DETERMINING MECHANICAL PROPERTIES AND TYPE OF FRACTURE OF OSSEOUS TISSUES FROM SPENT HENS

by

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(ABSTRACT)

This study was undertaken to evaluate shear, bending, and torsion test procedures on the radius, ulna, humerus, femur, tibia, and tarsometatarsus of 72 week old caged layers. Shear and bending properties were determined to evaluate the effects of juvenile protein feeding sequence, body size, and cage profile on the strength of these bones. Body size affected ($P < 0.05$) the ultimate shear forces of the femur, tibia, and tarsometatarsus. The ultimate bending force and shear modulus of the tibia were also influenced by body weight ($P < 0.05$). Juvenile protein sequence and body weight affected the torque and torsional shear stress of the tibia. No differences were noted in shear and bending strength when bone geometry as well as force was considered. The frequency pattern of the bending fractures occurring in

the radius, ulna, femur, and tibia were influenced by juvenile protein feeding sequence ($P < 0.05$). The bones from the control birds failed in bending due predominantly to a weakness of tensile stresses. The reversal in protein caused the bones to fail due to a combination of tensile and shear stresses. The torsion test of the tibia and radius showed the failure pattern to follow a 45 degree helix around the diaphysis of the bone which is indicative of tensile failures. The four point bending test was not desireable for determining the modulus of elasticity of poultry bones with a length to diameter ratio less than 10. No correlations were found between shear and bending properties of any of the bones. Similarly, the shear, bending, and torsion properties of the tibia were not related.

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"Few researchers are able to find the optimum. While hindsight is said to be keener than foresight, it appears that most researchers consistantly err in one direction or the other. Those who demand more completeness than is needed, do so repeatedly; those who rely too much on iterations, persist in doing so. Fortunately, one researcher's activities tend to balance another's. The reader will have to evaluate his own situation; hopefully he will use hindsight as a basis for corrective action."

Garrett and Goss

taken from ASAE Paper No. 72-825

Chapter 1

OVERVIEW AND BACKGROUND

The increasing cost of fuel and feed energy has made it necessary for the poultry industry to focus its attention towards raising broilers and layers in cages versus floor pens. Eleven years ago Lloyd (1972) stated that cages provide increased capacity in house, more production per year, more efficient use of space and equipment, and recovery of greater investment over a shorter period. However, broilers are still being raised in floor pens because of the higher incident of breast blisters and bone breakage during processing of cage reared birds (May et al., 1981; Merkley, 1981b; Andrews and Goodwin, 1972; Wabeck and Littlefield, 1972; Rowland et al., 1971). Until bone strength is improved through genetic improvement or through dietary means, broilers will continue to be raised in floor pens. However, layers are being grown in cages with downgrading due to broken bones considered to be an economical loss.

Wesley (1983) investigated a processing plant and found 15 percent of the broilers had broken bones or separated joints. The subsequent downgrading resulted in losing 11 cents per kilogram of liveweight. The economic value of spent hens is 18 to 22 cents per kilogram of liveweight

(Murch, 1983). These economical values are significant considering 7.00 Mt of poultry were processed and inspected in the United States in 1982 (Anon., 1983). Eighty percent of this processed meat was related to broilers, four percent to spent hens and breeders, and the remainder to other fowl such as turkeys and ducks. The economical loss to the poultry industry in 1982 was 7.5 million dollars if it is assumed five percent of the meat was downgraded at a cost of only two cents per kilogram. Kralis (1968) reported in extreme cases 85 to 95 percent of the carcasses of spent hens were not acceptable for deboning and further processing.

This tremendous economical loss has necessitated much research in evaluating the bone strength of caged and floor reared birds. Investigators have tried various diets, cage construction, bird density, exercise, and strain of bird for improvements in bone strength. Most of the dietary work was associated with feeding a particular diet at critical periods during a layer's life, such as during the latter part of the egg production cycle. Research with broilers has been directed towards development of an improved cage. With the exception of two studies (Andrews and Goodwin, 1973 and Adams et al., 1971), all research projects have shown the ultimate breaking force of bones from floor reared birds to be higher than those from caged birds.

Jackson (1967) stated that bone fragility resulted from lack of sex hormones, calcium deficiency, malnutrition, or lack of normal stimulation due to stress and/or strain. The general trend has been after a diet was formulated to produce eggs with maximum strength, no further mineral supplements were added to increase bone strength (Ferguson et al., 1974 and Yates and Rutherford, 1967). Moore et al. (1977) believed bone strength could only be improved by genetic selection or dietary supplements.

The emphasis of past research has been to evaluate the effectiveness of a diet based strictly on the ultimate breaking force of a particular bone. No consideration has been given to bone geometry, mechanical properties or fracture mode. Andrews and Goodwin (1973) noted differences in force-deformation curves during testing the tibia of caged and floor reared birds. This should be an indicator that certain bones were deforming more than others; therefore, the diet may have changed the mode of failure and the composition of the bone. However, the fracture mechanism was not investigated by Andrews and Goodwin (1973) or proceeding investigators.

The overall objective of this research was to consider mechanics of materials in evaluating the mechanical properties of bones from spent hens. Selected groups of birds were fed diets which contained various levels of crude protein during the juvenile growing period. Prior research has reported only maximum breaking load, however, effective evaluation of the bone strength requires a knowledge of mechanical properties as well as breaking loads. Further knowledge may be gained by inspecting the mode of failure and determining whether fractures are related to shear or tensile stresses. The specific goals of this research were to:

1. Evaluate test procedures for determining mechanical properties of bones from spent hens,
2. Determine mechanical properties of bones from spent hens which were grown on a reversed protein diet during the early stages of life (0 to 20 weeks),
3. Investigate whether bone fractures are related to shear or tensile stresses, and
4. Determine the effect of diet and body weight on the mechanical properties of bone.

Chapter 2

LITERATURE REVIEW

Osseous tissue or bone is the calcified connective tissue of the body. Bone provides protection, gives rigidity and form to the body, acts as a lever, stores minerals, and provides a site for blood formation. Osseous tissue has unique mechanical properties which enable the tissue to provide rigid kinematic links, attachment sites for muscle, and facilitate muscle action or body movement (Frankel and Nordin, 1980). For example, analysis of the femur requires consideration of eighteen forces exerted on the bone by muscle attachments, a ball and socket joint attachment to one bone, and hinge joint attachment with a second bone (Cowin, 1981). The following literature review looks at testing procedures and limitations in analyzing bones, and specific research projects on bone strength of layers and broilers.

2.1 FORCES AND MECHANICAL PROPERTIES

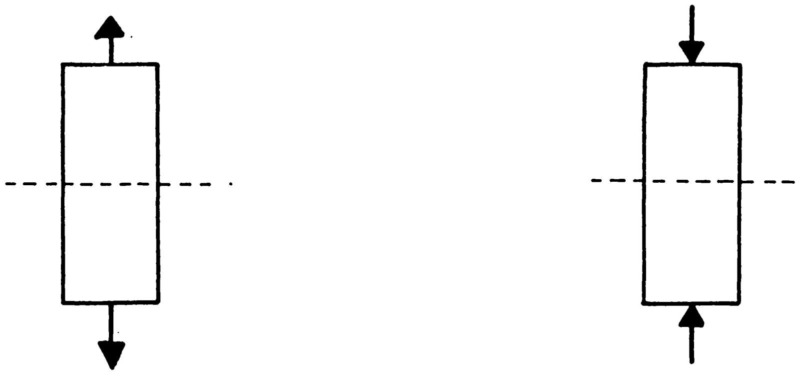
2.1.1 Determining Mechanical Properties of Bone

Nature has provided living organisms with the ability to resist normal forces exerted upon their skeletal system. Loading and geometrical configurations influence the mechanical properties of biological materials. The American Society for Testing and Materials (1966) defined mechanical properties as "those properties of a material that are associated with elastic and inelastic reaction when force is applied, or that involve the relationship between stress and strain". Examples of these properties includes all types of stress and strain, modulus of elasticity, creep, and others. These properties have been determined using one or more of five loading configurations: shear, tension, compression, torsion, or bending (three or four point, Figure 1).

2.1.1.1 Tension and Compression

Tension or compression results when a material has been loaded with equal forces applied at each end of the material. The tensile forces are opposite in direction (Figure 1a) and compressive forces are applied towards the surface of the material. Bones fail in tension due to the debonding of the cement lines and outward movement of the osteons. Compressive failures result in oblique cracking of the osteons and occur in the vertebrae (Frankel and Nordin, 1980).

Tensile and compressive loading have been used to show the anistrophic nature of bone. Simkin and Robin (1974) found the modulus of elasticity of bovine tibiae was 24.5 GPa for tensile loading and 6.4 GPa for compressive loading. The results showed a 75 percent difference in the tensile



a. Tension b. Compression

c. Three Point Bending d. Four Point Bending

e. Torsion f. Shear

Figure 1. Loading configurations used in determining the mechanical properties of bones.

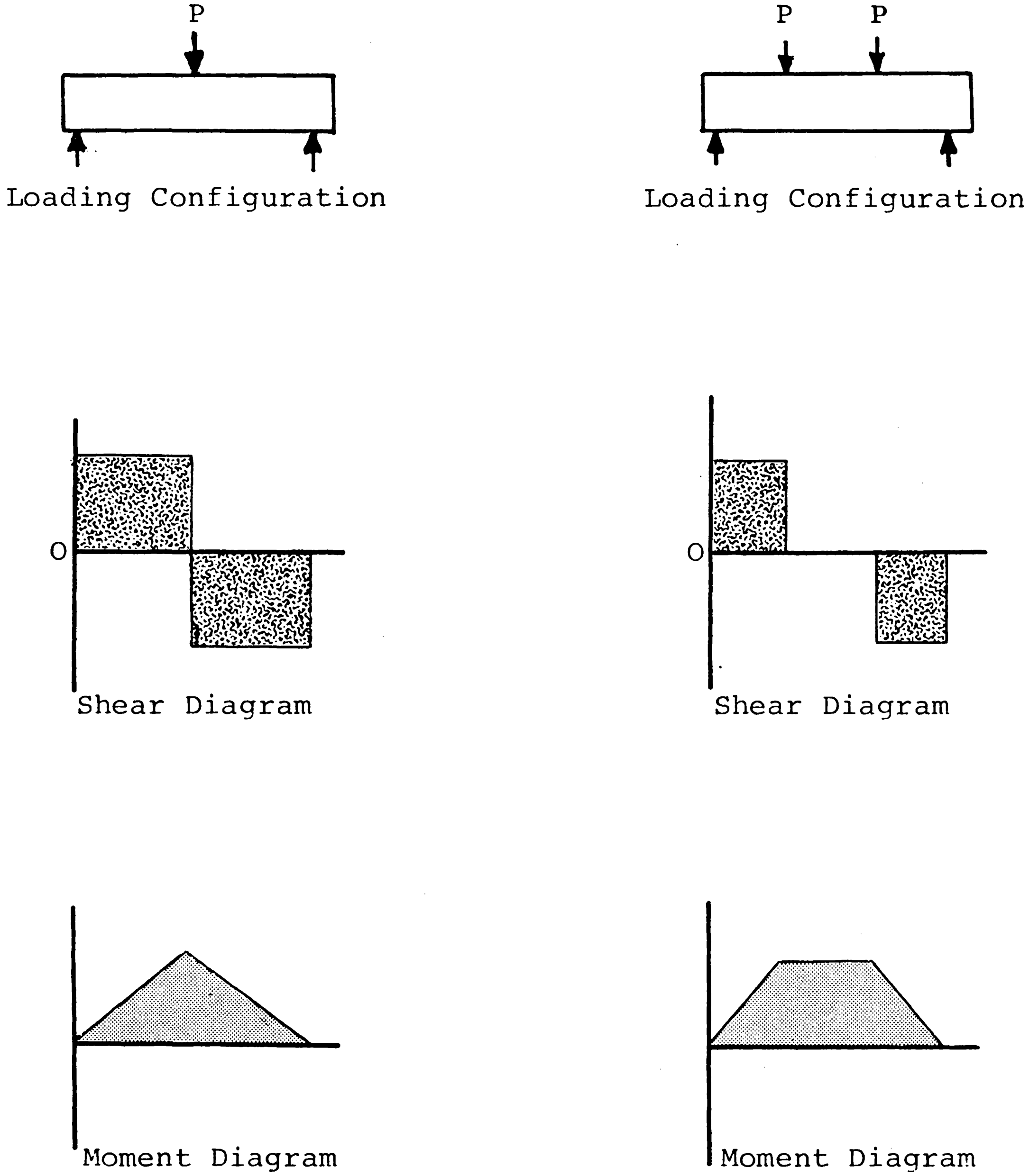
and compressive mechanical properties of bone. In another study involving bovine bones, Reilly and Burstein (1975) reported the modulus of elasticity and Poisson's ratio were not significantly different in the transverse and radial direction. The transverse and radial planes were planes of isotrophy regardless of the direction of loading. The strength ratio in the principal directions of longitudinal, transverse, and radial were 3:1:0.4, respectively. In the longitudinal direction, the ultimate strength of the bone was 33 percent weaker in tension than in compression. A 66 percent reduction was noted in the transverse direction. Reilly and Burstein (1975) concluded that in the longitudinal direction the bone behaved as a plastic material, however, this was not true in the transverse direction.

Reilly et al. (1974) used tension and compression loads to determine the elastic modulus of human and bovine bones Their research indicated that the modulus of elasticity was the same for both tensile and compressive loading, this was unique from other studies where these loading configurations produced remarkably different values for modulus of elasticity (Sweeny et al., 1965; Reilly and Burstein, 1975; Simkin and Robin, 1973). Smith and Walmsley (1959) used tensile and compressive loading techniques to determine the factors affecting the elasticity of bone. They concluded that the modulus of elasticity as determined with compressive loading was only three percent greater than that determined by tension loading.

Yamada (1973) reviewed numerous biomechanics studies from Japanese researchers. The ultimate tensile strength of wet compact bone was greatest in the femur of horses, and then in descending order, in cattle, deer, wild boars, pigs, and ostriches. The distal ends of the bones were found to be stronger than the proximal ends and the middle portion of the diaphysis was stronger than the proximal or distal ends. In the animal kingdom, the ultimate compressive strength of wet femoral bone was greatest in cattle and horses and then in deer, ostriches, wild boars, and pigs. The ultimate compressive strength was 30 to 40 percent higher than the ultimate tensile strength of wet bones.

2.1.1.2 Three and Four Point Bending

Bending results when a force is applied to a beam causing it to bend about its neutral axis. Three point bending results when three forces acting on a structure produce two moments (Frankel and Nordin, 1980). The material is forced to break at the application point of the center load where the moment is maximum (Figure 2a). Four point bending is a result of two force couples producing two moments on a beam.



a. Three Point b. Four Point

Figure 2. Comparison of shear and moment diagrams for three and four point bending loads.

The magnitude of the bending moment between the couples remains constant (Figure 2b). Therefore, the structure will break at the weakest point between the couples (Frankel and Nordin, 1980).

Baker (1976) used three point bending tests in verification of a finite element model on whole bone deflection. The Bernoulli-Euler deflection equation lacked sufficient precision for adequate results with the finite element model. However, the bone displacement could be predicted using the tapered Timoshenko beam element.

Bending tests were used by Burstein et al. (1972) to determine the effects of yielding on specimens from bovine femora. These tests demonstrated the effect of cross-sectional shape on initiation of plastic flow and tensile tests showed the amount of plastic deformation. The plasticity resulted in an increase in moment carrying capacity which meant the effects of yielding should be used in evaluating the mechanical properties of bone. Crenshaw et al. (1981) found diets with higher levels of calcium and phosphorus increased the mechanical and geometrical properties of bones from swine. However, there were no polynomial relationships between percentage of ash and any of the bone strength properties. Pope and Outwater (1974) concluded the fracture strength of the tibiae from Holstein cows was a function of the distance from the proximal to distal epiphysis. The bone was found to be nearly isotropic at the epiphysis and anisotropic over most of the diaphysis. Data analysis indicated there was no significant difference in fracture strength or modulus of elasticity over the middle two thirds of the diaphysis.

A summary of bending tests performed on bones from birds and mammals showed the humerus has higher breaking loads than other bones. The difference in breaking loads for bones of a particular animal range from 30 to 90 percent. In domestic fowls, the femur was the strongest and required a mean breaking force of 215.7 N, while the radius was the weakest and required only 33.3 N until failure. Domestic fowl had a modulus of elasticity of 98.0 MPa which was constant regardless of bone (Yamada, 1973).

2.1.1.3 Torsion

Torsion is a consequence of a force causing a structure to twist about an axis and maximum shear stresses on the extreme fibers. A structure loaded in torsion will have equal shear, tensile, and compressive stresses which allows failure in the weakest mode (Frankel and Nordin, 1980). The cross sectional area and distribution of bone tissue around a neutral axis affect the strength and stiffness in torsional loading.

