increase blood calcium in poultry; this increase is comparable to the naturally occurring increases noted when the hen comes into production. Jones et al. (1965) reported that subcutaneous injection of estradiol in laying hens caused increased blood calcium levels within 28 hours after injection. Riddle and Dotti (1945) also reported elevated blood calcium levels in young birds when estrogen was administered. Urist and Deutsch (1960b) reported that estrogen given without androgen did not give the hen production against osteoporosis. Fry and Stadelman (1958) reported, however, that diethylstilbestrol injection resulted in no significant increases in percent bone ash of growing broilers. They did show that age and sex had highly significant effects on the ash content of bones of 6-, 10-, and 14-week-old broilers even thought hormonization had little or no effect.

Urist (1959) found that hens and roosters deprived of calcium differed from growing chicks in that they were able to draw upon bone calcium supplies and maintain a blood calcium level of 10

milligrams per 100 cubic centimeters. It may be assumed that the skeletal system, particularly the larger bones, assists in maintenance of calcium homeostasis by resorption and deposition of calcium. Urist (1959) found that estrogen readily produced new bone formation in calcium-deficient hens, but that the deposition was intramedullary bone. This can be assumed to contribute little to bone strength since it is found only in the interior of the bones and is not part of the cortex. Urist and Deutsch (1960a) suggested that since the cortex of the long bones of the laying hen are normally thinner and more porous than in the rooster, a low grade form of osteoporosis is present in the hen laying at a high rate.

It is an established fact that the levels of dietary calcium and phosphorus can regulate bone ash content (Dilworth and Day, 1965). Harms et al. (1967) also found that various dietary calcium and phosphorus levels resulted in a variation in bone ash content. They reported that maximum growth could be attained with much lower levels of calcium and phosphorus than those indicated to be required (National Research Council, 1966), provided the calcium-phosphorus ratio was optimum. However, in many instances bone ash was increased by higher dietary levels of the two minerals than were required for normal growth.

In most calcium or phosphorus assays, tibia ash has been used as the main criterion for evaluation of dietary adequacy (Waldroup et al., 1965). It was found by Rowland et al. (1967) that the tibia breaking strength of chicks was highly correlated with tibia ash when compared at various dietary calcium and phosphorus levels. Since tibia breaking strength is correlated to bone mineralization the measurement of tibia strength has proved a useful tool in studying

bone fragility in laying hens (Rowland et al., 1968).

The purpose of the following experiments was to investigate further and define naturally occurring bone fragility in the domestic chicken, with special emphasis on the laying hen.

EXPERIMENT I

Comparison of Bone Strength of Caged and Floor Layers and Roosters

It is generally believed that hens kept in wire cages exhibit a condition of bone fragility which often results in bone breakage during processing, causing downgrading of the ready-to-cook hens. This phenomenon is not normally observed in floor layers. A review of the literature indicates that essentially no research has been reported comparing the bone strength of caged and floor layers. However, Adams et al. (1968) examined a small sample of hens obtained from a commercial processing plant, and reported that the breaking strength of bones from hens kept in cages was slightly higher than of those from hens maintained on the floor. The study reported herein was conducted to compare bone breaking strength of hens and roosters with a similar background when maintained in cages and in floor pens.

Experimental Procedure

An experiment involving two trials was conducted to compare the bone breaking strength of hens maintained in wire cages and in floor pens. DeKalb egg-production-type pullets were used in trial 1 and a Welp strain was used in trial 2. In each trial three pens of 15 pullets maintained on the floor and eight groups of five birds each maintained in individual cages were placed on each experimental treatment. In trial 1 two experimental diets were fed. These diets contained 0.8 and 1.2 percent phosphorus and each contained 3 percent calcium with 2046 kilocalories of productive energy per kilogram of

feed. In trial 2 only the diet containing 0.8 percent phosphorus was fed. The vitamin and trace mineral supplementation was similar to that used by Waldroup and Harms (1964). Two levels of phosphorus were used in the first trial since it had been previously shown that the phosphorus requirement differed for hens maintained in cages and in floor pens (Harms et al., 1961; Singsen et al., 1962), and it was later shown by Simpson et al. (1964) that a difference in phosphorus level in laying diets resulted in structural changes in the bones.

At the end of the fifth and tenth month of lay in trial 1, 12 hens in production were sacrificed from each treatment group for determination of bone breaking strength. In trial 2 all birds that were in production were sacrificed at the end of the fifth month for determination of bone breaking strength and percent bone ash. The breaking strength of the left tibia was determined by the procedure outlined by Rowland et al. (1967). Bone ash data obtained by the method described by the A.O.A.C. (1965) was determined for hens in trial 2.

One trial was conducted with broiler breeder males (Peterson) to determine the difference in bone strength of males maintained in cages and in floor pens. These males were given identical treatments during the seven week growing period. At the end of the seventh-week, one-half were placed in wire cockerel cages with wire floors, and were kept in these cages until the end of the trial. During the breeder period they received a diet containing 3 percent calcium and 0.8 percent phosphorus supplemented with vitamins and minerals identical to those used by Waldroup and Harms (1964). At 60 weeks of age 20 males were selected at random from the floor and 20 from cages, and breaking strength was measured by the same method used

in trial 1.

Results and discussion

Breaking strength of bones from hens maintained on the floor was significantly greater ($P < .005$) than for those birds maintained in cages (Table 1). Increasing the phosphorus level of the diet in trial 1 failed to affect breaking strength significantly. This would indicate that the decreased strength of bones of the birds cannot be corrected by feeding increased phosphorus levels.

Bone ash in trial 2 was significantly ($P < .005$) higher for hens maintained on floor when compared to those in cages (Table 1). This difference in bone ash is not considered to be the cause for the large differences in breaking strength of bones for the two groups, since larger differences in bone ash due to varying dietary calcium levels in previous studies did not result in such changes in breaking strength (Rowland et al., 1968).

The breaking strength of bones from roosters maintained on the floor was significantly greater ($P < .01$) than those given similar treatments and maintained in cages (Table 2). This would indicate that the bone fragility problem is not due solely to calcium depletion from egg shell formation.

These data confirm the assumption that bones from birds maintained on the floor are stronger than those maintained in cages when the two groups are fed the same diet and are of the same age and strain.