Evans (1978) used torsional testing to show differences in shear stresses depend upon the location on a femur from where test specimens were machined. Lakes et al. (1979) found similar results between human and bovine bones in viscoelastic properties using torsional and biaxial studies. They concluded that bone was viscoelastic and the degree varied within the individual bone in both time dependence and strain dependence of the shear modulus. Martens et al. (1980) evaluated the effect of strain rates on whole human femur and tibia bones by using torsional tests. The dispersion of energy absorption and angular deformation before fracture was induced by the variation in geometrical properties and material properties. Frankel and Burstein (1971) showed the shear stress at the proximal end was approximately half that at the distal end.

Reilly and Burstein (1975) found the ultimate longitudinal tension and compression stress were at least twice that of the ultimate torsional stress. Long bones tested at higher loading rates withstood a higher torque before failure in torsional test performed by Sammarco et al. (1971). They concluded failure was more dependent upon deformation than loading rate or stress. As the loading rates increased, the place of fracture shifted from the center of the bone to the epiphysis which was related to the energy absorption characteristics of cancellous bone.

Miller and Piotrowski (1974) studied the side to side (right and left hand side) variation in torsional properties of fibulae. The distribution of differences between sides showed no left-right bias and was independent of animal type. Side to side variation was also studied by Puhl et al. (1972) who examined biomechanical properties of paired fibulae using torsional testing. The data indicated paired fibulas exhibited varying degrees of difference depending upon the mechanical property. The conclusion drawn was that the right or left side of dog fibulas could not be used as a control when studying various alterations on the left or right fibula, respectively.

Yamada (1973) reported that torsional breaking moments were proportional to wall thickness. Among species tested, horses had the highest torsional breaking moment for all limbs while guinea pigs had the least. The femur had a higher ultimate breaking moment but a lower ultimate torsional strength than the other bones. The ultimate torsional strength was between 33.3 and 80.4 MPa regardless of animal or bone type.

2.1.1.4 Shear

Shear loads (Figure 1f) are applied perpendicular to the surface of a beam. Shear fractures are usually found in cancellous bone. Cortical bone is strongest in compression, then tension and then in shear (Reilly and Burstein, 1975).

Wilson and Baker (1979) used shear test to determine the shear strength of long shaft poultry bones. The shear stress averaged 39.6 MPa for the tibia and 7.7 MPa for the femur. Their results showed the importance of incorporating shear deflection in determining the mechanical properties of short-deep beams using the three point bending test They concluded if the ratio of length to diameter was less than 10, then shear deflection could not be neglected. When shear was neglected, the modulus of elasticity was 36 percent lower than when shear was incorporated in determining the moduli. Yamada (1973) reported the ultimate shear stress of wet compact bone ranged from 70.6 to 82.4 MPa with an average value of 77.5 MPa. The average ultimate shear stress of long bones was highest in horses which was 50 percent higher than the stresses in a pig. The average value in horses was 91.2 MPa and in pigs was 60.8 MPa. The shear strength of human bones was between those of wild boars and pigs.

2.1.1.5 Selection of Test Procedure

Materials which are homogenous, isotrophic and elastic will have the same mechanical properties regardless of test procedures used. Since bone is nonhomogenous and anistrophic, it has different mechanical properties depending upon load configuration and geometry. Other considerations include testing a bone wet or dry, frozen or rethawed, and machined or whole bone specimens. Burstein and Frankel (1971) ruled out tension and compression tests because of lack of clinical fractures. Mather (1967) indicated that bending tests should be used because clinical fractures were a result of this load. Smith and Walmsley (1959) noted differences in Young's modulus when using tension, compression and bending tests. They recommended bending tests because long bones were invariably subjected to bending stresses. The behavior and mechanical properties were more accurately reflected when bending tests were used (Smith and Walmsley, 1959). Baker and Haugh (1979) stated that the three point bending test was used because of simple testing procedures and large deflection. Burstein and Frankel (1971) criticized three and four point bending test because of application of loading force and bone geometry, respectively. They recommended torsional testing because the bone was subjected to equal stresses at every cross-section along its length. This test enabled the weakest section to be determined using a realistic loading mode (Martens et al., 1980).

2.1.1.6 Determining Cross-Sectional Area

The problem of determining the cross-sectional area and moment of inertia of a bone specimen has been eliminated in many investigations by using machined specimens (Martin and Atkinson, 1977; Reilly and Burstein, 1975; Pope and Outwater, 1974; and Smith and Walmsley, 1959). However, many methods have been developed for determining the geometrical properties of whole bones.

Lovejoy and Burstein (1977) used laminography to determine the geometrical properties of whole bones. The technique involved x-raying the specimen and then photographing the cross-section and the x-ray. The black and white slides were projected onto a 50 x 50 cartesian grid. The distribution of the cortical bone was an estimation of the amount occurring within the grid. The laminography technique produced areas within 4 percent of actual measured cross-sectional area. X-raying and radiographs were used by Minns et al. (1975) in studying the geometry of tibiae. The x-rays were enlarged three times and projected onto a cartesian grid of 30 x 30 where the major axes of the cross-sections were measured.

Another technique has been to measure the principle axes perpendicular to the long axis and model the bone as a standard geometrical shape. Martens et al. (1980) modelled the human femur as a circular cross-section and the tibia as triangular. Crenshaw et al. (1981) modelled the femur and humerus of swine as ellipses. The metacarpal, metatarsal, and rib were assumed to be elliptical quadrants. Standard equations were then used to determine the geometrical properties. Martin and Atkinson (1977) used silhouettes of the top and bottom of a cross-section from the human femur. The silhouettes were obtained by placing the cross-sections on a piece of photographic paper in a dark room. The picture was magnified four times and a ruler was used to measure external and internal dimensions of an assumed circular cross-section. Mather (1967) used a planimeter to determine the area of a photograph of the cross-sectional areas from whole bones. In a comparison of the moment of inertia based on using Mohr's method (Andrews, 1932) and a planimeter versus assuming the cross-sectional perpendicular to the long axis of the femur was an ellipse. He found the error ranged from 1.10 to 38.0 percent. Piziali et al. (1980) and Piziali et al. (1976) determined the cross-sectional area of various bones with a square presmatic epoxy mold. The geometry was determined using an optical digitizer and then a computer program converted the data to cross-sectional area and moment of inertia.

2.1.1.7 Effects of Drying and Freezing on Mechanical Properties

Literature shows consideration has been given to the condition of bones at testing. The mechanical properties are different for dry versus wet or frozen versus thawed specimens.

Rowland (1967) developed a procedure for testing poultry bone. After defleshing a bone, it was placed in boiling water for six minutes and then air dried at room temperature for 48 hours. Merkley and Wabeck (1975), Merkley (1981a), and Cox and Balloun (1971) used an oven to dry the bones at 95°C for 24 hours prior to testing. Smith and Walmsley (1959) concluded moisture evaporation from a bone followed a logarithmic curve and Young's modulus was seven percent higher during the first hour of testing. Evans and Lebow (1951) found Young's modulus of human femur was 18 percent higher for an air-dried bone. Weir et al. (1949) found only a two percent difference in Young's modulus of a rat femur tested wet and dry. Kornegay et al. (1981) found drying of the metacarpals from swines increased the stiffness, flexural modulus and Young's modulus when compared to those bones tested wet. Evans (1973) reported all investigators studying the problem have found drying increased the tensile and compression strength characteristics, modulus of elasticity, and hardness of bone. Drying decreased the mechanical characteristics of shearing strength in a direction perpendicular to its long axis and its energy absorbing capacity. Merkley (1981a) compared results of bone which were dried at 95°C for 18 hours and wet bones and found the wet bones required more breaking force than dried bones. These results were in agreement with Kornegay et al. (1981). However, the overall conclusions of Merkley et al. (1981a) were not affected by drying the bones. Wet bones deviated from a straight line at the proportional limit while dry bones tended to have a linear stress-strain relationship (Evans, 1973 and Yamada, 1973).

Smith and Walmsley (1959) found an inverse relationship between temperature and modulus of elasticity. As the temperature increased, the elasticity decreased proportionally. Merkley and Wabeck (1975) looked at the effect of freezing bones prior to testing. The humeri and tibiae both had a 61 percent reduction in breaking force when the bones were tested frozen. There was a significant reduction in strength; however, for comparative purposes within a particular study, the freezing did not affect the results. Seldin (1965) tested machine specimens and found no statistical difference in mechanical properties of bones which had been frozen and then thawed. He concluded bones could be frozen at -20°C without changes in strength characteristics after thawing.

2.1.2 Summary of Part One Literature Review

The anistrophic nature of bone has been established using machined specimens which were tested in tension and compression. Mechanical properties of whole bones were determined using bending test which provided a more realistic loading mode. Machined samples have eliminated the need to develop procedures for determining the geometrical properties. However, the structural characteristics of milled specimens cannot be directly related to whole bones. This portion of the literature review has tried to convey the importance of considering bone condition at testing (moisture content, temperature, and size), types of loading, and types of results being reported by other researchers.

2.2 BONE STUDIES IN POULTRY

The second part of the literature review deals specifically with studies involving bone strength of chickens (broilers and layers). Test procedures, bone types, and general results are presented.

2.2.1 Layers

The effects of sodium flouride on egg production, quality and bone strength of caged layers were investigated by Merkley (1981a). After 25 weeks of production, the birds were sacrificed and the humeri and tibiae were removed. The breaking load of the humeri ranged from 74.5 to 195.4 N for test one and 66.6 to 114.7 N for test two. The highest breaking strength occurred when the birds received flouridated water for 45 weeks. The tibia breaking strength ranged from 80.6 to 121.5 N and from 64.6 to 122.1 N for two similar experiments. Similar to the humeri, the tibia was stronger in those layers receiving flouride for 45 weeks. The results also showed that it is more important to add flouride to the water during the formation of the bone.

Engster et al. (1981) investigated feeding layers a diet with increases in flouride due to either sodium flouride or soft rock phosphate. Inclusion of sodium flouride in the diet increased the bone strength by nine percent over the control group. A 14 percent decrease was noted in bone strength of birds fed a diet containing soft rock phosphate. The bone ash was significantly increased with those rations supplying 250 ppm of flouride. The flouride levels in the bone were increased by a factor of ten in those birds recieving the soft rock phosphate. The sodium flouride increased the flouride in the bones by a multiple of 20.

Rowland et al. (1972) found differences in the strength of the tibia based on strain of layer. The breaking strength was from 215.5 to 340.1 N with a difference observed between strains. The strength of the tibiae from caged layers varied from 139.9 to 178.7 N and floor reared layers ranged from 161.2 to 212.1 N. A difference between caged and floor reared layers existed in all strains, however, no difference in percent bone ash. However, no relationships existed between body weight, breaking strength, tibia ash, and egg production.

The bone strength of caged and floor reared layers and roosters fed diets containing different levels of phosphorus was evaluated by Rowland et al. (1968). The tibiae from caged reared hens were significantly weaker than the tibiae from floor reared hens. The left tibia of twelve birds sacrificed after 5 and 10 months of production were removed and tested according to procedures outlined by Rowland et al. (1967b). The breaking strength of the tibiae varied from 133.4 to 199.1 N. There was no significant difference noted based on the level of phosphorus contained in the diet. A decrease was noted in bone ash; however, this was not considered to be the contributing cause towards a reduction in breaking strength of the tibiae in caged layers. The breaking strengths of the tibia from the roosters were 611.9 and 718.8 N for cage and floor reared birds, respectively. This indicated the decrease in bone strength of caged birds was not solely attributed to a depletion of calcium during egg production.

The effect of different types of housing (pens, floored pens, or cages) was investigated by Rowland and Harms (1970). The tibiae were significantly weaker in caged layers versus the tibiae from floor reared birds. The addition of 10 percent feces to the diet did not influence body weight, tibia strength, or ash content. The breaking strength varied from 140.8 to 198.5 N depending upon housing conditions. The breaking force of tibiae from caged layers was lower but not different in a similar experiment. Rowland and Harms (1970) concluded the difference in tibia breaking strength of layers in cages was related to confinement and lack of exercise rather than to the lack of recycled fecal nutrients.

Rowland and Harms (1972) examined the time required to develop bone fragility in floor and cage reared laying hens by extracting the tibia from 20 hens every four weeks after production began. After four weeks of production, there was a significant difference in breaking strength of the tibia between the layers grown on the floor and those grown in cages. Breaking strength gradually decreased in layers raised in cages during the entire five months. The difference in tibia breaking strengths remained constant after the eighth week. There was an increase in the strength of the tibia of floor birds during the first three months and then a gradual decrease. Tibia breaking force varied from 149.1 to 155.0 N for caged layers and 156.1 to 192.7 N for floor reared birds. The experiments showed bone strength decreased rapidly after the sixteenth week of production. Rowland and Harms (1972) concluded the cages prevented the skeletal system from developing sufficient strength to allow a gradual decrease in bone mass during the laying period and yet remain strong enough to prevent shattering in the processing plant.

In an earlier study on bone fragility of caged layers by Rowland et al. (1967a), an increase in dienestrol diacetate in the diet resulted in significant increases in tibia strength and ash content compared to the control group. Results of their experiments showed there was statistical difference in breaking force based on deformation rate. At a rate of 0.27 mm/s, the breaking force was 209.8 N versus 232.4 N for the higher speed of 6.25 mm/s. At the 1.0 and 6.0 percent calcium levels, the breaking force of the tibia was significantly lower and higher, respectively, than the 3.0 percent group. A correlation between bone ash and breaking strength of tibiae and higher levels of dietary calcium and the cessation of egg production resulted in higher breaking strengths and bone ash. However, this relationship between ash content and bone strength was not supported in later research (Rowland et al., 1972).

The possibility of increasing bone strength of caged layers by feeding a diet which included portland cement or sodium biocarbonate was studied by Ferguson et al. (1974). The breaking strength of the radii did not differ significantly; however, there was an increase in strength from 31.4 to 34.0 N in the radii from birds fed the sodium bicarbonate. An increase in percent cement also showed an increase in strength since the forces were 31.9, 33.2, and 34.0 N for the three increasing levels of cement in the diet. The conclusion drawn was that the additional calcium was necessary for increasing the breaking strength of the radii.

Moore et al. (1977) examined the radius and egg characteristic of laying hens fed different diets and living in different types of housing. No statistical difference was found in breaking strength of the radii at four months of age as a result of dietary supplements. The bones of the floor birds had a higher breaking strength than birds housed at densities of one or three per cage. The average breaking force prior to placing the layers on the experimental diets was 30.2 N. The radii of the floor pen birds had a breaking force of 31.0 and 31.2 N for the fourth and twelfth production periods, respectively. The breaking force of the radii from caged layers varied from 18.2 to 30.7 N. Significant increases in breaking strength of radii from caged layers was observed for those groups fed supplements of vitamin D_3 or calcium and phosphorus. However, hens raised in floor pens showed no response in breaking strength of the radii when their diet contained extra calcium and phosphorus.

Bone breakage as affected by type of housing or exercising of the layers was examined by Meyer and Sunde (1974). Layers maintained on litter and fed calcium carbonate versus dolomitic limestone had significantly higher breaking forces and percent ash for the tibiae. Birds which were transferred from cages to floor pens during the last two or four weeks had no broken wings. Floor reared birds had greater breaking strength at the $P < 0.01$ level than any cage reared bird regardless of diet supplement. The breaking strength of the tibia ranged from 197.1 to 247.1 N for experiment one. Exercising significantly increased the breaking strength of the humeri and reduced the number of broken bones in the wing. The force required to break the humerus from a floor reared bird was twice that of any group raised in cages (regardless of exercise). Minor differences were noted in the breaking force of tibia from the exercised birds grown in cages. Tibia from floor birds had a breaking force of 252.0 N and tibia of caged birds required a force of 182.4 N. Exercising decreased the incidence of broken bones during processes; however, bone strength was not increased.

Processed birds from floor and cage reared birds were used to evaluate procedures for studying bone breakage and strength (Adams et al., 1971). Results showed the mean breaking strength of the femur was greatest in caged birds with no broken bones during processing and the floor birds had the weakest femurs. There were no statistical differences in the breaking forces which varied from 141.4 to 157.5 N. A similar trend was observed for the tibia with the breaking strength ranging from 220.6 to 245.1 N. The wing bones (radius, ulna, and humerus) from caged birds were weaker but not statistically different than floor reared birds. The breaking forces ranged from 187.1 to 242.2, 144.1 to 164.7, and 52.9 to 65.7 N for the humerus, ulna, and radius, respectively. The higher value was the mean value for floor reared birds and the lower value for caged birds which had bones broken during processing. The clavicle was observed to be the most frequently broken bone and had a breaking force ranging from 45.1 to 54.9 N, however, there were no significant differences. They recommended using the radius as an indicator of the strength of the clavicle since it was easy to remove with minimum damage to the carcass.

2.2.2 Broilers

The effects of flouridated water on the bone strength of caged broilers was studied by Merkley (1976). The humeri was significantly stronger in all experiments with birds drinking the flouridated water. The ultimate breaking force of the humeri from the control group ranged from 113.7 to 136.3 N. Humeri from treated birds had maximum breaking loads of 136.3 to 160.8 N depending upon the level of flouride treatment. Tibiae breaking strength ranged from 61.8 to 144.1 N in control groups and 94.1 to 167.7 N in treated groups. Flouride increased the bone strength from 16 to 40 percent and was a function of sex and level of treatment.