Table 1. Tibia breaking strength and bone ash from hens fed two levels of phosphorus with 3 percent calcium when maintained in individual cages and in floor pens (Experiment 1)

	% Phosphorus	Breaking Strength (lbs) Trial 1 5 months of lay[2]	Breaking Strength (lbs) Trial 1 10 months of lay[2]	Breaking Strength (lbs) Trial 2 5 months of lay[3]	Bone Ash (%) Trial 2 5 months of lay[3]
Cage	0.8	30.0[a1]	30.0[a]	31.7	53.9
	1.2	32.1[a]	31.4[a]	-	-
Floor	0.8	44.6[b]	42.1[b]	44.7*	56.9*
	1.2	40.9[b]	39.9[b]	-	-

[1]Numbers with different subscripts are significantly different at the .01 level of probability based on Duncan's multiple range test.

[2]Twelve hens per treatment group.

[3]Thirty hens per treatment group.

*Significantly different at the .005 level from other treatments in the same column.

Table 2. Breaking strength of bones from broiler males maintained in individual cages and floor pens (Experiment 1)

	Tibia Breaking Strength (lbs.)
Cages	137.4
Floor	161.6*

*Significantly different at the .01 percent level of probability.

EXPERIMENT II

The Effect of Wire Pens, Floor Pens, and Cages on Bone Characteristics of Laying Hens

Several factors which may lead to osteoporosis or bone fragility include a deficiency of sex hormones, calcium deficiency, malnutrition, or a lack of normal stimulation due to stress and/or strain such as encountered in prolonged recumbency, plaster casts, etc. (Jackson, 1967). It has been well established by Trueta (1968) that immobilization, bed rest, and lack of muscular activity are all factors contributing to osteoporosis in humans. It was also demonstrated that bone rarefaction occurred in rabbits whose feet and legs were immobilized by plaster casts.

It was found in Experiment 1 that hens maintained in cages had a lower bone breaking strength and a lower tibia ash than those kept in floor pens. It is possible that hens kept in cages develop osteoporosis or bone fragility more rapidly than floor birds due to a lack of exercise. Therefore, these studies were conducted to determine if exercise could be a factor in regulating osteoporosis in hens.

Experimental procedure

Trial 1. A total of 165 commercial egg-production-type pullets (Welp) 21 weeks of age were randomly divided into four groups. These birds received a basal diet (Table 3) containing calcium and phosphorus levels of 3 percent and 0.7 percent, respectively, during a five-month treatment period. Forty-five pullets were equally divided among three floor pens 1.52 meters X 1.68 meters with peanut hulls for

Table 3. Composition of diet (Experiment 2)

Ingredient	% of diet
Yellow Corn	69.90
Soybean Meal (50% protein)	19.00
Alfalfa Meal (20% protein)	2.50
Ground Limestone	5.90
Defluorinated Phosphate (18% P and 32% Ca)	1.95
Iodized Salt	0.25
Micro-ingredients*	0.50

*Supplied per kg. of diet: 6600 I.U. vitamin A, 2200 I.C.U. vitamin D_3, 500 mg. choline chloride, 40 mg. niacin, 4.4 mg. riboflavin, 13 mg. pantothenic acid, 22 mcg. vitamin B_{12}, 22 mg. ethoxyquin, 20 mg. iron, 2 mg. copper, 198 mcg. cobalt, 1.1 mg. iodine, 99 mcg. zinc, 83.6 mg. manganese, and 2.2 mg. menadione.

litter; 45 were equally divided among three similar floor pens which had a raised wire floor (2.5 centimeters X 5.2 centimeters mesh) which prevented the recycling of fecal material; 75 pullets were placed in individual cages 20.3 centimeters X 45.7 centimeters in size. Twenty-five of the caged pullets received the basal diet to which 10 percent fecal material from under their cages was added in order to simulate coprophagy which occurs in floor pens.

At the end of the five-month feeding period the hens were sacrificed and the left tibia was removed from each bird. These were defleshed after cooking for approximately five minutes in boiling water, air dried at room temperature for 48 hours, and broken on the Allo-Kramer Shear Press using the procedure described by Rowland et al. (1967). After breaking, the tibiae were ashed according to the procedure as outlined by the A.O.A.C. (1965). Specific gravity was measured on all eggs laid one day prior to terminating the experiment. Body weights of all hens were obtained at the end of the experiment.

Trial 2. In this trial, 140 Babcock pullets 24 weeks of age were divided into three groups. Forty-five pullets were divided equally among three floor pens with wire floors; 45 were assigned to floor pens with peanut hulls for litter; 50 pullets were placed in individual 20.3 centimeters X 45.7 centimeters cages. The birds were fed the same basal diet (Table 3) as previously used. They were sacrificed after five months of lay and breaking strength and bone ash determined as outlined in trial 1.

Statements of probability are based on Duncan's multiple range test (1955).

Results

Trial 1. Hens maintained in cages for five months had significantly

lower tibia breaking strength, tibia ash percent, and body weights than hens maintained on the floor and in wire pens (Table 4).

The addition of 10 percent feces to the diet did not significantly influence bone strength, tibia ash percent, or body weight of hens maintained in cages; however, they consumed approximately 10 percent more feed. The specific gravity of eggs from the cage, cage plus manure, floor, and wire floor pen hens was 1.082, 1.093, 1.085, and 1.083, respectively. These values did not differ significantly.

Trial 2. In the repeat study using a different strain of pullets, a similar trend was observed; the caged hens has numerically, but not significantly, lower tibia breaking strengths and tibia ash (Table 4). Again no differences were found between the floor hens and those maintained in wire floor pens.

Discussion and summary

Two trials were conducted to study the influence of wire floor, cage confinement, and feeding feces on bone strength and percent bone ash of chickens. Hens confined to cages, thus with restricted activity, had a lower bone ash and bone strength than floor hens. It should be noted, however, that the birds on the floor had 1702 square centimeters while those in cages had only 928 square centimeters. These data indicate that a wire floor *per se* does not account for a decrease in strength in cage hens, nor does recycling of nutrients greatly influence bone characteristics as measured in this study.

In the first trial a greater difference between groups was found than in the second trial. A spectulative explanation might be tied in with seasonal effects. The first trial was terminated in the warm weather while the second trial was terminated in cool weather. It is also possible that a strain difference may exist.