Supplementing the diet of broilers with sodium flouride or sodium silicate in the drinking water was investigated by Merkley and Miller (1981). The breaking strength of the humeri from the treated birds drinking the water with sodium flouride showed a 40 percent increase in strength ($P < 0.01$) and sodium silicate showed a 21 percent ($P < 0.05$) when compared to the strength of the humeri from a control group. The difference in breaking strengths of the tibiae between birds on the control water and those receiving the sodium flouride in the water was significant at the $P < 0.01$ level.

Andrews and Goodwin (1973) studied the performance of broilers reared in cages with different types of flooring materials. No significant difference existed between caged and floor reared birds in the maximum breaking force of the tibia. They concluded this was a possible result of a rubber-nylon floor used in the cages which may have produced a trampoline effect. The significant difference in breaking force of the humerus was attributed to lack of wing exercise.

The effect of cage density on bone strength of broilers was examined by Merkley and Wabeck (1975). The control group was reared on the floor at a density of 0.09 m^2 per bird. The densities of the caged birds were 0.04, 0.06, and 0.09 m^2 per bird for zero to eight weeks. The breaking strengths of the humeri were significantly greater for the floor reared versus caged birds in two of the three experiments. The humeri had a breaking force of 221.2 to 214.9 N for floor reared birds and 101.8 to 238.6 N, 91.5 to 178.8 N, and 98.8 to 221.0 N for cage density of 0.09, 0.06, and 0.04 m^2 per bird. Using combined results of the three trials, there were no differences in the breaking strengths of the humerus or the tibia based on cage density.

Wabeck and Littlefield (1972) looked at bone strength of broilers reared in floor pens and in cages with different bottoms. Floor pen birds showed no wing breakage while caged birds showed 10 to 30 percent breakage. The breaking strength of the humeri ranged from 156.4 to 199.6 N for caged birds and 344.9 to 373.5 N for floor reared birds. There were no differences in breaking force of humeri based on type of cage bottom. Significant difference was found between cage and floor treatments. The tibiae had breaking forces of 237.6 to 329.0 N for caged birds and 296.8 to 330.8 N for floor birds. Similar to humeri, no differences were found based on type of cage bottom and a significant difference occurred between caged and floor birds.

A comparison of bone strength of broilers reared under various conditions in coops and floor pens was performed by Merkley (1981b). The right side set (tibia, humerus, and radius) of bones were tested fresh and the left side were oven dried for 24 hours. No numerical or statistical trends were noted in the strength of fresh or dried wing bones from birds reared in fan-equipped and control coops. Placement of a screen ceiling at a height of 0.23 m above the floor significantly reduced the breaking strength of fresh and dried humeri. The strength of the radius displayed trends similar to the trends of the humerus. The screen reduced the strength of the radius to levels found in the coop reared birds. An increase in floor space along with the screen ceiling did not increase the strength of the radius. When the ceiling height was raised to 0.46 m, the humerus and radius both displayed strength characteristics similar to floor pens with no ceiling restriction. The ceiling height affected only the breaking force of the wing bones and not the tibia. Drying the bones reduced the breaking force but did not affect the overall conclusion of the experiment.

The results from a study involving the relationship of pen height to bone strength of broilers were presented by May et al. (1981). The humerus strength was significantly lower for birds raised in floor pens with a ceiling height of 0.33 m versus a height of 0.64 m or 2.54 m (conventional ceiling height). Wire flooring or restriction of pen floor did not further reduce the bone strength. The ultimate stress of the bones from birds in cages decreased as the ceiling height decreased and was not affected by type of flooring. The stress of the tibia was not statistically different in any of the tests regardless of floor type or ceiling height.

Rowland et al. (1971) compared bone characteristics of floor and battery grown broilers in three experiments with similar diets. They found no statistical differences between the strength of the tibia from floor and battery birds in two of the three experiments. They concluded that the breaking strength and ash content of the tibia were not different for caged versus floor grown broilers.

2.2.3 Summary of Poultry Studies

The review of literature showed many studies involved in determining the effects of diet or cage construction on the bone strength of layers and broilers. These projects are summarized in Tables 1 and 2. Table 1 shows the studies involving bone characteristics of layers. A prominent investigator was Rowland who used a modified Allo-Kramer Shear Press (three point bending test). His procedure was to dry the tibia for 48 hours at room temperature and then perform the test. Merkley has been involved in many of the studies related to broilers (Table 2). He used an Instron Universal Testing machine in his research and tested dry, wet, and frozen bones in three point bending. Except for one study, May et al. (1980), the reported results were maximum breaking force and no consideration was given to the geometrical properties. Bones of caged layers were weaker than those of floor reared birds in many studies. Results of studies shown in Tables 1 and 2 showed that the tibia was studied in 17 of 18 investigations and the humerus in 9 of 18. Other bones studied were the ulna, femur, radius, and clavicle.

Table 1. Summary of literature on studies of bone breakge and strength of layers.

Investigators	Bone	Objective	Conclusion
Merkley, 1981	Humerus Tibia	Effect of sodium flouride	Flouride increased bone strength for both the humerus and tibia.
Moore, et al., 1977	Radius	Effect of calcium and phosphorus	Dietary supplements had no effect on the breaking strength of the radii.
Meyer and Sunde, 1974	Humerus	Effect of housing type	Exercise increased humerus breaking load and reduced wing breakage in processing.
Ferguson, et al., 1974	Radius	Portland cement and sodium biocarbonate effects	Neither portland cement or sodium biocarbonate had any effect on radii bone strength.
Rowland and Harms, 1972	Tibia	Time to develop bone fragility	At four weeks of age, floor reared birds had higher tibia breaking loads than caged birds.
Rowland, et al., 1972	Tibia	Difference in strains	Significant difference in tibia breaking loads between strains.
Adams, et al., 1971	Femur, tibia radius, humerus, ulna, clavicle	Determine bone to use for future fragility studies	Use the radius as an indicator of clavicle strength, the most frequently broken bone during processing.
Rowland and Harms, 1970	Tibia	Effect of pen type	Breaking strength of floor reared birds was greater than caged birds.
Rowland, et al., 1968	Tibia	Effect of dienestrol dialetate	Increased levels of dienestrol diacetate resulted in an increase in bone strength.
Rowland, et al., 1968	Tibia	Compare effect of phosphorus on floor vs. caged birds	Breaking strength of floor reared birds was greater than cage reared birds.

Table 2. Summary of literature on studies of bone breakage and strength of broilers.

Investigators	Bone	Objective	Conclusion
May, et al., 1981	Humerus Tibia	Effect of cage pen height	Breaking strength of the humerus decreased as cage height decreased.
Merkley, 1981	Humerus Radius Tibia	Effect of cage height and air movement	Air movement had no effect on bone strength, decreasing cage height decreased strength of humerus and radius, no effect on tibia.
Merkley, 1976	Humerus Tibia	Effect of flourided water	Flourided water caused an increase in bone strength.
Merkley and Wabeck, 1975	Humerus Tibia	Effect of cage density	Cage density had no effect on bone strength.
Andrews and Goodwin, 1972	Humerus Tibia	Effect of cage floor	Humeri breaking strength was higher for floor reared birds, no difference in tibia breaking strength.
Wabeck and Littlefield, 1972	Humerus Tibia	Effect of cage floor type	Type of cage floor, wire, flexible tubing, mylar mat or steel mat, had no effect on bone strength.
Rowland, et al., 1971	Tibia	Comparison between floor and battery grown birds	Strength of tibia was similar between floor and battery birds.

The majority of the studies have shown the breaking force of bones from cage reared birds were weaker than those bones from floor reared birds. The restriction in cage height influences the structural characteristics of living organism, such as bone, in chickens. The experiments indicate that denial of wing mobility affects the breaking force of the bones more than lack of minerals. Diet supplement or redesign of cages have not improved the strength characteristics of bones from birds grown in cages. However, with the exception of one study, only the breaking force was considered and not the influence that the bone geometry may have had on the ultimate loads.

2.3 FRACTURE MECHANISMS

There was minimal information found in the literature on the type or location of fractures occurring in bones of spent hens or layers. Prior research has focused on evaluating the effects of some parameter on the ultimate force. However, an alternative involves defining the fracture based on the atomic structure, the shear stresses, and the tensile stresses. Since it appears that this concept has not been applied to bone fractures, a brief overview of the subject is presented.

Any structural material is composed of crystals or grains which are built from atoms. Knowledge of the inter-ionic potentials and the energy levels of the bonding electrons provide a better understanding of why a material possesses certain strengths or resistance to fractures (Knott, 1978). A fracture occurs because of the crystals debonding (tensile) or two planes of crystals sliding (shear). The failure mechanism may be described in terms of the atomic slip, the effect of dislocations in atomic structure, or the micro-crack formulation. Determining the exact conditions under which the combined stresses cause a failure is difficult. However, the fracture may be described as brittle (cleavage) or ductile (Ugural and Fenster, 1975).

2.3.1 Brittle Fractures

A brittle fracture is the result of an elongation in the atomic bond between two planes. The material deforms elastically and displays no plastic deformation or yielding under stress. This fracture separates along crystallographic plane of the atomic bonds. Theoretical stresses required to separate the planes of atoms or crystals are higher in magnitude than normal stresses. In conventional materials, the cohesive forces between matrix and particles are large which reduces the chance of failure due to separation of molecular bonds. However, normal tensile stresses are sufficient for debonding, an indication that structural defects in the crystals play an important role in the fracture mechanism. The crystal lattice is composed of large particles resulting in average stresses being able to debond the atomic bonds. In summary, brittle fractures are a separation or cleavage of fibers which display minimum yielding before failure.

2.3.2 Ductile Fracture

A ductile material shows considerable plasticity and yielding before permanent deformation. Yielding is the result of two planes of atoms slipping or the displacement between two sections of a crystal. The crystallographic plane where slip occurs is known as the shear or slip plane. Those planes with the highest number of atoms per unit area are the easiest to slip. The total effect of slip occurring along many randomly oriented planes is represented by yielding. The amount a crystal yields is affected by those interstitial solutes which are in the solvent lattice. Ductile fractures are due to a slip displacement of two planes of atoms.

The fracture mechanism is a function of the amount of yielding. Brittle fracture requires the cohesive forces between atoms to be broken. A ductile fracture is a result of shear stresses being able to disorder the crystals and cause movement in planes of atoms.

The fracture mechanism was not discussed in reviewing the strength characteristics of bones from broilers or layers. Although the analysis of fractures in machined samples from bovine and human bones has been reported, there were no discussions of the fracture mode of whole bones.

Bonfield et al. (1978) investigated the fracture mechanic parameters of the bovine femora. A proportional relationship occurred between crack velocity and the speed at which the load was being applied. An increase in crack velocity was associated with a critical strain energy release rate and a vertical stress intensity factor. Wright and Hayes (1977) used linear elastic fracture mechanics to study longitudinal crack propagation in compact bovine bone. They found a five percent increase in bone density resulted in a 30 percent increase in the critical stress intensity factor. However, no relationship was found between specimen thickness and stress intensity.

Evans (1978) found a correlation between torsional properties of bones and the histological components in the break area. The torsional shear stress, shear modulus, and energy absorbed to failure were increased with an increase in the percentage of complete osteons in the break area. However, an increase in the percentage of osteon fragments reduced the shear stress and modulus. In this study, the fractures were related to shear stresses rather than tensile stresses. The fractures were perpendicular to the long axis rather than a spiral around the long axis. While the importance of the fracture type was not discussed, Evans (1978) provided a complete discussion on the percentage of primary and secondary osteons and their relationship to the torsional properties. Pfafrod et al. (1975) found that torsional fracture was dependent upon the direction of load relative to the orientation of the osteons. They also noted mechanical properties were decreased with an increase in void spaces in the bone matrix. These results were in agreement with Sammarco et al. (1971) who indicated bone failure was more dependent on deformation than stress when subjected to torsional loading.

2.4 IMPORTANCE OF PROTEIN IN EARLY DIET

Bone has unique carbohydrate-protein complexes which could be associated with the initiation of calcification by acting as an anion-cation buffer. There is an intense and rapid calcification along with an increase in non-collagenous protein during bone formation. The calcification front is characterized by amphorous calcium phosphate with the crystal resulting latter on. Vaughan (1975) postulated the bone serum albumin in the bone tissue fluid was in contact with the cells on the surface of the bone and then became incorporated into the matrix structure. These vesciles in the bone tissue could then burst and liberate amorphorous calcium apatite. The matrix molecule, sialoprotein, acts as an ion buffer and releases anions and cations with the apatite crystals attaching to these nucleation sites. The protein-polysaccharides, associated with the hypertrophic cells, could then release calcium ions in sufficient amounts to induce further calcification on the nucleation sites. Thus, the protein in the early diet is needed not only for the nucleation site for initial apatite crystal growth but also for storage and transport of the calcium ion during bone formation.

Mariotti (1976) found the growth rate of bone was highest in the proximal metaphysis regions, lowest in the mid-diaphysis region, and intermediate in the proximal epiphysis. The ossification procedure results in an increase in the mean length of the apatite crystal without changing the diameter (Currey, 1969a). The longer apatite crystals had an increase in strength and stiffness which permitted a greater portion of the crystal structure to resist the shear stresses. In the present investigation of the fracture of the tibia, the speculated regions with the longest apatite crystals, i.e. region of bone with less nucleation sites, were the areas where the bone failed due to tensile stresses. The tensile strength of collagen fibers was to be of the same order as that of bone (Currey, 1969b). However, the apatite crystal, calcium-phosphate compounds, act as stiffeners in the bone matrix. The calcium compounds are removed during the egg production cycle, therefore, weakening the structural characteristics of bones from layers. Tam et al. (1976) found bone formation in rabbits to follow a biorhythm pattern rather than constant growth. Assuming this occurs in the ossification of bones from other animals, it becomes rather difficult to predict when high levels of protein intake are needed for maximum bone strength. Different protein compounds are involved with the nucleation sites, transporting the calcium to the nucleation site, and direct participation in the structural mineralization of bone. Protein is important in all phases of bone formation and the literature tends to suggest that reversal of protein in the juvenile growing diet will affect the structural characteristics of bones.

2.5 CONCLUSION OF LITERATURE REVIEW

Mechanical properties of osseous tissues have been determined using one of six loading configurations. The research has established the anistrophic nature of bone by using machined test specimens. Mechanical and geometrical properties of bone vary between the distal and proximal epiphyses and in a cross-sectional direction. The difficulty arises when trying to apply these results to whole bones. Poultry researchers have evaluated the ultimate bending force of whole bones from chickens, quail, and turkeys. However, stress or the relationship between geometry and force was not considered in these tests. No attempts were made to determine if the reduction in breaking force of bones from cage reared birds was accompanied with a reduction in cross-sectional area of the bone. Nature has the unique ability to resorb bone tissue if the osseous tissue is not being properly utilized.

Chapter 3

EXPERIMENTAL PROCEDURES

Cowin (1981) emphasized the importance of not only knowing the breaking load but also the mechanical properties and type or mechanism of fracture. Emphasis was also placed on determining the effects of change in diet or environment on mechanical characteristics of bone. This approach has not been considered in analyzing strength of bones from spent hens or broilers.

The project presented herein was the result of analyzing bones from spent laying hens using a biomechanics approach. One of the goals was to determine the mechanical properties of various bones rather than merely the breaking load. Another objective was to define the mode of failure of the bones using three point bending and torsion tests. Statistical analysis was performed on all results to determine the effects of a restricted protein diet and body weight on the mechanical properties. Thus, the overall approach was to determine breaking load, mechanical properties and mode of failure, and the effect of diet or body weight on these parameters.

3.1 BACKGROUND OF STUDY

A study evaluating the influence of reverse protein feeding regimes during the juvenile period of chickens was undertaken by the Poultry Science Department of Virginia Polytechnic Institute and State University in 1980. The project goals were to determine the effects of restricted protein in the juvenile diet on maturity, egg production, and egg shell strength of 576 single comb white leghorn chickens. During the first 20 weeks, a standard diet (control) and three different diets (Table 3) with a reversed protein regime were fed to groups of 144 chicks. The 144 layers per treatment were subdivided into small, medium, and large (48 birds/group) based on body weight at 20 weeks of age (Table 4). Birds were then placed on similar diets (Table 5) and into production for 52 weeks with their egg production and growth characteristics being evaluated every 28 day period. After 52 weeks of production, the birds were reweighed (Table 4) and twelve birds were randomly selected from each diet treatment and body size and then sacrificed. Two sets, a right and left set, of bones were removed from each bird. Each set consisted of the radius, ulna, humerus, femur, tibio-tarsus (tibia), and tarsometatarsus. After defleshing, cleaning, and labeling, the bones were placed into cold storage at -4°C and remained there until time of testing. The bones were thawed before testing.

Table 3. Sequence of percent crude protein in juvenile diet by age (weeks).

Juvenile Protein Sequence	Age in Weeks							
	0-1	1-2	2-3	3-4	4-6	6-8	8-14	14-20
Control	18	18	18	18	18	15	15	12
One	18	18	18	12	12	12	15	18
Two	18	18	12	12	12	12	15	18
Three	18	12	12	12	12	12	15	18

Table 4. Mean body weight (kg) of the layers at 20 and 72 weeks of age.

Age (weeks)	Body Size	Juvenile Protein Sequence			
		Control	One	Two	Three
20	Small	1174.25	1142.42	1145.50	1150.42
	Medium	1266.08	1258.42	1257.50	1233.17
	Large	1389.25	1361.83	1370.33	1365.00
72	Small	1696.25	1667.08	1705.42	1616.67
	Medium	1860.41	1852.50	1848.75	1790.00
	Large	1977.50	2055.83	2050.83	2037.08

Table 5. Composition of the diets during the grower, prelayer, and layer periods.

	Period							
Ingredient	Grower			Prelay	Layer	Layer	Layer	Layer C[2]
% Protein in Diet	18%	15%	12%	18%	18%	17%	16%	16%
Ground Corn	66.67	72.08	77.49	61.79	61.79	64.29	66.80	
Soybean Meal	21.28	14.14	7.00	25.47	25.47	22.97	20.46	
Rice Hulls	3.35	5.65	7.26	4.00	1.35	1.35	1.35	
Fish Meal	2.50	2.50	2.50	---	---	---	---	
Alfalfa Meal	2.50	2.50	2.50	2.00	2.00	2.00	2.00	
Limestone	.50	.50	.50	4.34	6.75	6.75	6.75	
Defl. Phosphate	1.72	1.77	1.82	1.50	1.75	1.75	1.75	
Vitamin Premix	.50	.50	.50	.50	.50	.50	.50	
Trace Mineral Premix	.05	.05	.05	.05	.05	.05	.05	
Salt	.25	.25	.25	.30	.30	.30	.30	
DL-Methionine	.05	.05	.05	.04	.04	.04	.04	
L-Lysine	---	.01	.08	---	---	---	---	
Metabolizable Energy (kwl/lb)	1318	1318	1318	1253	1253	1262	1270	1260
Calcium	1.00	1.00	1.00	2.25	3.25	3.25	3.25	3.5-3.75
Available Phosphorus	.50	.50	.50	.40	.45	.45	.45	.45
Methionine	.39	.34	.30	.36	.36	.35	.35	.28
Cysteine	.39	.35	.30	.37	.37	.35	.35	
Methionine & Cysteine	.78	.69	.60	.73	.73	.70	.68	
Lysine	.96	.75	.60	ok				
Tryptophan	.23	.19	.15	ok				
Arginine	1.26	1.01	.77	ok				
Threonine	.77	.65	.53	ok				

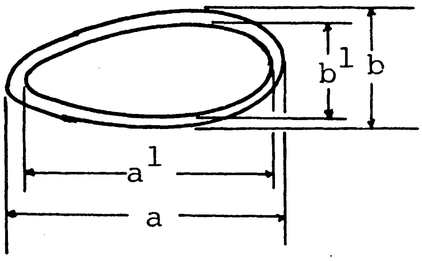
3.2 GENERAL TEST PROCEDURES

3.2.1 Bone Selection

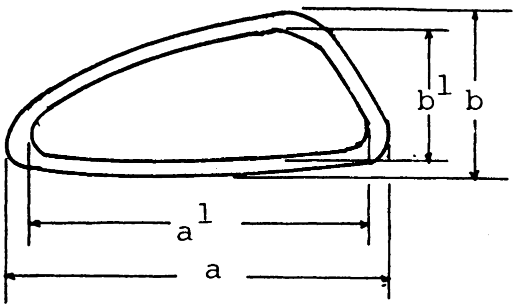
Each diet treatment contained 72 sets of bone (24 sets per body size) which included a right and left set from 36 birds. All bones were located in a common area and then randomly selected. Approximately the same number of right and left hand sets were selected from each treatment until the desired number of sets per test was reached. The bones for each test were regrouped and randomly tested.

3.2.2 Bone Geometry and Modeling

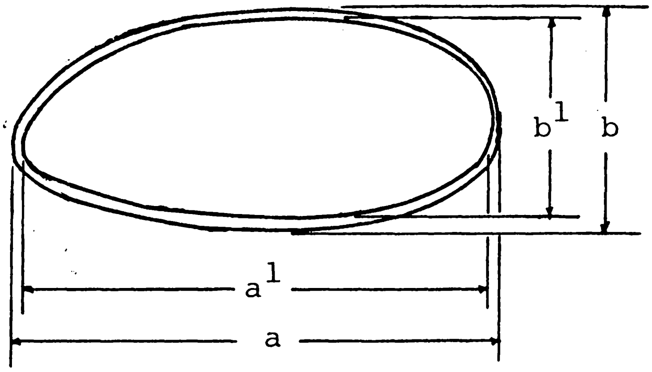
The method selected for determining the geometrical properties of the different bones was to model them as known geometrical shapes. Because of the quantity of bones being tested, photographing or x-raying the cross sections was not feasible. The bones were sawn at the center of the diaphysis after loading to failure. The exterior and interior dimensions were measured along the principal axis perpendicular to the long axis. Typical cross sections and measurements of the wing bones are shown in Figure 3. Figure 4 shows representative cross sections of the leg bones. The dimensions of each bone were measured with a vernier caliper having an accuracy of ± 0.0254 mm. The length and weight of each bone was also measured and recorded.



a) Radius

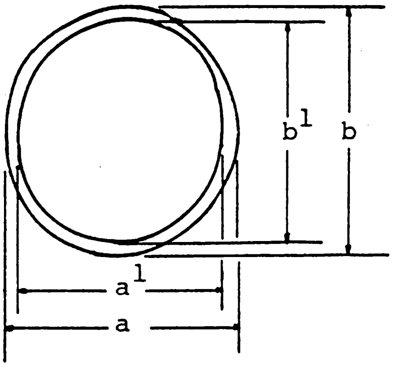


b) Ulna

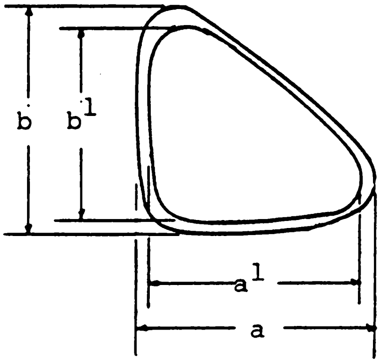


c) Humerus

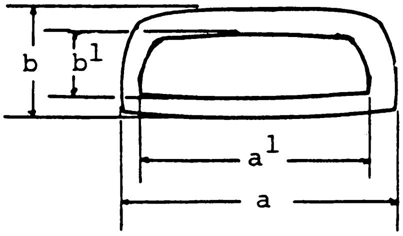
Figure 3. Typical cross sections of the wing bones.



a) Femur



b) Tibia



c) Tarsometatarsus

Figure 4. Typical cross sections of the leg bones.

3.2.3 Loading Configurations

Five loading configurations have been used in evaluating the mechanical properties of bone. Four of the five procedures were used in this experiment. Tension and compression were not ulitized as standard machined specimens could not be prepared because of the thin walls of the bones.

3.2.3.1 Shear Test

Shear tests were performed using a double shear block (Figure 5). The shear force was exerted over a 12.7 mm section located at the center of the shaft. The test position of each bone was such that the thinnest axis of the cross section perpendicular to the diaphysis was parallel to the direction of loading. These tests resulted in the ultimate shear force and shear stress being evaluated for each bone. Controls and loading rates used with the Instron are summarized in Table 6.

3.2.3.2 Three Point Bending Test

The three point bending test was chosen because fractures were related to this loading and the frequency with which poultry researchers used this test. The ultimate bending force, bending stress, and flexural modulus of elasticity were determined using the bending test. Figure 6 shows the

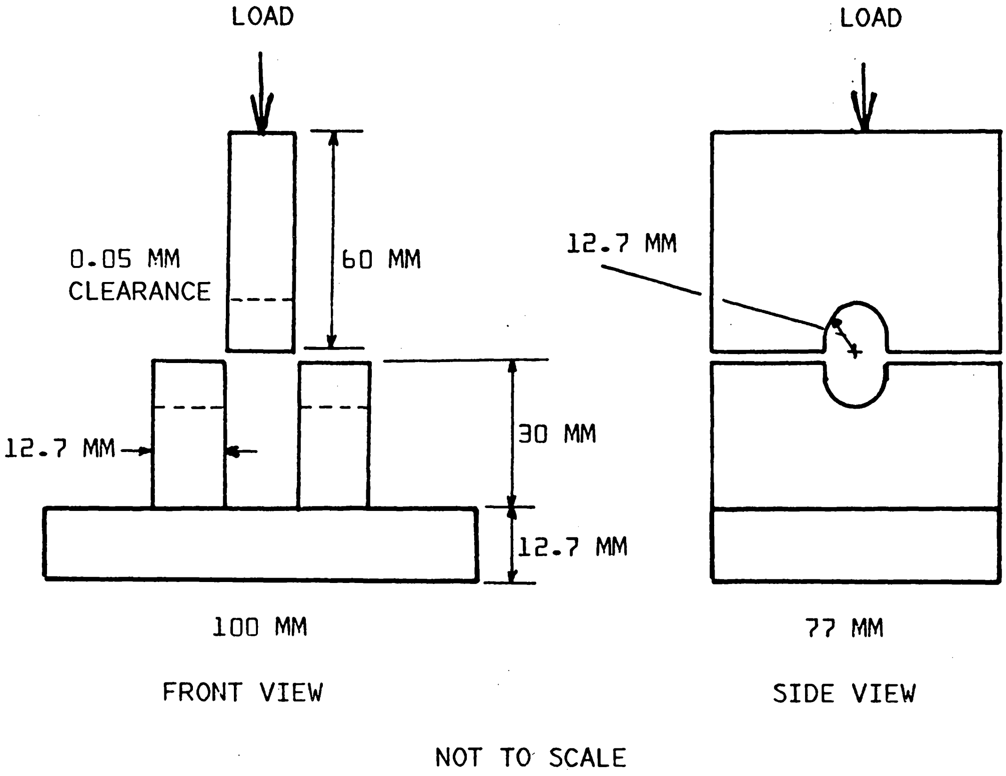


Figure 5. Illustration of the double shear test fixture used with Universal Instron Test Machine.

Table 6. Control speeds of the Instron testing machine and support distances of the test fixtures for the different bones.

Type of Test	Crosshead Speeds (mm/min)	Support Distance (mm)	Bones
Shear	5.08 (254)[1]	12.7	Radius Ulna Humerus Tibia Femur T.mt.
Three Point Bending	12.7 (508)	76.2	Tibia
	12.7 (508)	38.1	Radius T.mt. Humerus
	12.7 (1270.0)	50.8	Femur Ulna
Four Point Bending	5.08 (1270.0)	50.8	Femur Ulna
Torsion	12.7 (50.8)	Length of Diaphysis	Tibia
	12.7 (12.7)	Length between Molds	Radius

[1] Chart speed (mm/min)

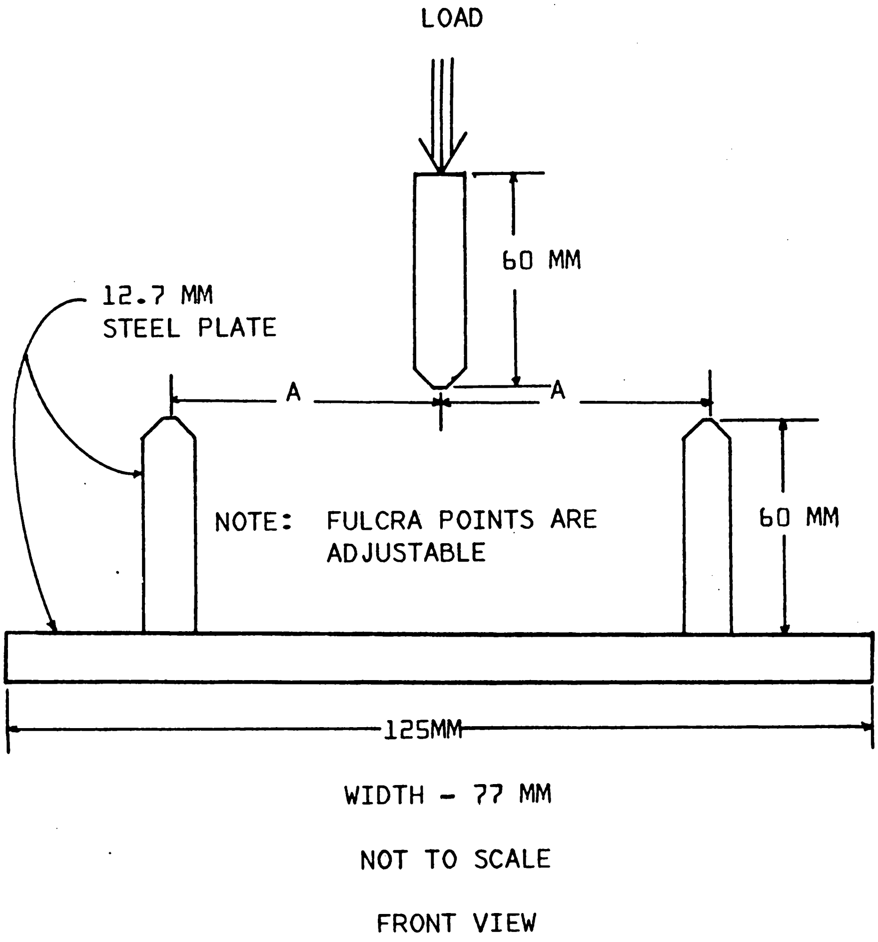


Figure 6. Schematic of the three point bending test fixture used with the Instron.

test fixture used with three point bending. The adjustable supports permitted the different bones to be tested in bending and maintain a L/D ratio equal to or greater than ten. The controls used with the Instron are summarized in Table 6. The three point bending test was calibrated using a material with a known modulus of elasticity. These results are presented in Table 7. Eighteen bones from each diet treatment (six per body size) were tested by applying a load at the center of the diaphysis. Four sets of bones from each cell were tested in three point bending to determine the mode of failure due to bending force. The load for each test was applied parallel to the smaller axis of the cross section that was perpendicular to the long axis.

3.2.3.3 Four Point Bending Test

A four point bending test was developed for determining the modulus of elasticity of bones with L/D ratios less than 10. Figure 7 illustrates the effects of neglecting shear when the L/D ratio is less than 10 in a round hollow tube. With four point bending, shear equals zero between the force couples (Figure 2b). The ulna and femur were found to have smaller L/D ratios than the other bones and were used in this test. One of the difficulties with this test was determining the deflection at the center of the bone. The

Table 7. Calibration data of the different test fixtures using materials with known mechanical properties.

Test Fixture	Mechanical Property	Sample Type	Predicted Value	Known Value[1]
Three Point Bending	Modulus of Elasticity (GPa)	6.27 mm Steel Rod	179.1 185.1 181.3	193.0
Four Point Bending	Modulus of Elasticity (GPa)	Aluminum Flat Bar, 12 mm x 25 mm x 77 mm	62.8	68.4
		9.5 mm Steel Rod	179.3 187.5 201.3	193.0
Torsion	Shear Modulus (GPa)	Brass Bar, 12.7 mm x 17.7 mm x 25.4 mm	44.3	38.6
		Aluminum Bar, 12.9 mm x 12.7 mm x 25.4 mm	25.5 27.2	26.7

[1] Known values taken from Eshbach (1975).