Table 4. Tibia breaking strength, body weights, and percent tibia ash of hens[1] (Experiment 2)

Treatment	Trial 1			Trial 2	
	Breaking Strength (lbs.)	Tibia Ash (%)	Body Weight (gms.)	Breaking Strength (lbs.)	Tibia Ash (%)
Floor Pen	44.61[a]	56.17[a]	1588[a]	47.82[a]	59.55[a]
Wire Pen	44.73[a]	56.93[a]	1548[a]	49.78[a]	59.48[a]
Cage	31.65[b]	53.88[b]	1370[b]	44.52[b]	57.77[a]
Cage + M[2]	34.13[b]	54.34[b]	1304[b]	-	-

[1]Different superscripts within a column indicate a significant difference at 0.05 level according to Duncan's multiple range test.

[2]Ten percent manure on an air dried basis was mixed with the basal.

It is concluded that the differences in tibia ash and tibia breaking strength of hens in cages is due to confinement and probably by lack of exercise and/or floor space and not to recycling of fecal nutrients.

EXPERIMENT III

Influence of Protamone and Dienestrol Diacetate on Bone Fragility of Caged Layers

The effect of various thyroproteins on laying hens has been studied fairly extensively since 1923 when Crew and Huskley first attempted to demonstrate that thyroid feeding had an effect on egg production. Turner et al. (1945a, 1945b) found that Protamone,* an iodinated casein with high thyroidal activity, increased egg production when added to the diet. These workers concluded that an optimum level of Protamone in the feed ranged between five and 10 grams per 100 pounds of feed. Additional work by Turner et al. (1946) demonstrated that Protamone fed continuously at 10 grams per 100 pounds of feed prevented a decrease in egg production during the summer months. Work by Singh and Shaffner (1950) indicated that thyroprotein elevated the basal metabolic rate. However, Lillie et al. (1952) questioned whether it improved egg production although the hen's basal metabolism was increased.

The use of estrogens to prevent further degeneration and relieve pain in humans with vertebral osteoporosis has been suggested by Jackson (1967). Dienestrol diacetate has been shown to increase blood calcium (Jones et al., 1965); therefore, it might be expected to influence bone strength. Rowland et al. (1968) reported that the addition of high levels of dietary calcium during the last two weeks

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*Registered trademark for Iodinated Casein, Agri-Tech., Inc., Kansas City, Mo.

before slaughter, or a cessation of egg production resulting from feeding high levels of iodine or dienestrol diacetate, increased the breaking strength of bones from laying hens.

Since the feeding of thyroprotein increases the hen's metabolism it could increase the hen's mobilization of minerals and speed up replacement of bone calcium lost during egg shell formation. Therefore, this experiment was conducted to determine the effect of Protamone on bone strength of caged layers.

Experimental Procedure

Trial 1. Sixteen roosters and 32 hens (commercial egg-production-type 64 weeks of age) were divided into four equal groups containing four males and eight females each. The groups were further divided into two males and four females per floor pen. These birds had been receiving the basal diet (Table 2) for the previous 10-month period. The following four treatments were given for a two-week period: (1) basal diet for two weeks; (2) 1408 milligrams dienestrol diacetate per kilogram for two weeks; (3) basal diet for first week and 622 milligrams of Protamone per kilogram of feed during the second week; and (4) 1408 milligrams dienestrol diacetate for one week followed by 1408 milligrams dienestrol diacetate plus 622 milligrams of Protamone per kilogram for an additional week. At the end of the two-week feeding period the left tibia was removed from each bird, defleshed after cooking for approximately five minutes in boiling water, air dried at room temperature for 48 hours, and broken on the Allo-Kramer Shear Press using the procedure described by Rowland et al. (1967).

A second test was conducted which was an exact replicate of the first except that 14 males and 14 females were used in each treatment group. These were further divided into eight pens, each containing seven

males and seven females.

Analysis of variance (Snedecor, 1956) indicated that there were no significant treatment X test interactions; therefore, the data for the two tests were combined for presentation. Statements of probability are based on Duncan's multiple range test (1955).

Trial 2. In view of the fact that Protamone increased the breaking strength of the hen tibia in the first trial, an additional trial was conducted to determine the optimum level of Protamone for this purpose.

In the first test, 75 individually caged egg-production-type laying hens were used. These hens had been receiving the basal diet (Table 2) for 10 months and were laying at approximately 60 percent. They were randomized into 15 equal groups containing five birds each. The groups were fed diets containing the following levels of Protamone: 0, 622, 1244, 1866, and 2488 milligrams per kilogram of diet. At the end of a three week feeding period, the left tibia was removed from each bird, and broken in the manner described in trial 1.

In the second test, 84 egg-production-type birds maintained in individual cages were randomized into 21 groups of four hens each. These birds had also been in production for 10 months, and had been receiving the basal diet shown in Table 2. The diets containing the various levels of Protamone used in the first test were again fed. In addition, two other diets were fed. One contained 6 percent calcium as compared to 3 percent calcium in the basal diet, and the second contained 6 percent calcium with 1866 milligrams of Protamone per kilogram of diet. These diets were included to determine if the beneficial effects from Protamone were additive to the improvement from feeding high levels of calcium as previously reported by Rowland

et al. (1968). At the end of a two-week feeding period, the left tibia was removed and the procedure followed in earlier trials was used for determining breaking strength. After breaking the tibia, ash was determined according to the procedure as outlined by the A.O.A.C. (1965).

Results and discussion

Trial 1. The addition of Protamone to the diet resulted in significantly increasing breaking strength of bones in both males and females (Table 5). The addition of dienestrol diacetate to the diet of the hens resulted in a numerical increase in breaking strength which approached the 5 percent level of probability. The fact that dienestrol diacetate strengthened bones in laying hens agrees with a previous report by Rowland et al. (1968). The increase in bone strength from dienestrol diacetate and Protamone was found to be additive with the laying hen. However, the addition of dienestrol diacetate to the diet of the rooster resulted in significantly decreasing the bone breaking strength. This resulted in a sex X dienestrol diacetate interaction which approached significance at the 5 percent level of probability. This sex X dienestrol diacetate interaction was rather interesting since the estrogenic treatment of bone disorders in humans is controversial (Jackson, 1967).

Trial 2. The addition of 622 milligrams of Protamone per kilogram of feed resulted in significantly increased bone breaking strength (Table 6). Increasing the level of Protamone in the diet resulted in a numerical but not statistically significant increase in tibia breaking strength.