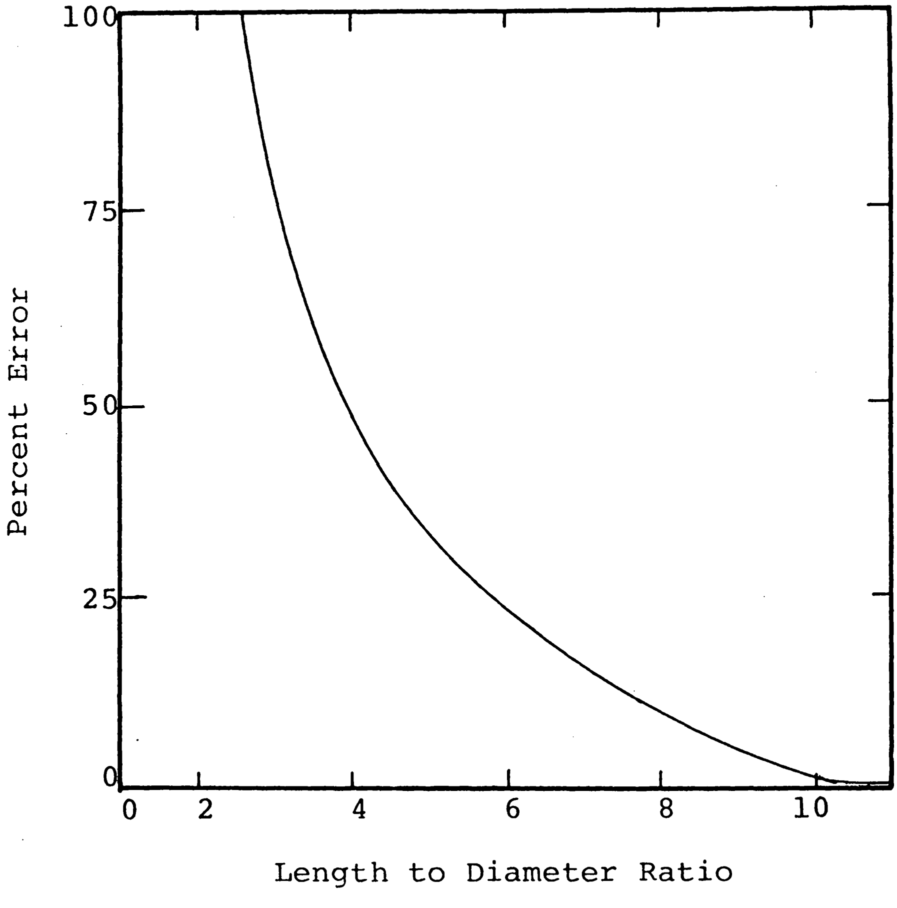


Figure 7. Percent error introduced in the calculation of the modulus of elasticity of a round thin walled beam if the length to diameter ratio is less than 10 (adapted from Mabie, 1956).

force-deformation curve of the Instron recorded deflection at the point of load application. However, a deflection device was developed to measure the deflection at the center of the bone and is shown in Figure 8. The deflection arm transfers a deflection value twice the actual deflection since the ratio of the arm from the pivotal point to the dial is twice the amount from the pivotal point to the point of measurement. This improved the accuracy of the fixture in determining the small deflections. The Instron controls and calibration data of the four point bending test are presented in Table 6 and 7, respectively.

The bone was placed on the supports (Figure 9) and then a small load was applied and the dial deflection gage was set to zero. The load was then applied until yield point was approached where once again the loading was stopped and the amount of deflection was recorded.

3.2.3.4 Torsion Test

Torsion was selected to define whether fractures were related to tensile or shear stresses. Testing was performed on the tibia and radius because they had straight diaphyses with uniform cross sections. The front view of the torsional test fixture is shown in Figure 10. A bone was placed in the 12.7 mm square slots and clamped down to prevent the

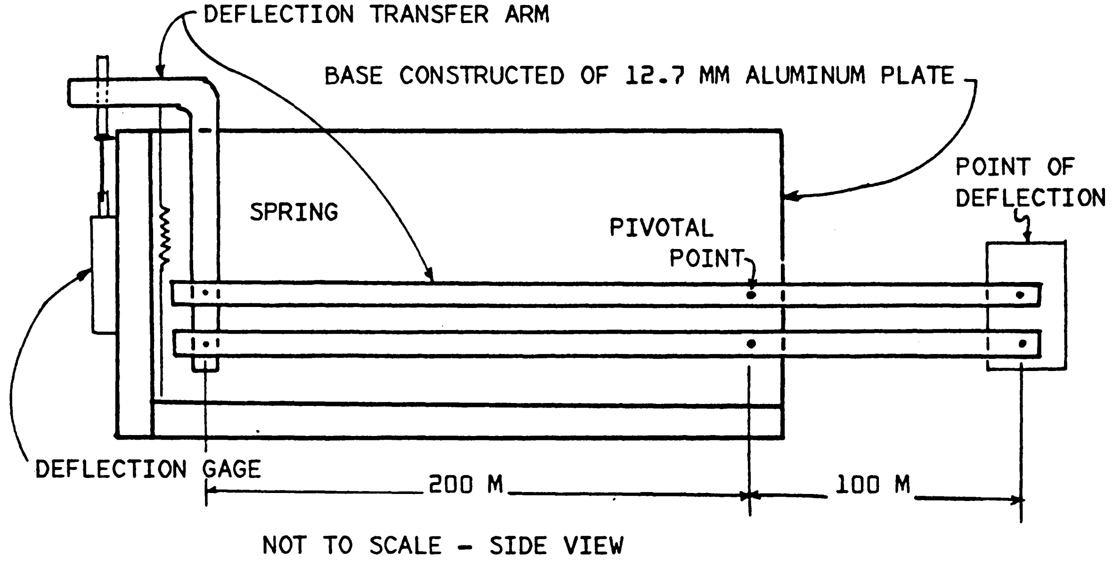


Figure 8. Schematic of the deflection measuring device used with the four point bending test.

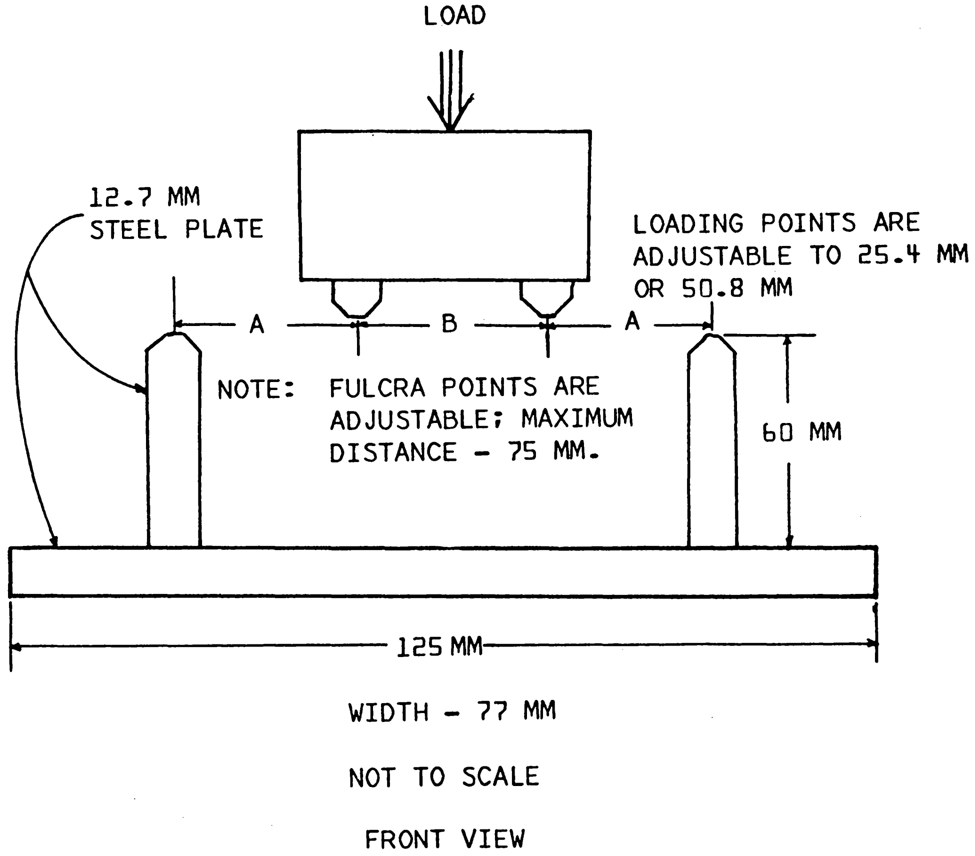
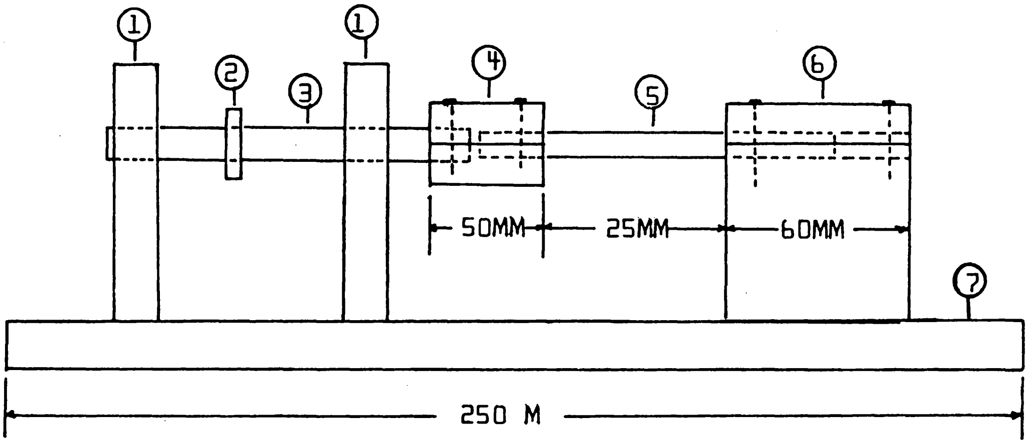


Figure 9. Illustration of the four point bending test fixture used with Instron Testing Machine.



SIDE VIEW - NOT TO SCALE

PARTS IDENTIFICATION LEGEND

1. ALUMINUM BEARING BLOCK
2. 89.2 MM LEVER ARM, CONNECTED TO INSTRON
3. 15 MM DIAMETER SHAFT
4. SPECIMEN HOLDER, KEYED TO ROTATE WITH SHAFT
5. TEST SPECIMEN WITH 12.7 MM SQUARE ENDS
6. SPECIMEN HOLDER, REMAINS STATIONARY
7. BASE WIDTH - 77 MM

Figure 10. Schematic of the torsional testing apparatus used with Universal Instron Machine.

condyles from twisting. The epiphyses of the tibia had to be sanded to perform the test. The radii were placed in molds and their epiphyses were then mounted in a plastic liquid. After this material hardened, the molds were sanded to a 12.7 square mm^2. Table 6 presents the controls and loading rates used with the Instron. The apparatus was calibrated using materials with a known shear modulus and Table 7 presents the results of this calibration using aluminum and brass bars.

3.2.3.5 Summary of Test Procedures

In summary, three of the five possible loading configurations were selected in testing the bones of spent laying hens. Each scheme provided different mechanical properties and an insight into the strength characteristics of bones. These tests produced results for determining maximum breaking load, mechanical properties, and mode of failure for the individual bones.

Testing was performed using an Instron Model TTL Universal Testing Machine (Figure 11) which recorded a force-deformation output curve. The actual deformation

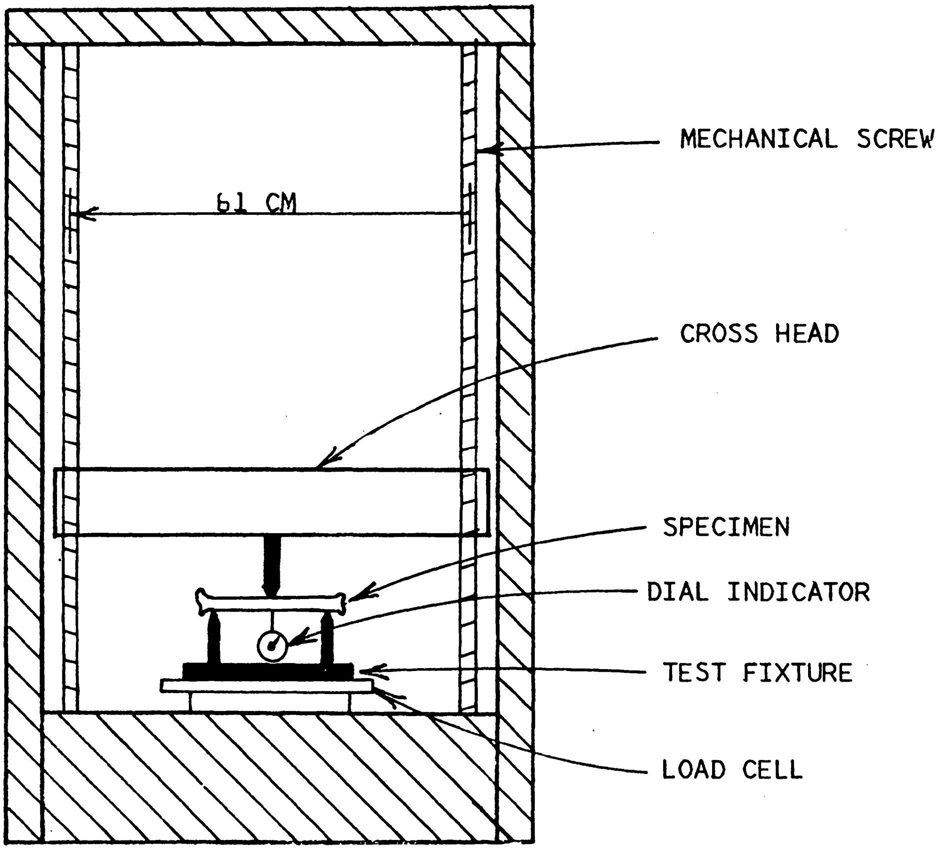


Figure 11. Illustration of Universal Instron Testing Machine.

was a function of crosshead and chart speeds and was a function of the magnification ratio defined by the two speeds. The force was a function of full load scale and load cell size. The output could be transformed to stress-strain relationships for evaluating the strength characteristics.

3.3 STATISTICAL ANALYSIS

The statistical analysis of data could involve parametric or nonparametric statistics. Parametric statistics involves a known distribution and the ability to define the continuous function. Nonparametric statistics is a procedure to handle a data set in which the underlying distribution is not easily specified. This form of analysis compares distribution rather than the parameters describing the continuous function. Nonparametric statistics have advantages which include:

1. quick and easy to learn and apply,
2. reduction in work of collecting data,
3. sampling procedures may include several populations with very little assumed, and
4. probability statements are not qualified as severely as with parametric procedures.

However, if the form of the parent population is reasonably close to the distribution for normal theory then nonparametric procedures should not be used (Steel and Torrie, 1960). The review of literature gave no indication as to the type of expected distribution of the mechanical properties of bone.

Two way analyses of variance and covariance tests were performed on all mechanical properties and ultimate forces. The general linear model program (SAS, 1982) was used to statistically analyze the data sets. Outliers were considered to be any data point falling outside the 99th percentile. A data point was replaced if it was determined to be an outlier since the data point would skew the results. The effects of body weight and protein sequence were analyzed using the concepts of orthogonality.

3.4 SUMMARY

Different bones were tested in shear, torsion, and bending (three and four). Mechanical properties were determined using equations from strength of material. A statistical analysis was performed on the various data sets to determine the effects of juvenile protein sequence and body size. The fracture mode was determined using the three point bending and torsional test results.

Chapter 4

DATA REDUCTION AND ANALYSIS

The testing procedures resulted in a force-deformation curve and measurements of physical dimensions for each bone. Data were transferred to the computer for final reduction and determination of the mechanical properties. Presented herein are the procedures for determining the geometrical and mechanical properties, statistical analysis, and fracture mechanism.

4.1 GEOMETRICAL PROPERTIES

4.1.1 Bone Geometry and Modeling

The evaluation of ultimate stresses, modulus of elasticity, and shear modulus required that the bone geometry be known. The cross sectional area, moment of inertia, and polar moment of inertia were needed to evaluate these properties.

The bones were modelled as known geometrical shapes. The exterior and interior dimensions were measured along the principal axes perpendicular to the long axis. The cross sections perpendicular to the long axis of the radius, ulna, humerus, and femur were considered elliptical. The tibia cross section was represented as a quadrant of an ellipse and a rectangle was used to model the cross section of the tarsometatarsus. Typical cross sections of the wing bones are shown in Figure 3 and Figure 4 shows representative cross-sections of the leg bones. Table 8 presents the equations for determining the area and moment of inertia for each geometrical shape (Souders, 1975). Other geometrical properties, such as length or extreme distance from neutral axis to outer fiber, were measured directly or determined using the dimensions of the cross-section of a bone.

4.2 MECHANICAL PROPERTIES

4.2.1 Shear Mechanical Properties

Mechanical properties were determined using the force-deformation curves and geometrical properties. The necessary information from each chart was removed for data reduction. Strength of material equations (Popov, 1976; Roark and Young, 1975) were used to evaluate the properties. The shear force-deformation curve provided the ultimate breaking load and enabled ultimate shear stress to be determined. The ultimate shear force was the maximum peak in the horizontal direction of the output curve. The ultimate shear

Table 8. Equations used for determining the cross-section area and moment of inertia for the different geometrical shapes of the bones.

Geometrical Shape	Equation of Cross-Sectional Area	Equation of Moment of Inertia	Bones Modelled
Hollow Ellipse	$0.7857\ (a * b - a' * b')$[1]	$0.049\ [a * (b)^3 - a'(b')^3]$	Radius Humerus Ulna Femur
Hollow Quadrant Ellipse	$0.7857\ (a * b - a' * b')$	$0.0549\ [a * (b)^3 - a'(b')^3]$	Tibia
Hollow Rectangle	$(a * b - a' * b')$	$0.083\ [a * (b)^3 - a'(b')^3]$	T.mt.

[1] a, b - exterior dimensions.

a',b' - interior dimensions.

stress was a function of shear force and cross-sectional area or:

$$\tau = P/(A * 2) \qquad 4.1$$

where:

τ - ultimate shear stress (MPa),
P - maximum shearing load (N), and
A*2 - shear cross sectional area (m^2)
for double shear.

The data were analyzed according to diet and body size with each combination containing a sample size of 12.

4.2.2 Bending Mechanical Properties

The three point bending test was used to determine the maximum bending force, ultimate bending stress, and flexural modulus of elasticity. The ultimate force was the peak value of the force-deformation curve. Two mechanical properties were determined from the three point test results. The ultimate bending stress was determined from

$$\sigma = MC/I = (P * L * C)/4 * I \qquad 4.2$$

where:

σ - maximum bending stress (MPa),
M - bending moment (N - m),
C - distance from neutral axis to outer fiber (m),
I - moment of inertia (m^4).
P - maximum breaking load (N), and

L - length between supports (m).

The equation for moment of inertia for each geometrical shape is presented in Table 8. The constant C equals one-half the diameter for an ellipse. For a quadrant ellipse, C equals:

$$C = d - (4 * d)/(3 * \pi) \qquad 4.3$$

where:

d - length of axis parallel to load.

For a rectangle, C equalled one half the width of the smaller axis perpendicular of the longitudinal axis. The modulus of elasticity represented the stiffness of the bone to an imposed load. This property was determined in the elastic region where upon removal of the load the material would return to its original shape. The modulus of elasticity is determined from

$$E = (\Delta P * L^3)/[48 (\delta) * I] \qquad 4.4$$

where:

E - modulus of elasticity (MPa),

ΔP - change in force in elastic region (N),

L - length between supports (m),

δ - deflection as result of ΔP (m), and

I - moment of inertia.

If the L/D ratio was significantly less than 10, then the modulus of elasticity was determined using a four point bending test. The modulus of elasticity was determined using

$$E = [\Delta P * L^3/(48 * \Delta\delta * I)] * (3 * a - 4 * a^3) \qquad 4.5$$

where:

ΔP - force resulting in $\Delta\delta$,

$\Delta\delta$ - deflection indicated by dial gage divided by two, and

a - distance from the support to point of loading.

4.2.3 Torsional Mechanical Properties

Torsion testing was performed on the tibiae and radii to determine angle of twist to failure, shear modulus, shear stress, and torque at failure. The angle of twist was the degree to which the bone was twisted before failure. This value is a function of displacement on the output curve and the moment arm and was approximated by

$$\gamma = La/\delta \qquad 4.5$$

where:

γ - angle of twist in radians,

La - length of moment arm (88.9 mm), and

δ - total deflection (m).

The torque at failure was a function of breaking force at failure and moment arm

$$T = P * La \qquad 4.6$$

where:

T - torque (N-m), and

P - load at failure (N).

The shear modulus equation assumed the shaft has a thin walled, uniform cross-sectional area, and is a homogenous istropic material (Roark and Young, 1975). This was evaluated using

$$G = \Delta T * L/(K * \theta) \tag{4.7}$$

where:

G - shear modulus (MPa),

ΔT - change in torque or twist (N-m),

L - length of shaft (m),

K - constant depending on cross-sectional shape, and

θ - angle of twist resulting from ΔT.

The ultimate shear stress was defined as

$$\tau = T/(z * t * A) \tag{4.8}$$

where:

τ - shear stress (MPa),

T - torque (N-m),

t - wall thickness (m), and

z - section modulus (m^4),

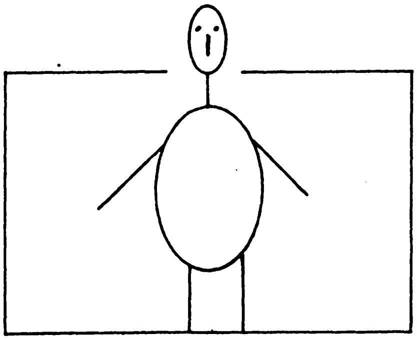
A - cross-section area within mean boundary (m^2).

4.3 STATISTICAL ANALYSIS

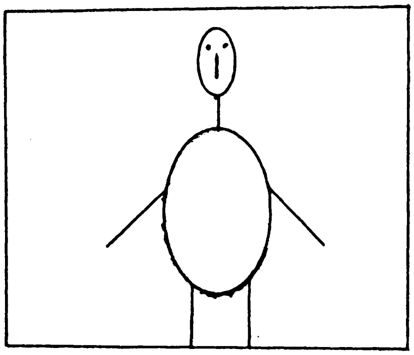
Statistical analyses were performed using SAS (SAS, 1982). The programs used included the general linear model, correlation coefficient, categorical responses, and a linear regression model.

4.3.1 Three Way Analysis of Variance

Data were analyzed to determine the effects of juvenile protein sequence, body size, and cage profile. Since not enough data could be obtained to determine a type of distribution, it was assumed the data followed a normal distribution. The birds were reared in cages with two different ceiling heights (Figure 12). A high profile cage had a ceiling height of 625 mm and permitted the birds to stand up in the cage. The ceiling of a low profile cage was 400 mm high. In this cage, a bird was able to protrude its head through the top of cage. These differences in cages influenced the stance and mobility of the wings. Therefore, a three way analysis of variance was used to analyze the data sets. This analysis was used to model the independent variable as a function of the main effects. The model for the



a) Low Profile



b) High Profile

Figure 12. Cross sections of the different cages in which the 72 week old spent hens were raised.

three factor experiment was given by

$$Y_{ijkl} = \mu + PS_i + SZ_j + CP_k + (PS * SZ)_{ij} + (PS * CP)_{ik} + (SZ * CP)_{jk} + (PS * SZ * CP)_{ijk} + \varepsilon_{ijkl} \qquad 4.9$$

where:

Y_{ijkl} - independent variable ($l = 1,2,3,\ldots$),

μ - overall mean of data set,

PS_i - effect of the ith protein sequence ($i = 1,2,3,4$),

SZ_j - effect of the jth size ($j = 1,2,3$),

CP_k - effect of the kth cage profile ($k = 1,2$),

$(DT * SZ)_{ij}$ - interaction of the ith sequence and jth body size,

$(DT * CP)_{ik}$ - interaction of the ith sequence and kth cage profile,

$(SZ * CP)_{jk}$ - interaction of the jth body size and kth cage profile,

$(DT * SZ * CP)_{ijk}$ - interaction of the ith sequence, jth body size and kth cage profile, and

ε_{ijkl} - deviation of the observed Y_{ijkl} from the population mean μ.

The model used the least square means in the analysis of variance since the cell sizes were unequal. Orthogonal contrasts were used to compare the control protein sequence to sequences one, two, and three, sequence one to sequences two and three, and sequence two to sequence three. Orthogonal polynominals were used to determine if a linear or quadratic relationship existed between the independent variable and body size. The F-test was used to determine statistical differences in cage profiles. The general linear model procedures developed by SAS (1982) were used for the statistical analysis.

An analysis of covariance was also used to analyze the data. In this analysis, the body size was replaced by the actual body weight of the birds at the 72 week of age. Therefore, body weight became the covariant variable.

The effects of protein sequence on the type of fracture was analyzed using the chi-square test procedures for evaluating the hypothesis of independence of two variables. This hypothesis is known as marginal homogenity or the true response probabilities are the same across the population (juvenile protein sequence). Another statistical procedure was used to determine the correlation coefficient between the shear and bending properties. Bones were randomly selected from right and left hand sets and then tested in bending and shear test. The properties were paired and a correlation coefficient was determined. It was assumed no differences existed in the strength characteristics of the bones from the right and left hand sides of the birds.

4.3.2 Determining Outliers

There was no obvious reason to believe any outliers existed since no unusual observations were seen during the tests. However, some factors not so obvious, such as lack of feed or water during growth, might have produced unreasonable or unrealistic test values. The data was assumed to follow a log-normal distribution. An outlier was considered to be any test value greater than the 99th percentile. Any value of X was discarded if

$$\Phi\left(\frac{\ln X - \lambda}{\xi}\right) = 0.99 \qquad 4.10$$

where:

X is independent variable,

λ is population mean, and

ξ is standard error.

Using a log-normal table to determine the value of Φ at the 99th percentile, the equation was rewritten as

$$(\ln X - \lambda)/\xi = 2.366 \qquad 4.11$$

or

$$\ln X = \lambda + 2.326\xi \qquad 4.12$$

where:

2.326 is the critical value of 99th percentile.

The value X was considered an outlier if

$$X > X_{crt} \qquad 4.13$$

where:

$$X_{crt} = \exp\,(\lambda + 2.326\xi)$$

If X was an outlier, it was discarded and test procedures were repeated on another bone from the same size bird fed the same diet and growing in identical cages using identical methods. There were only five values determined to be outliers in all of the results.

4.4 ANALYSIS OF FRACTURE

The type of fracture was categorized as either brittle or ductile. Brittle fracture required forces which exceed the cohesive forces between atoms to be broken. A ductile fracture was a result of shear stresses being able to disorder the crystals. The fracture mode was determined from bones loaded in torsion and bending. Visual inspection was made of each bone to determine the type of fracture. Torsional loading of the tibia permitted the bone to fail anywhere between the proximal and distal epiphyses. The radius was forced to fail along the diaphysis during torsional testing because of the plastic molds. In bending tests, bones were required to fail at the center of the diaphysis where the moment was maximum (Figure 2a). The location of fracture was also defined for the tibiae tested in torsion.

4.5 SUMMARY

The mechanical properties for the different bones of 72 week old caged layers were evaluated. Three-way analyses of variance and covariance were used to determine the effects of protein sequence, body size, and cage profile. Orthogonal contrasts were used to compare sequences and orthogonal polynominals were used to evaluate the effects of body size. Influence of cage profile was determined by using the F-test. The location and type of fracture was defined for the bones loaded to failure in torsion and bending tests.

Chapter 5

PRESENTATION OF RESULTS

The results of data reduction and analysis are presented with a complete discussion provided in Chapter 6. The least square means and standard errors of the properties from the shear, bending, and torsional tests are presented in Appendices A, B, and C, respectively. The data were analyzed using three way analysis of variance and analysis of covariance. Body size was replaced by the actual body weight at 72 weeks of age in the analysis of covariance. Figures are used to show the interaction patterns which occurred in the statistical analysis. Other results presented are the analyses of fracture patterns which occurred in the bones tested in bending and torsion.

5.1 SHEAR TEST RESULTS

The ultimate shear forces of the radius, ulna, humerus, femur, tibia, and tarsometatarsus are presented in Tables A1 through A6, respectively. Numerically, the maximum shear forces of the leg bones tended to be similar for different diets. The shear forces were nearly equal in the ulna and humerus while the shear force for the radius was 15 percent less. The shear strength in all of the bones, except the ulna, tended to increase proportionally with body size. The ulna was strongest in the smaller birds. The birds housed in high profile cages tended to have stronger bones than those birds housed in low profile cages. The low profile cages caused a reduction in the ultimate shear force of the wing bones of about 10 to 20 percent, whereas, the leg bones showed a reduction in strength of less than six percent.

Table 9 presents a summary of the statistical analyses of the ultimate shear force of the different bones. The shear force of the radius showed no difference ($P < 0.05$) based on the protein sequence, body size, and cage profile. Orthogonal contrasts showed significant difference in the strength of the radius from birds fed juvenile protein sequence (JPS) one versus two and three. However, there was no difference between the control and the other three diets. The ultimate shear force of the ulna was affected by cage profile ($P < 0.01$), however, no differences based on protein sequence and body size were observed. The significance of cage profile was removed when body weight was used as a covariant (Table 9). The shear force of the humerus was influenced at the $P < 0.01$ level by both JPS and cage profile and at the $P < 0.05$ level by body size. However, these results were skewed at the 0.05 level due to an interaction between protein sequence and body size (Figure 13). The pattern of the

Table 9. Summary of statistical analyses of the ultimate shear force of the different bones from 72 week old caged layers.

Type of Bone	Analysis of Variance			Analysis of Covariance		
	Juvenile Protein Sequence[a]	Body Size[b]	Cage Profile[a]	Juvenile Protein Sequence[a]	Body Weight[b]	Cage Profile[a]
Radius	NS[c]	NS	NS	NS	NS	0.01
Ulna	NS	NS	0.01	NS	NS	NS
Humerus	0.01[d,e]	0.05[d,f]	0.01	NS	NS	NS
Femur	NS	0.01[f]	NS	NS	0.05	NS
Tibia	NS	0.01[f]	NS	NS	0.05	NS
T.mt.	NS	0.01[f]	NS	NS	0.05	NS

[a] See Table 3 and Figure 12, respectively, for protein sequence and cage profile.

[b] Body size categorized as small, medium, or large and body weight is the actual weight of the hens at 72 weeks of age.

[c] Level of significance (NS - not significant, $P > 0.05$).

[d] Interaction at $P < 0.05$.

[e] Contrast differences in protein sequences two versus three.

[f] Linear relationship between size and force at $P < 0.05$.

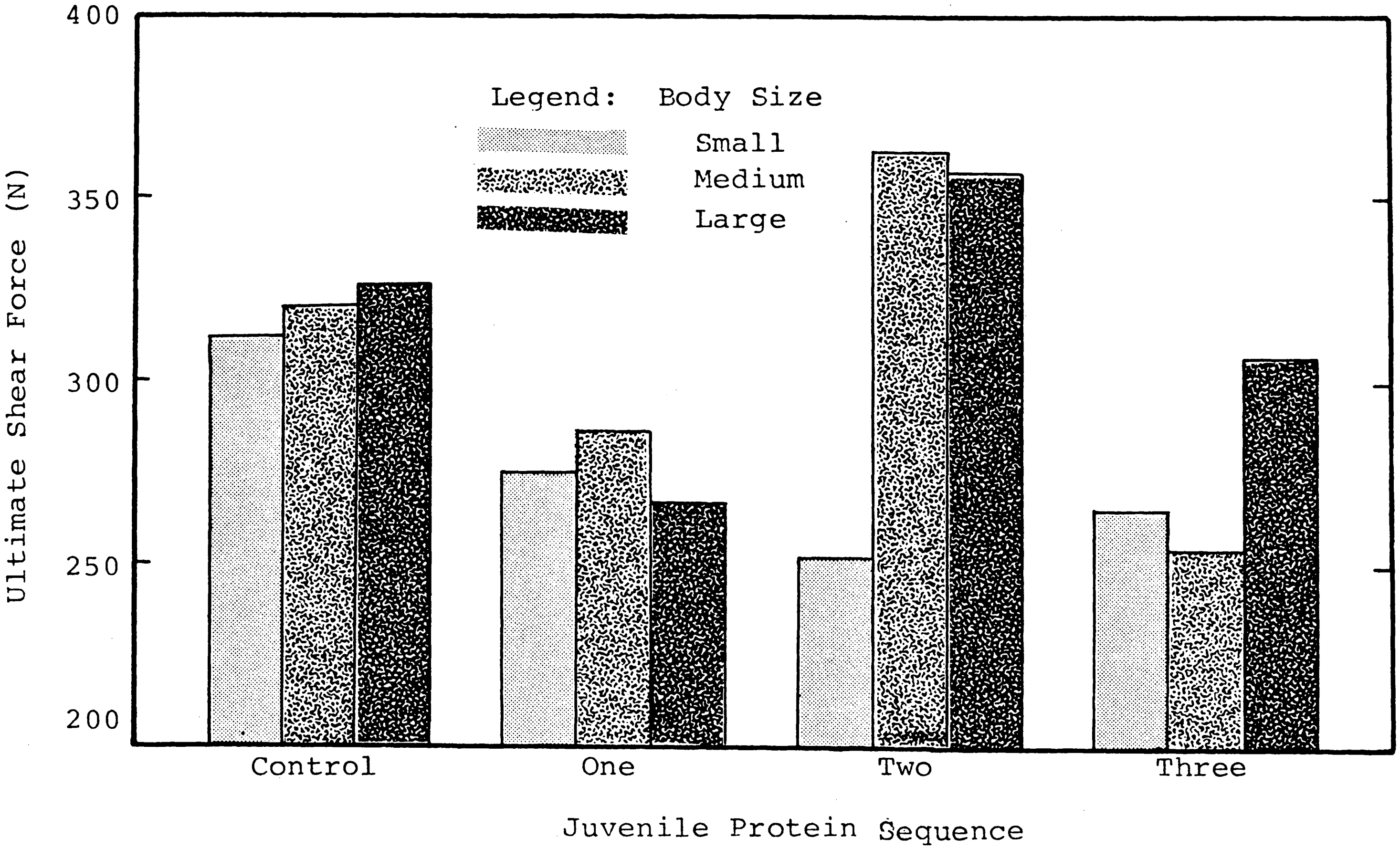


Figure 13. Histograms showing the effects of protein sequence and body size in the statistical analysis of the ultimate shear forces of the humeri.

histograms in Figure 13 would have been similar if no interaction occurred between variables. The smaller birds had the lower breaking forces in all sequences but JPS three and ultimate shear force of the humerus from medium and large birds was influenced by protein sequence two. The control diet shows minimum difference in shear force based on body size. At a $P < 0.01$ level, there was a difference between JPS two and three, however, other orthogonal contrasts did not indicate any statistical differences in protein sequences. Even though no significant differences in the ultimate shear force of the wing bones were observed based on body size, a linear relationship existed between body size and shear force for the humerus. The analysis of covariance (Table 9) shows that the significance which were present in the analysis of variance did not occur when body weight was used as a covariant rather than body size as a category. Body size significantly ($P < 0.01$) influenced the ultimate shear force of the femur, tibia, and humerus. The orthogonal polynomials showed that body size was linearly proportional to the shear force. Body weight was significant ($P < 0.05$) when using analysis of covariance.