Increasing the level of calcium from 3 to 6 percent resulted in a numerical increase in bone breaking strength (Table 6). This

Table 5. Force required to break tibiae from roosters and hens as influenced by dienestrol diacetate or Protamone (Experiment 3, Trial 1)

Treatment		Bone Strength (lbs.)*	
First Week	Second Week	Male	Female
Control	Control	89.85	51.19
Dienestrol Diacetate	Dienestrol Diacetate	86.20	53.33
Control	Protamone	96.78	55.59
Dienestrol Diacetate	Dienestrol Diacetate + Protamone	90.86	57.49
- Protamone		88.02^{a}	52.27^{r}
+ Protamone		93.83^{b}	58.85^{s}
- Dienestrol Diacetate		93.32^{b}	54.34^{r}
+ Dienestrol Diacetate		88.53^{a}	55.42^{r}

*Means with different superscripts are significantly different at the .05 level according to Duncan's multiple range test.

Table 6. Force required to break tibiae and tibia ash as influenced by dietary treatment (Experiment 3, Trial 2)

Treatment	Tibia Breaking Strength (lbs.) Test 1	Test 2	Average of 2 Tests*	% Tibia Ash Test 2
Control	33.90	31.55	32.74[a]	60.68
622 mg./kg. Prot.	36.67	34.41	35.55[b]	60.04
1244 mg./kg. Prot.	34.30	37.55	36.06[b]	59.24
1866 mg./kg. Prot.	37.25	36.45	36.85[b]	61.91
2488 mg./kg. Prot.	37.86	37.55	37.71[b]	61.04
6% Ca	-	33.55	-	63.05***
6% Ca + 1866 Prot.	-	39.29**	-	63.28***

*Means with different superscripts are significantly different at the .05 level, according to Duncan's multiple range test.

**Significantly different from diet containing 6 percent calcium.

***Diets containing 6 percent calcium significantly different from those containing 3 percent calcium.

finding agrees with a previous report by Rowland et al. (1968). Supplementing the diet with 1866 milligrams of Protamone per kilogram of diet with a level of 6 percent calcium resulted in a further improvement in bone breaking strength. This indicates that improvement from Protamone and increased calcium levels are additive.

The addition of Protamone to the diet did not influence tibia ash (Table 6). Increasing the level of calcium from 3 to 6 percent resulted in significantly increasing bone ash. The fact that the addition of Protamone to the diet did not increase bone ash and the increasing of calcium did increase bone ash would indicate that the two factors are working in a different manner. This might account for the fact that the responses from the two methods are additive.

Summary

Two trials were conducted to study the influence of dienestrol diacetate and Protamone on bone strength of chickens. In the first trial it was found that feeding a level of 622 milligrams of Protamone per kilogram of diet resulted in significantly strengthening bones of both male and female chickens. The addition of 1408 milligrams of dienestrol diacetate per kilogram of diet significantly increased breaking strength of the bones of males.

In a second trial it was found that increasing the level of Protamone from 622 milligrams per kilogram to 2488 milligrams resulted in a numerical but not significant improvement in bone breaking strength. Increasing the dietary calcium level from 3 to 6 percent resulted in increasing bone strength. The increases from dietary calcium and the addition of Protamone were found to be additive. Supplementing the diet with Protamone did not influence

the tibia ash; however, increasing the calcium level from 3 to 6 percent resulted in an increase in tibia ash.

EXPERIMENT IV

Differences in Tibia Strength and Bone Ash Among Strains of Layers

It has been found in the foregoing experiments (Rowland et al., 1968) that caged hens tend to develop this condition more often than floor hens; however, conflicting evidence was presented by Adams et al. (1970) which indicated that caged hens have stronger bones than floor hens. One of the variables which might explain why hens, at times, exhibit bone fragility while at other times none is apparent, could be strain difference. Strain differences have been demonstrated for many of the hen's nutritive requirements such as protein (Harms et al., 1966; Balloun and Speers, 1969), amino acid requirements (Krautman, 1969), egg weights (Kondra et al., 1968), fat utilization, and other traits. The purpose of the three trials reported herein was to determine if there were any differences in bone characteristics of spent hens due to strains.

Experimental Procedure

Approximately 200 pullets per strain were housed in four replicate pens (366 X 366 centimeters) of 50 birds each in trials 1, 2, and 3. In trials 2 and 3, 200 pullets per strain were also housed two per cage in 25.4 X 45.7 X 45.7 centimeters cages. The birds were grown for the Fifteenth, Sixteenth, and Seventeenth Florida Random Sample Poultry Tests (O'Steen et al., 1968; Christmas et al., 1969; Christmas et al., 1970) in floor pens with cane pumice litter. The chicks were fed a starter diet (Table 7) for six weeks, a grower diet until 20 weeks of

Table 7. Composition of basal diets (Experiment 4)

Ingredient	Chick Starter (%)	Grower (%)	Laying (%)
Yellow Corn	55.85	73.85	67.58
Soybean Meal (44% protein)	35.00	17.00	13.10
Fish Meal (60% protein)	3.00	3.00	2.50
Meat Scraps (55% protein)	-	-	5.00
Alfalfa Meal (17% protein)	3.00	3.00	5.00
Defluorinated Phosphate	1.20	1.20	0.40
Ground Limestone	1.30	1.30	5.50
Iodized Salt	0.40	0.40	0.40
Vitamin Premix	0.25*	0.25*	0.50**
Manganese Sulfate	-	-	0.02

*Each kg. of diet contained: vitamin A - 2200 USP units; vitamin D_3 - 550 I.C. units; riboflavin - 1.65 mg.; d-pantothenic acid - 4.4 mg.; niacin - 8.8 mg.; choline chloride - 330 mg.; vitamin B_{12} - 3.3 mcg.; procaine penicillin - 13.07 mg.; manganese sulfate - 30.5 mg.

**Each kg. of diet contained: riboflavin - 110 mg.; d-pantothenic acid - 220 mg.; niacin - 495 mg.; vitamin B_{12} - 33 mcg.; vitamin D_3 - 6600 USP units; vitamin A - 22000 USP units; choline chloride 2200 mg.

age, and were fed a commercial-type layer diet for a 400-day laying period. All three trials were on a 15-hour lighting regime. Pullets were housed at 20 weeks of age on August 5, 1966, March 5, 1968, and May 2, 1969, respectively, for trials 1, 2, and 3.

At the termination of the laying period 20 hens, which were in production, were randomly sacrificed from each strain in trial 1. In trials 2 and 3, 40 hens per strain, representing both caged and floor groups were sacrificed. The left legs were removed, the tibiae were defleshed after boiling for approximately six minutes and then air dried at room temperature for 48 hours in an air-conditioned room. The force required to break each tibia was measured on a modified Allo-Kramer Shear Press (Model #SP12) by a previously developed procedure (Rowland et al., 1967). A downspeed of one centimeter each 2.5 seconds was used and the supports upon which the bone rested were 87 millimeters apart. In trials 2 and 3, the tibia fragments were collected in groups of five bones and tibia ash values were determined according to standard A.O.A.C. procedures (A.O.A.C., 1965).

Analysis of variance revealed a significant treatment X trial interaction; therefore, the data for the three trials were not combined.

Results and discussion

Trial 1. In trial 1, 12 strains were utilized for measurement of tibia breaking strength, which ranged from 48.38 pounds to 76.30 pounds (Table 8) with a statistical difference at the .05 level among three groups of strains.

Trial 2. Ten strains were utilized in this trial and again there were statistically differing breaking strengths among several strains (Table 9). There was a highly ($P < .01$) significant difference

Table 8. Tibia breaking strength and egg production by strains (Experiment 4, Trial 1)

Strain	Breaking Strength (lbs.)[1]	Total Egg Production[2]
Kimber K-137	48.38[a]	250.6
True Line #365-B	50.69[a]	256.7
White Leghorn	53.22[a]	258.7
Welp Line 937	53.42[ab]	240.1
Randombred	53.94[ab]	206.6
H & N "Nick Chick"	54.77[ab]	273.8
Honegger Layer	54.91[ab]	258.5
Shaver Starcross 288	55.29[ab]	251.9
Garber G-200	59.81[b]	279.4
Hy-Line Hy-934 E	60.82[b]	256.7
Hi-Cash	62.56[b]	253.6
Babcock 300	76.30[c]	226.3

[1]Means with different superscripts are significantly different at .05 level according to Duncan's multiple range test.

[2]Based on total eggs laid on a pullet-housed basis over a 400-day laying period.

Table 9. Tibia breaking strength, percent tibia ash, and egg production by strains (Experiment 4, Trial 2)

Strain	Tibia Breaking Strength (lbs.)			% Tibia Ash		Total Egg Production[2]	
	Cage	Floor	Average[1]	Cage	Floor	Cage	Floor
Hy-Line Hy-934 E	32.76	44.44	38.60[a]	59.67	61.10	225.2	243.3
Welp Line 937	38.53	43.36	40.95[ab]	62.08	61.68	203.6	224.3
Garber G-200	39.94	44.54	42.24[ab]	60.26	60.81	199.7	208.0
Babcock B-300	37.95	47.41	42.68[ab]	62.89	62.80	227.3	252.2
True Lines 365-H	41.25	44.51	42.88[ab]	61.10	61.83	199.5	215.2
IBX Hi-Cash	37.47	48.89	43.18[ab]	59.85	60.82	203.2	214.8
Honegger Layer	35.67	51.10	43.39[ab]	60.27	61.81	247.3	248.3
Kimber K-137	38.71	48.63	43.67[b]	60.35	61.94	234.2	223.8
Shaver S.C. 288	39.91	47.72	43.82[b]	60.24	62.60	248.0	237.8
W. L. Randombred	48.35	50.99	49.67[c]	61.44	60.99	189.7	197.0

[1]Means with different superscripts are significantly different at .05 level according to Duncan's multiple range test.

[2]Based on total eggs laid on a pullet-housed basis over a 400-day laying period.

between caged and floor birds with the caged hens having a lowered breaking strength in every group than did the corresponding hens of the same strains which were maintained on the floor. There were no significant differences in tibia ash among strains or between caged and floor birds. In most strains, the floor hens laid a few more eggs than did the caged birds.

Trial 3. The third trial utilized only seven strains and again there were significant differences among strains (Table 10) with a tibia strength range of 32.93 pounds to 43.95 pounds. Within every strain there was again a statistically significant difference between caged and floor hens with the caged hens exhibiting a lower tibia breaking strength. In this trial the caged hens had a significantly ($P < .05$) lower tibia ash value than did the floor birds. With one exception, egg production was again higher in floor birds than in those maintained in cages. Shaver Starcross caged hens laid 274.1 total eggs as compared to 270.6 eggs from the same strain on the floor.

Summary

Three trials were conducted utilizing 12 strains of S.C.W.L. pullets to determine if tibia breaking strength and tibia ash varied among strains. These data indicate that there was a significant strain difference in tibia strength but there were no apparent differences in tibia ash among strains. It was found that caged hens had a significantly lower tibia breaking strength when compared to floor hens of the same strain; there were, however, some strains of pullets that were maintained in cages that had a higher breaking strength than other strains maintained on the floor. Floor hens were observed to lay at a slightly higher rate than the caged birds and in the third trial had higher tibia ash values.

Table 10. Tibia breaking strength, percent tibia ash, and egg production by strains (Experiment 4, Trial 3)

Strain	Tibia Breaking Strength (lbs.)			% Tibia Ash		Total Egg Production[2]	
	Cage	Floor	Average[1]	Cage	Floor	Cage	Floor
Welp Line 937	31.46	36.68	32.93[a]	53.97	58.33	243.2	277.4
Babcock B-300	34.68	41.98	38.38[b]	56.87	59.02	270.5	278.0
Kimber K-137	36.58	44.25	40.43[bc]	55.87	59.17	251.9	261.5
Hy-Line Hy--934 E	37.80	44.23	41.03[bc]	54.12	57.30	247.9	259.2
Garber G-200	35.68	47.20	41.45[bc]	54.19	58.47	226.5	250.3
Shaver S.C. 288	39.90	45.90	42.95[bc]	55.74	57.46	274.1	270.6
Randombred	40.12	47.68	43.95[c]	56.65	57.72	209.3	232.1

[1]Means with different superscripts are significantly different at .05 level according to Duncan's multiple range test.

[2]Based on total eggs laid on a pullet-housed basis over a 400-day laying period.

EXPERIMENT V

Time Required to Develop Bone Fragility in Laying Hens

Bone fragility has been demonstrated to occur in laying hens maintained in cages (Experiment 1). The bone mass loss which accompanies bone fragility occurs with aging, and is particularly prevalent in caged layers even though it does occur in floor hens to a lesser degree.