The least square means shown in Tables C7 through C12 are the ultimate shear stresses for the radius, ulna, humerus, femur, tibia, and tarsometatarsus, respectively. Numerically, the shear stresses tended to be higher in all bones, except the tibia, from the birds fed the control sequence in which case the tibia had the lowest shear stress. The leg bones of birds housed in low profile cages had greater stress values than those bones from birds which were housed in the high profile cages. The wing bones had higher shear stresses in birds housed in the high profile cages.

Analysis of the ultimate shear stresses (Table 10) showed interactions existed between protein sequence and body size for stress of the radius and humerus. Figure 14 and 15 helps explain the interaction occurring in the radius and humerus, respectively. Juvenile protein sequences one and two show an inversely proportional relationship between weight and shear stress in the radius (Figure 14). The control sequence and JPS three show different relationships occurring between size and stress. The ultimate shear stress of the humerus has three different patterns (Figure 15). The protein sequences, control and three, could be categorized as either proportional or inversely proportional depending upon which body sizes are being examined. There were no differences in the shear stress of the ulna based on protein sequence, body size or weight, and cage profile.

Table 10. Summary of statistical analyses of the ultimate shear stress of the different bones from 72 week old caged layers.

	Analysis of Variance			Analysis of Covariance		
Type of Bone	Juvenile Protein Sequence[a]	Body Size[b]	Cage Profile[a]	Juvenile Protein Sequence[a]	Body Weight[b]	Cage Profile[a]
Radius	NS[c,d]	NS[d]	NS	NS	NS[d]	NS[d]
Ulna	NS	NS	NS	NS	NS	NS
Humerus	0.01[d,e]	NS[d,f]	0.01	NS	NS	NS
Femur	NS	NS[g]	NS	NS	NS	NS
Tibia	NS	NS	NS	NS	NS	NS
T.mt.	NS	0.01[g]	NS	NS	NS	NS

[a] See Table 3 and Figure 12, respectively, for protein sequence and cage profile.

[b] Body size categorized as small, medium, or large and body weight is the actual weight of the hens at 72 weeks of age.

[c] Level of significance (NS - not significant, $P > 0.05$).

[d] Interaction at $P < 0.05$.

[e] Contrast differences between control protein sequence versus one, two, and three.

[f] Linear relationship between size and stress at $P < 0.05$.

[g] Quadratic relationship between size and stress at $P < 0.05$.

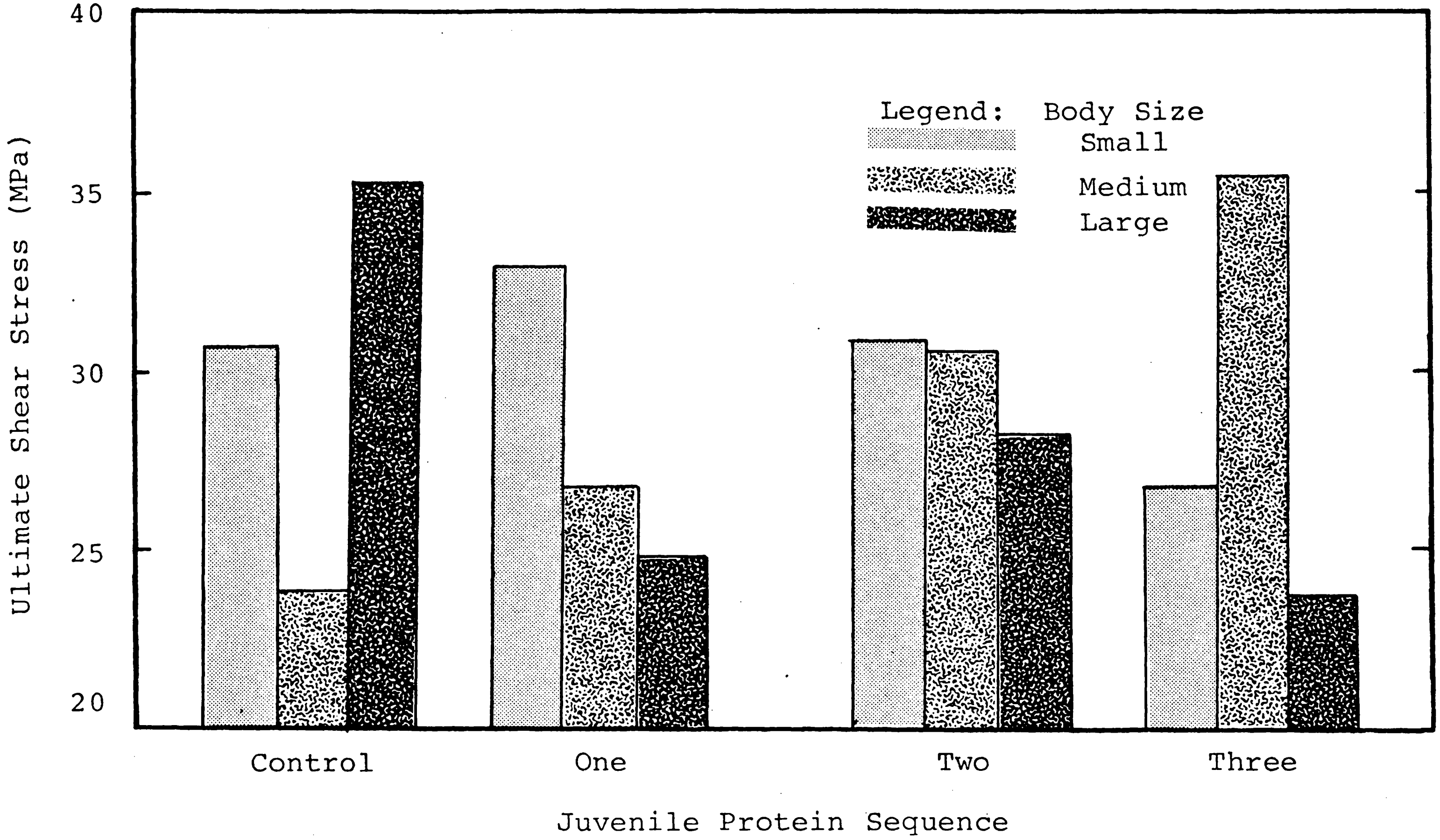


Figure 14. Histograms showing the effects of protein sequence and body size in the statistical analysis of the ultimate shear stress of the radius.

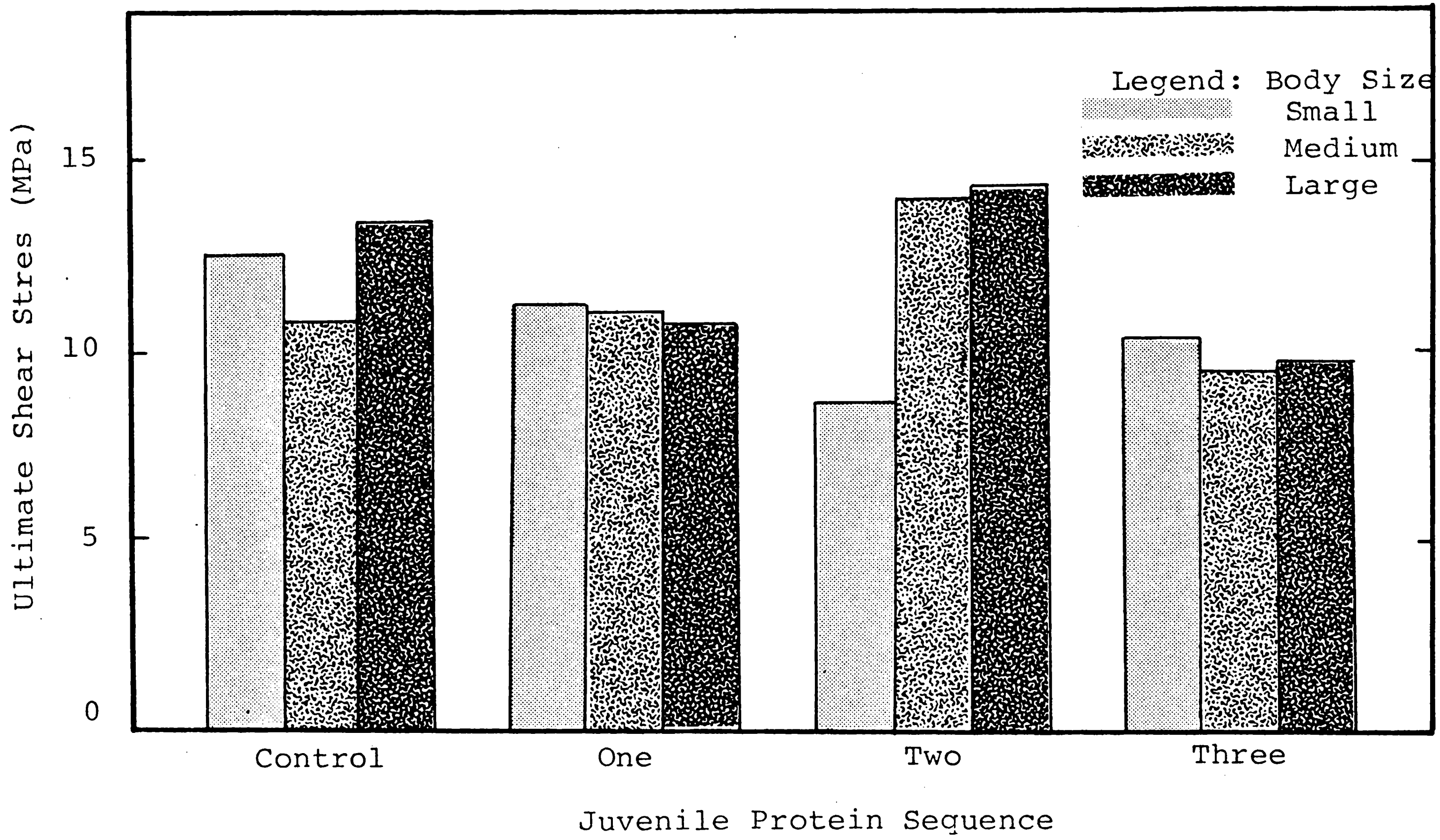


Figure 15. Histograms showing the effects of protein sequence and body size in the statistical analysis of the ultimate shear stress of the humerus.

The shear stress of the tibia was not influenced by protein sequence, body size, or cage profile. Analysis of the ultimate shear stresses of the femur also showed no significance. The stress of the tarsometatarsus was significantly affected by body size ($P < 0.01$) but not by protein regime or cage profile. The orthogonal polynominals showed the relationship between body size and shear stress to be quadratic rather than linear as observed in the ultimate shear force. No statistical differences or interactions occurred in the ultimate shear stress of any of the wing or leg bones when the analysis of covariance was used (Table 10).

In summary, the ultimate shear forces were influenced by body size and cage profile when analyzed by a three way analysis of variance. However, when body weight was used as a covariant these differences were not present. The ultimate shear stresses of the wing bones were affected by the interaction of protein sequence and body size. The stresses of leg bones were not affected by protein feeding level, body size or cage profile. Regardless of main effects, the radius had the highest shear stress, and in descending order, the ulna, tibia, femur, tarsometatarsus, and humerus.

5.2 THREE POINT BENDING RESULTS

The ultimate three point bending force for the radius, ulna, humerus, femur, tibia, and tarsometatarsus is presented in Table B1 to Table B6, respectively. Numerically, the variation in protein sequences had minimum effect on the mean breaking forces of the different bones. There was up to a 16 percent difference in the breaking forces of the different bones based on diet. The bones of the larger birds withstood more bending force before failure in all bones except the tarsometatarsus. Similar to the shear force, the smaller bird always had the weaker bones. The birds from high profile cages tended to have stronger bones than the birds from the low profile cages.

Analyses of the data at a 5 percent level of significance indicated the body size and cage profile had significant effects on the ultimate bending forces of the wing bones (Table 11). Orthogonal contrast indicated a linear relationship existed between body size and ultimate bending force for the radius, ulna, and humerus. A difference ($P < 0.05$) was noted when JPS one was contrasted to two and three in ultimate force of the humerus. The bending force of the humerus was also affected by cage profile ($P < 0.01$). Differences were also noted in the bending forces of the radius and ulna based on cage profile. These effects were not observed in

Table 11. Summary of statistical analyses of the ultimate bending force of the different bones from 72 week old caged layers.

Type of Bone	Analysis of Variance			Analysis of Covariance		
	Juvenile Protein Sequence[a]	Body Size[b]	Cage Profile[a]	Juvenile Protein Sequence[a]	Body Weight[b]	Cage Profile[a]
Radius	NS[c]	0.05[d]	0.05	NS	NS	NS
Ulna	NS	0.05[d]	0.05	NS	NS	NS
Humerus	NS[e]	0.01[d]	0.01	NS	NS	NS
Femur	NS	0.01[d]	NS	NS	NS	NS
Tibia	NS	0.05[d]	NS	NS	0.05	NS
T.mt.	NS[f]	NS[f]	NS	NS	NS	NS

[a] See Table 3 and Figure 12, respectively, for protein sequence and cage profile.

[b] Body size categorized as small, medium, or large and body weight is the actual weight of the hens at 72 weeks of age.

[c] Level of significance (NS - not significant, $P > 0.05$).

[d] Linear relationship between size and force at $P < 0.05$.

[e] Contrast differences between protein sequence one versus two and three at $P < 0.05$.

[f] Interaction at $P < 0.05$.

the analysis of covariance of the ultimate bending force of the wing bones. The bending forces of the femur and tibia were affected by body size ($P < 0.01$). As with other tests and bones, there was a linear relationship between size and breaking force. The effects of size on the maximum bending force of the tarsometatarsus were skewed by an interaction between protein sequence and body size. This interaction is explained in Figure 16. The type of relationships which possibly exist between body size and diet were not apparent in Figure 16. Overall, the ultimate bending forces of the leg bones were not influenced by protein sequence or cage profile. The only difference noted in the analysis of covariance was that body weight influenced the ultimate bending force of the tibia ($P < 0.05$, Table 11).

Tables B7 through B12 give the ultimate bending stresses of the radius, ulna, humerus, femur, tibia, and tarsometatarsus, respectively. Table 12 summarizes the statistical analyses of the ultimate bending stress of the different bones. Bones from birds fed the control sequence tended to have higher stresses than the bones from birds fed one of the three reversed protein diets. An inversely proportional trend occurred between body size and stress for the six bones. The bones of birds housed in high profile cages tended to have higher ultimate bending stresses than those bones from birds housed in low profile cages.

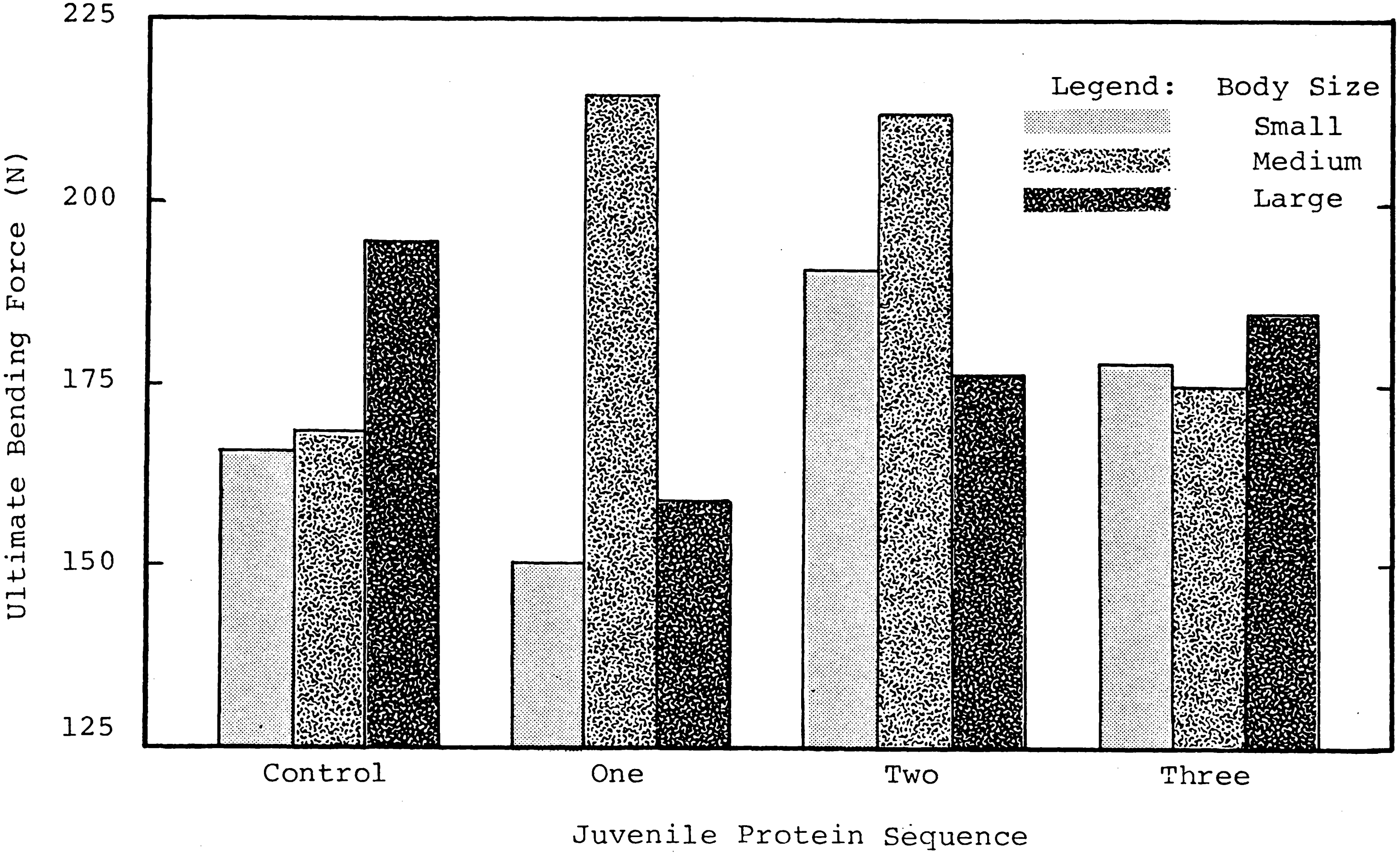


Figure 16. Histograms showing the effects of protein sequence and body size in the statistical analysis of the ultimate bending force of the tarsometatarsus.