Cox and Balloun (1971) demonstrated that bone mineral depletion commenced with the first egg laid and progressed rapidly for the first 30 eggs laid. This process continues until a point is reached beyond which a further reduction in bone mass results in a bone fragility problem which can be of economic importance to the processor of spent hens. A prime consideration in setting up experiments to study this condition is that of experiment length. It was found in Experiment 1 that there was no significant difference in tibia breaking strength between hens maintained in cages for 10 months and birds maintained five months. This would indicate that bone fragility develops very rapidly in newly housed pullets up to a certain point and then plateaus.

The following trials were conducted to determine the length of time necessary to study bone fragility and produce classic symptoms, i.e., lowered tibia breaking strength and lowered tibia ash.

Experimental Procedure

Two trials were conducted, each utilizing 260 20-week-old

S.C.W.L. pullets (Kimber K-137). Chicks were hatched on August 21, 1969, and September 9, 1969, respectively, for trials 1 and 2. They were grown in floor pens with peanut hulls used as litter, and were fed a commercial-type starter diet containing 21.6 percent protein, 0.73 percent phosphorus, and 1.10 percent calcium until eight weeks of age. From eight weeks to 20 weeks a typical corn-soy-type grower diet containing 14 percent protein, 1.07 percent calcium, and 0.66 percent phosphorus was fed.

In each trial the pullets were divided into two groups at 20 weeks of age. The first group contained 100 pullets housed in individual 20.3 X 45.7 X 45.7 centimeters cages (0.093 square meters) with 2.54 X 5.08 centimeters wire mesh floors. The second group of 100 pullets was equally divided and housed in three concrete floor pens with peanut hull litter at the rate of one pullet per 0.232 square meters. Twenty pullets which were grown on the same regime were sacrificed at the time of housing to determine base breaking strength and tibia ash values. Twenty hens were thereafter sacrificed from each group every four weeks. All pullets were on the same 15-hour light regime and received a commercial corn-soy-type diet (Table 11) for the duration of the study. This diet contained approximately 16.3 percent protein, 3 percent calcium, 0.70 percent phosphorus, and 2042 kilocalories of productive energy per kilogram of feed.

At the end of each four-week period 40 pullets (consisting of 20 pullets each from the cage and floor) were sacrificed and the left leg from each removed. The tibiae were defleshed after cooking for approximately six minutes in boiling water and then air dried for 48 hours at room temperature in an air-conditioned room. The force

Table 11. Composition of laying diet (Experiment 5)

Ingredient	% of Diet
Yellow Corn	69.85
Soybean Meal (50% protein)	19.00
Alfalfa Meal (20% protein)	2.50
Ground Limestone	6.00
Defluorinated Phosphate (18% P & 32% Ca)	1.90
Iodized Salt	0.25
Premix[1]	0.50

[1]Activity per kg. of diet: vitamin A - 6600 I.U.; vitamin D_3 - 2200 I.C.U.; vitamin B_{12} - 22 mcg.; riboflavin - 4.4 mg.; niacin - 40 mg.; manganese - 83.6 mg.; ethoxyquin - 0.0125%; iron - 19.8 mg.; copper - 1.98 mg.; iodine - 1.1 mg.; zinc - 99 mcg.; cobalt - 198 mcg.; pantothenic acid - 13 mg.; menadione - 2.2 mg.; choline chloride - 500 mg.

required to break each tibia was measured on a modified Allo-Kramer Shear Press (Model #SP12) by a procedure which had been developed previously (Rowland et al., 1967). A downspeed of one centimeter per 2.5 seconds was used and the supports upon which the bone rested were 87 millimeters apart. Each group of 20 pullets was divided into four groups of five hens each and tibia ash determined according to standard A.O.A.C. procedures (A.O.A.C., 1965).

Analysis of variance revealed no treatment X trial interaction; therefore, the data for both trials were combined.

Results and discussion

Within four weeks after housing there was a highly significant difference in breaking strength between floor and caged pullets (Table 12). This difference in breaking strength increased from the fourth to eighth week and then remained approximately constant throughout the remainder of the study. The data suggest that pullets placed in cages had a gradual decrease in breaking strength over the entire five-month period. Pullets on the floor had an increase in their initial tibia breaking strength which continued to rise for three months, and then had a gradual decrease in bone strength. Breaking strength was also determined at the end of the twentieth week in trial 2. The difference between caged and floor birds (30.45 vs. 37.27 pounds) was the same as the sixteenth week. In the two trials both groups had declined severely in the sixteenth week. This would suggest that placing pullets in cages prevents their skeletal systems from developing sufficient strength to allow a gradual decrease in bone mass during the laying period and still remain strong enough to prevent shattering in the processing plant.

Tibia ash (Table 13) increased significantly during the first

Table 12. Tibia breaking strength (pounds) of pullets at various times after housing at 20 weeks of age when maintained in cages and floor pens (Experiment 5)

Treatment	Initial	4 Weeks	8 Weeks	12 Weeks	16 Weeks
Cage	-	31.92**	28.48**	30.55**	30.00**
Floor	31.27	37.45	36.36	37.85	38.08

**Significantly different ($P < .01$) from floor hens.

Table 13. Tibia ash from pullets at various times after housing at 20 weeks of age when maintained in cages and floor pens (Experiment 5)

Treatment	% Tibia Ash			
	Initial	4 Weeks	8 Weeks	12 Weeks	16 Weeks
Cage	-	59.64	60.27	58.04	57.22
Floor	55.28	60.00	62.00	60.16	59.41

month of lay for both floor and caged pullets. At the end of the first four weeks there was no significant difference between tibia ash of floor and caged birds, although the caged pullets had numerically weaker bones. Tibia ash increased in both groups between the fourth and eighth week and subsequently showed a slight decrease each successive month. The tibia ash of pullets kept in cages was lower than those on the floor by the end of the eighth week and remained lower throughout the experiment.

Summary

Two trials were conducted to determine the length of time necessary to produce bone fragility in caged layers. Tibia ash and bone breaking strength of caged and floor pullets were found to be higher in floor pullets after four weeks, with a maximum difference between groups occurring at the end of eight weeks. Both groups exhibited a slight decrease in both tibia ash and bone breaking strength which was not statistically significant with each successive month of lay. It was further observed that tibia ash was closely associated with breaking strength, but was not as sensitive a measurement of bone fragility as was breaking strength.

EXPERIMENT VI

Comparisons of Bone Characteristics Between Floor and Battery-Grown Broilers

Recent developments in the broiler industry, namely increased labor costs and disease problems, have initiated interest in growing broilers in colony cages and coops. Lloyd et al. (1970) reported 22.2 percent bone breakage from broilers grown in plastic coops as compared to 8.6 percent for those grown in floor pens.

It has been reported by Trueta (1968) that lack of muscular activity can be a factor in the development of osteoporosis in humans. Data from Experiment 2 demonstrated that using pens with wire floors did not decrease either tibia ash or tibia breaking strength; however, breaking strength was decreased for those hens maintained in cages. It was suggested that exercise might play a role in decreasing bone strength.

The purpose of this study was to determine what effect raising broilers in wire batteries had on bone characteristics.

Experimental Procedure

Trial 1. Sixty day-old broiler chicks, hatched December 4, 1969, (Peterson X Peterson) were divided into two groups of 30 chicks each. One group was housed in 1.5 X 1.5 meter floor pens with peanut hulls used for litter. Each chick was provided 880 square centimeters of floor space. The second group was grown in batteries. The finisher batteries, used after four weeks, measured 60 X 88 X 32 centimeters high, and also provided 880 square centimeters per chick. The battery

floors were composed of 2.2 X 2.2 centimeters wire mesh.

All birds received a basal diet (Table 14) containing 2381 kilocalories of productive energy per kilogram, 0.6 percent phosphorus, and 0.8 percent calcium, from the first day throughout the eight week feeding period. Both groups received continuous light throughout the trial.

Body weights were obtained at the termination of the trial and the birds were processed by a local processor using an Ashley Sur-Pick Model #SP38, batch-type picker. After processing, the birds were examined for breast blisters and broken bones. The left leg was removed from each bird, cooked for approximately six minutes in boiling water, and the tibia defleshed. The tibiae were then air dried at room temperature for 48 hours. The force required to break each tibia was determined using the modifications to the Allo-Kramer Shear Press described by Rowland et al. (1967). The maximum force to break each bone was recorded and the bone fragments were collected, defatted, dried, and ashed (in groups of five) as outlined in A.O.A.C. procedures (A.O.A.C., 1965).

Trial 2. Eighty broiler chicks (Shaver X Hubbard), hatched on March 2, 1970, were utilized in this trial. They were fed the same diet (Table 14) as in trial 1, and were sacrificed at the end of eight weeks. Procedures identical to those outlined previously were used and the same measurements obtained.

Trial 3. Eighty broiler chicks (Shaver X Hubbard), hatched on May 27, 1970, were divided into the same experimental groups as trials 1 and 2, and housed under the same conditions. However, the diet used in this trial (Table 14) contained 2337 kilocalories of productive energy per kilogram, 0.8 percent phosphorus, and 1 percent

Table 14. Composition of basal diets (Experiment 6)

Ingredient	Trials 1 & 2 (%)	Trial 3 (%)
Yellow Corn	57.90	51.46
Soybean Meal (50% protein)	30.60	35.33
Animal Fat	6.00	3.00
Alfalfa Meal (20% protein)	2.50	6.50
Ground Limestone	.90	.47
Defluorinated Phosphate	1.10	2.17
Iodized Salt	.40	.40
dl-methionine	.10	.12
Micro-ingredient Mix[1]	.45	.50
Zoamix[2]	.05	.05
Calcium	0.8	1.0
Phosphorus	0.6	0.8

[1]Supplied per kg. of diet: 6600 I.U. vitamin A, 2200 I.C.U. vitamin D_3, 500 mg. choline chloride, 40 mg. niacin, 4.4 mg. riboflavin, 13 mg. pantothenic acid, 22 mcg. vitamin B_{12}, 125 mg. ethoxyquin, 20 mg. iron, 2 mg. copper, 198 mcg. cobalt, 1.1 mg. iodine, 100 mcg. zinc, 71 mg. manganese, and 2.2 mg. menadione sodium bisulfite.

[2]Trade name of Dow Chemical Company, containing 25% zoalene (3,5-dinitro-o-toluamide).

calcium. It was fed from one day of age until the birds were sacrificed.

An analysis of variance (Snedecor, 1956) showed a significant treatment X trial interaction; therefore, the data were not combined and each trial is discussed separately.

Results

Breast blisters occurred in less than 2 percent of the birds in each trial and no significant difference was found between the battery and floor birds. A total of three bones were broken due to processing: one broken wing bone in trial 2 from the battery birds, and two broken wings in trial 3, one from each treatment group.

Trial 1. Breaking strength of bones from the battery-grown broilers was not statistically different (Table 15) from those of the floor birds even though they were numerically weaker. The battery-grown birds were slightly heavier than the floor broilers. The males were significantly heavier in both groups. There was a statistically significant difference ($P < .01$) between the males and females with the females having a lower tibia breaking strength, while there was no significant difference between males and females as measured by tibia ash.

Trial 2. There was no significant difference (Table 15) between treatment groups when breaking strength, tibia ash and body weights were compared. The males in both treatment groups had significantly ($P < .01$) higher bone breaking strength and larger body weights than the females, while they were slightly lower in tibia ash values.

Trial 3. Breaking strength of bones from the battery-grown birds was significantly lower ($P < .01$) than those of the floor-grown broilers and tibia ash was approximately the same for both groups.

Table 15. Body weight, breaking strength, and tibia ash for broilers grown in batteries and in floor pens (Experiment 6)

	Body Weight (gms.)		Pounds Tibia Breaking Strength		% Tibia Ash	
	Male	Female	Male	Female	Male	Female
Trial 1						
Battery	1610	1364	32.82	19.73	47.8	51.2
Floor	1432	1209	35.33	21.12	46.4	48.0
Trial 2						
Battery	1839	1490	31.28	20.42	44.2	46.0
Floor	1821	1422	29.26	20.44	44.8	45.5
Trial 3						
Battery	1765	1493	35.35	27.90	46.2	46.6
Floor	1894	1453	44.20	30.38	46.0	47.1

There was no significant difference in body weight.

Discussion

The data from three trials indicated that there was not a large difference between floor-grown and battery-grown broilers when tibia breaking strength, tibia ash, and body weights were the criteria used for comparisons. However, the floor-grown broilers in the third trial had a significantly greater breaking strength than those grown on wire.

Although the battery-grown broilers in trial 3 had weaker bones, this did not appear to have any substantial effect on condition after processing. This difference could be due to a seasonal effect, since a greater difference has been reported for caged hens in summer and winter (Experiment 2). Also, there was considerable difference in feed conversion for floor broilers in trials 1 and 2 as compared to trial 3 (2.35, 2.32, vs. 2.09). Feed consumption was not measured for battery-grown birds. In the third trial the diet contained 1 percent calcium and 0.8 percent phosphorus; therefore, it should have been adequate. However, increasing the level of either or both minerals might have overcome the small decrease of bone strength of cage-reared broilers, since Rowland et al. (1967) found that increasing levels of either mineral increased bone strength.

Summary

Bone breaking strength and tibia ash of broilers grown on wire were not substantially different from those of broilers grown in floor pens in two of three trials. This would indicate that bone breakage, which is characteristic of caged layers does not occur to any large extent in eight-week-old battery-grown broilers. Breaking strength was significantly lower for battery-grown birds in the third trial.

This trial was conducted during hot weather, resulting in a decrease in feed intake. To compensate for the expected lower feed intake the calcium and phosphorus were increased; therefore, it should not have been a factor.

SUMMARY

Six experiments were conducted to study the phenomenon of bone fragility in chickens. In the first experiment a comparison was made in tibia breaking strength between caged and floor layers and roosters. It was found that the breaking strength of bones from hens and roosters maintained on the floor was significantly greater than for those birds maintained in cages. Bone ash was also significantly higher for the floor birds.

In Experiment 2, two trials were conducted to determine the effects of wire pens, floor pens, and cages on tibia breaking strength and tibia ash. In the first trial, it was found that hens maintained in individual cages had significantly reduced bone strength and tibia ash values when compared to hens maintained either in pens with peanut hulls used for litter or raised wire floors. Recycling manure did not significantly improve bone characteristics of the caged hens. In the second trial, it was found that caged hens exhibited a numerically lower tibia breaking strength and bone ash than those in floor pens. There was no difference between floor pens with peanut hull litter and those containing raised wire floors.

The third experiment consisted of two trials which were conducted to study the influence of dienestrol diacetate and Protamone on bone strength of chickens. In the first trial it was found that feeding a level of 622 milligrams of Protamone per kilogram of diet

resulted in significantly strengthening bones of both male and female chickens. The addition of 1408 milligrams of dienestrol diacetate per kilogram of diet significantly increased breaking strength of the bones of hens; however, it decreased the breaking strength of the bones of males.

In a second trial it was found that increasing the level of Protamone from 622 milligrams per kilogram to 2488 milligrams resulted in a numerical, but not significant, improvement in bone breaking strength. Increasing the dietary calcium level from 3 to 6 percent resulted in increased bone strength. The increases from dietary calcium and the addition of Protamone were found to be additive. Supplementing the diet with Protamone did not influence the tibia ash; however, increasing the calcium level from 3 to 6 percent resulted in an increase in tibia ash.

In Experiment 4, three trials were conducted utilizing 12 strains of egg-production-type pullets to determine if tibia breaking strength and tibia ash varied among strains. The data indicate that there was a significant strain difference in tibia strength but that there was no apparent difference in tibia ash among strains. It was found that caged hens had a significantly lower tibia breaking strength when compared to floor hens of the same strain; there were, however, some strains of pullets that were maintained in cages that had a higher breaking strength than other strains maintained on the floor. Floor hens were observed to lay at a slightly higher rate than the caged birds, and in the third experiment had higher tibia ash values.

The fifth experiment was conducted in order to determine the length of time necessary to develop bone fragility in laying hens. The time required to develop a difference between bone characteristics

of cage and floor pullets was found to be four weeks, with a maximum difference between groups occurring at the end of eight weeks. Longer periods of time did not change the difference of approximately seven to eight pounds between the cage and floor bird; however, both groups did exhibit a slight decrease which was not statistically significant with each successive month of lay. It was further observed that tibia ash was closely associated with breaking strength but was not as sensitive a measurement of bone fragility as was breaking strength.

In the last experiment, three trials were conducted to determine the effect of raising broilers in wire batteries on bone characteristics. In two of these trials, bone breaking strength and tibia ash were not substantially different when compared to broilers grown in floor pens. This would indicate that bone breakage, a characteristic of caged layers, does not occur to any large extent in eight-week-old battery-grown broilers. Breaking strength was significantly lower for battery-grown birds in the third trial. This trial was conducted during hot weather, resulting in a decreased feed intake. To compensate for this expected lower feed intake, the dietary calcium and phosphorus were increased; therefore, it should not have been a factor.

From the data obtained in the preceding experiments it is evident that caged layers develop bone fragility within four weeks after housing. The reason for this decrease in bone strength, which is not observed to such a large extent in floor birds, is still unknown, but is apparently due to some degree to lack of exercise and not to being kept on wire. Even though additional calcium and hormones can increase bone strength to a certain degree, the final solution has not been found. Perhaps future work should be directed toward preventing this condition from occurring rather than trying to correct it after it has progressed to a harmful degree.

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BIOGRAPHICAL SKETCH

Lenton O. Rowland, Jr., was born September 29, 1943, in Mobile, Alabama. One year later he moved to Lumberton, North Carolina, where he completed his early education. He moved to Gainesville, Florida, in 1955 and graduated from Gainesville High School in 1961.

Prior to entering the University of Florida he enlisted in the United States Army Reserve, spent six months on active duty and completed his military obligation during his undergraduate years. He received a Bachelor of Science in Agriculture with a major in Poultry Science, December, 1965.

After working for a feed company as a general pullet serviceman for nine months he entered the Graduate School of the University of Florida and received a Master of Science in Agriculture in Poultry Nutrition, August, 1967.

He has been a member of the Gator Band, Poultry Science Club, Poultry Science Association, Triangle Flying Club, was on the 1965 Poultry Judging Team, held the H. R. Mehrhof Scholarship, was the recipient of the Poultry Health Service Scholarship, a member of Sigma Phi Sigma, Alpha Zeta, Sigma Xi, Student Agricultural Council, and has held NIH and Agricultural Experiment Station Assistantships since his entrance in Graduate School.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Robert H. Harms

Robert H. Harms, Chairman
Professor of Poultry Science

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Jack L. Fry

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Professor of Poultry Science

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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