Table 12. Summary of statistical analyses of the ultimate bending stress of the different bones from 72 week old caged layers.

Type of Bone	Analysis of Variance			Analysis of Covariance		
	Juvenile Protein Sequence[a]	Body Size[b]	Cage Profile[a]	Juvenile Protein Sequence[a]	Body Weight[b]	Cage Profile[a]
Radius	NS[c,d]	NS[d]	NS	NS	NS	NS
Ulna	NS	NS	NS	NS	NS	NS
Humerus	NS[d]	NS[d]	0.05	NS	NS	NS
Femur	NS	NS	NS	NS	NS	NS
Tibia	NS	NS	NS	NS	NS	NS
T.mt.	NS[e]	0.01[e,f]	NS	NS	NS	NS

[a] See Table 3 and Figure 12, respectively, for protein sequence and cage profile.

[b] Body size categorized as small, medium, or large and body weight is the actual weight of the hens at 72 weeks of age.

[c] Level of significance (NS - not significant, $P > 0.05$).

[d] Contrast differences between protein sequences one versus two and three.

[e] Interaction at $P < 0.05$.

[f] Linear relationship between size and stress at $P < 0.01$.

The main effects did not influence the ultimate bending stress of the radius or the ulna in the analyses of variance and covariance. Cage profile affected ($P < 0.05$) the ultimate stress of the humerus. However, this difference was not observed when using the analysis of cova riance to evaluate the main effects on the bending stress of the wing bones. Bending stresses of the femur and tibia were not influenced by protein regime, body size, or cage profile. An interaction between diet and body size skewed the statistical differences of cage profile on the ultimate stress of the tarsometatarsus (Figure 17). No apparent relationships were observed from Figure 17 between body size and diet for the stress of the tarsometatarsus.

The flexural modulus of elasticity for the radius, ulna, humerus, femur, tibia, and tarsometatarsus is presented in Tables B13 through B18. When using the analysis of variance, the moduli of elasticity of the bones, with the exception of the tarsometatarsus, were not influenced by protein sequence, body size, or cage profile (Table 13). The modulus of elasticity of the tarsometatarsus was affected by body size. An inversely proportional relationship existed between body size and the modulus of elasticity. An interaction between body size and protein sequence existed in the analysis of the modulus of elasticity of the humerus

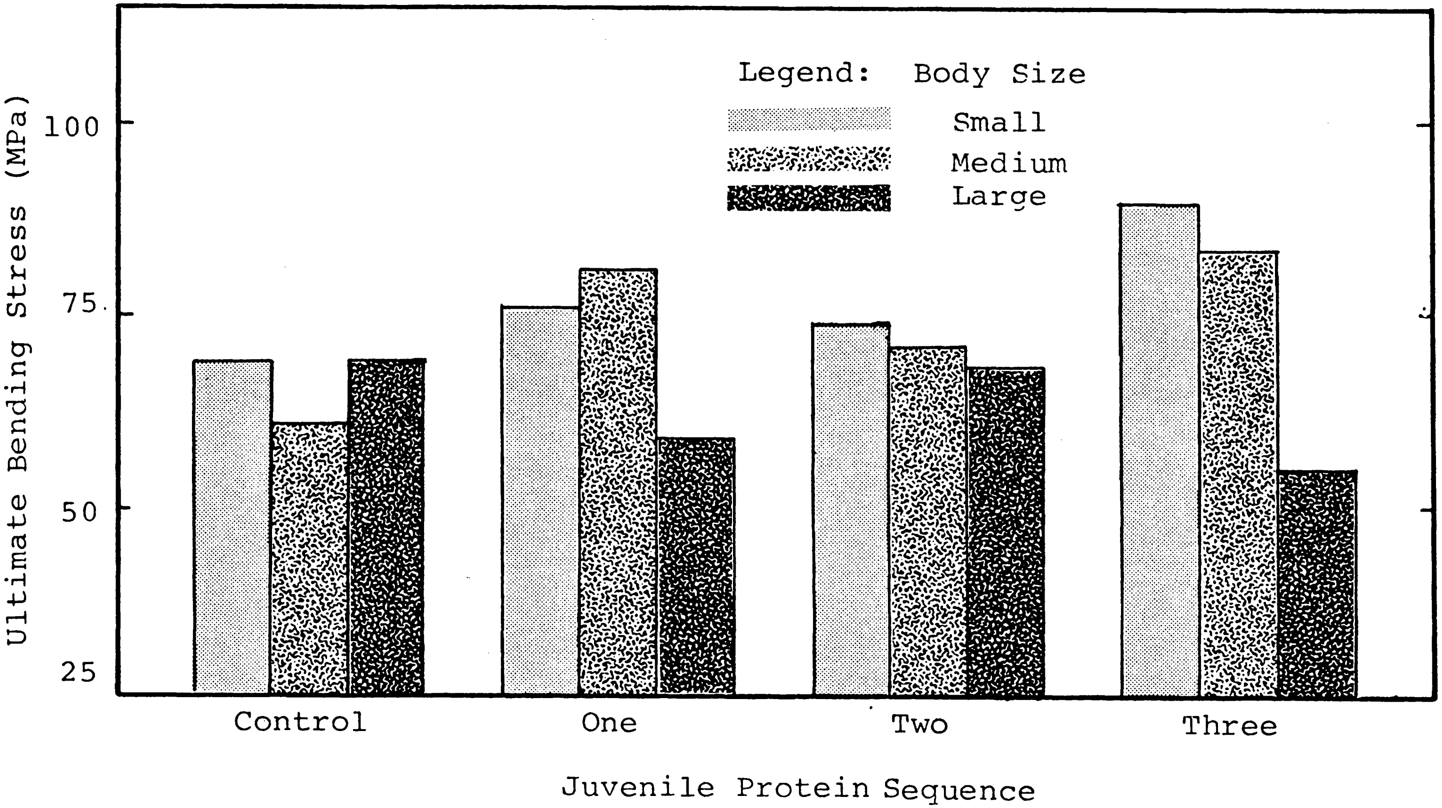


Figure 17. Histograms showing the effects of protein sequence and body size in the statistical analysis of the ultimate bending stress of the tarsometatarsus.

Table 13. Summary of statistical analyses of the modulus of elasticity of the different bones from 72 week old caged layers.

	Analysis of Variance			Analysis of Covariance		
Type of Bone	Juvenile Protein Sequence[a]	Body Size[b]	Cage Profile[a]	Juvenile Protein Sequence[a]	Body Weight[b]	Cage Profile[a]
Radius	NS[c]	NS	NS	NS	NS	NS
Ulna	NS	NS	NS	NS	NS	NS
Humerus	NS[d]	NS[d,e]	NS[e]	NS	NS	NS
Femur	NS	NS	NS	NS	NS	NS
Tibia	NS	NS[f]	NS	NS	NS	NS
T.mt.	NS	0.01[g]	NS	NS	NS	NS

[a] See Table 3 and Figure 12, respectively, for protein sequence and cage profile.

[b] Body size categorized as small, medium, or large and body weight is the actual weight of the hens at 72 weeks of age.

[c] Level of significance (NS - not significant, $P > 0.05$).

[d] Interaction at $P < 0.05$.

[e] Interaction at $P < 0.05$.

[f] Quadratic relationship between size and modulus of elasticity at $P < 0.05$.

[g] Linear relationship between size and modulus of elasticity at $P < 0.05$.

(Figure 18). However, when body weight was used as a covariant these effects vanished.

In summary, Appendix B contains the results of the three point bending test. When using the analysis of variance, the ultimate bending force of the bones was influenced by body size and cage profile. Body size also affected the ultimate bending stress of the different bones. The modulus of elasticity was not influenced by protein sequence, body size or cage profile. However, no significant differences were observed when body weight was used as a covariant in the statistical analysis.

5.3 FOUR POINT BENDING RESULTS

Difficulties occurred during the test procedures using the four point bending test. Because of the geometrical shapes, the loads were not applied symmetrically on the diaphysis of the femur. Table 14 compares the deflection of the dial and chart during the test of 12 femora. The dial should have measured the deflection at the center of the bone and the chart deflection at the points of loading. The percent difference between the readings varied from -23 to 32 percent (Table 14). Using strength of material equations for four point bending loads, the deflection readings from the dial should have been 27 percent higher than the

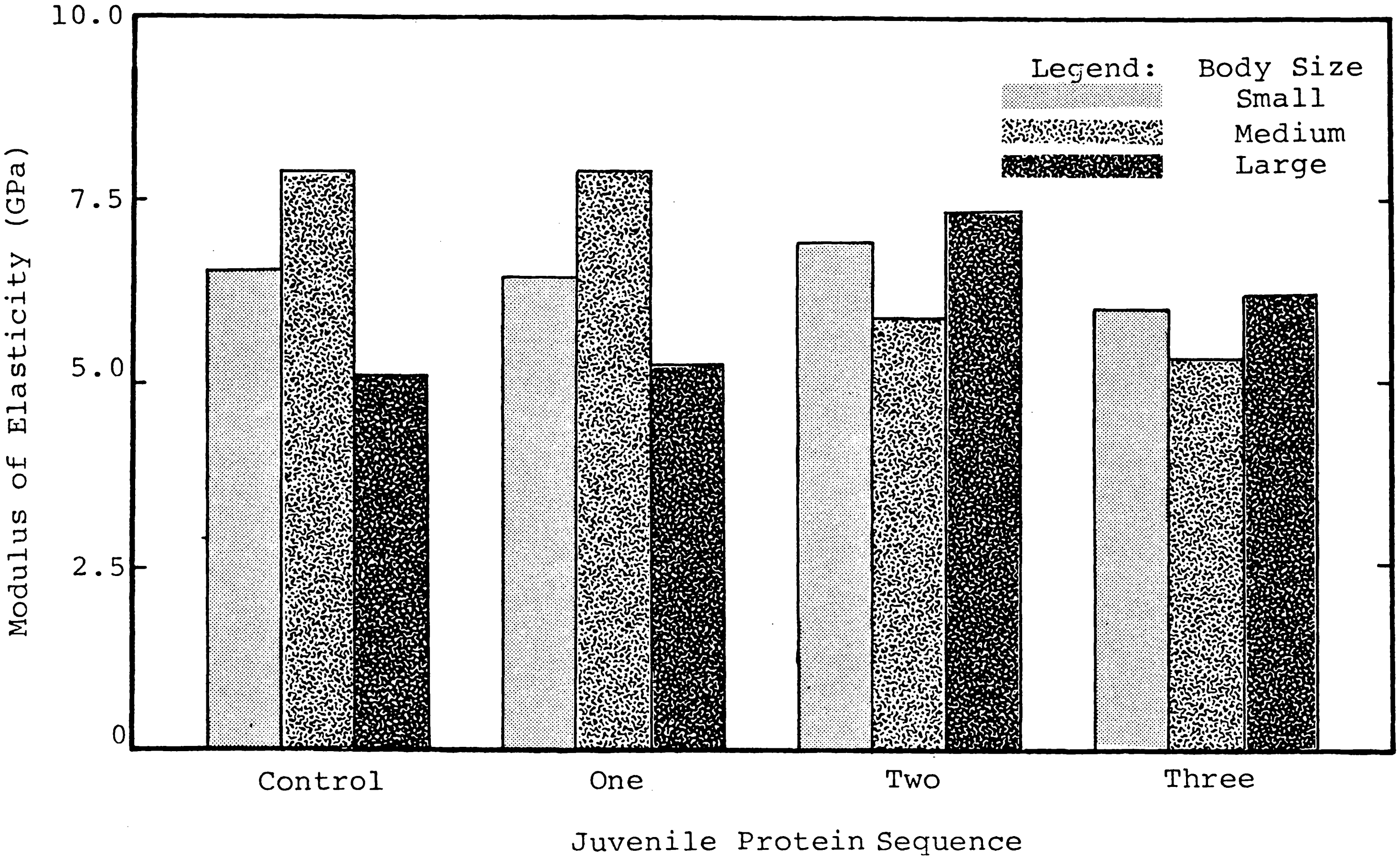


Figure 18. Histograms showing the effects of protein sequence and body size in the statistical analysis of the modulus of elasticity of the humerus.

Table 14. Comparison of the deflection readings taken from the deflection dial and the Instron chart while using the four point bending test.

Test Run No.	Dial Reading (mm)	Chart Reading (mm)	Percent Difference[1]
1	0.127	0.112	+12
2	0.127	0.086	+32
3	0.076	0.074	+ 3
4	0.083	0.102	-23
5	0.083	0.091	-11
6	0.127	0.112	+12
7	0.165	0.117	+29
8	0.089	0.081	+ 8
9	0.064	0.061	+ 4
10	0.074	0.076	- 3
11	0.083	0.099	-20
12	0.070	0.076	- 9
Ave	0.097	0.091	+ 6

[1] Using strength of material equations there should be a +27 percent difference.

chart deflection. This was only observed in 2 of the 12 four point bending tests (Table 14). In these two tests, the moduli of elasticity of the femur were 7.5 and 8.3 GPa. These are comparable to the modulus of elasticity of the femur obtained using three point bending test (Table B19). The moduli of elasticity for test runs 4 and 5 were 56 and 43 GPa, respectively. In these two tests, the deflection measured by the chart was considerably more than the dial deflection. Nonuniformity and length of the diaphysis caused the loads to be applied near the epiphyses. In this region, the bone would change from compact to cancellous which could result in different mechanical properties. Because of the uncertainity of the measured dial and chart deflections, this test procedure was not performed on any other bones.

5.4 TORSIONAL TEST RESULTS

The main objective of the torsional test was examination of the mode of failure. However, the force-deformation curve was also used to determine the angle of twist, ultimate torque, shear stress, and shear modulus of the tibia and radius. The properties of the tibia are presented in Tables C1 through C4 and those of the radius in Table C5.

Table C1 shows the angle of twist of the tibia as a function of protein sequence, body size, and cage profile. There were no differences ($P < 0.05$) noted in the angle of twist based on protein sequence, body size, or cage profile when using analysis of variance or covariance (Table 15). The results of analyzing the torque on the tibia at failure are shown in Table C2. An increase in body size or change in cage profile tended to cause an increase in the ultimate torque. Protein sequence ($P < 0.05$) and body size ($P < 0.01$) influenced the ultimate torque of the tibia in the analysis of variance. These significant differences remained when body weight was used as a covariant (Table 15). The ultimate shear stress of the tibia was given in Table C3. The stress of the tibia from birds fed the JPS two was 25 percent higher than the stress of the tibia from other birds. The tibia of smaller birds tended to have a lower shear stress than the tibiae of medium or larger birds. There were no differences in the stress as influenced by the main effects when using the analysis of variance but orthogonal contrasts showed a difference in the stress of tibia from birds fed JPS two and three. The analysis of covariance showed protein and body weight influenced ($P < 0.05$) the shear stress of the tibia. Cage profile did not affect the shear stress of the tibia in either analyses. Table C4

Table 15. Summary of statistical analyses of torsional properties of the tibia from 72 week old spent hens.

Torsional Property	Analysis of Variance			Analysis of Covariance		
	Juvenile Protein Sequence[a]	Body Size[b]	Cage Profile[a]	Juvenile Protein Sequence[a]	Body Weight[b]	Cage Profile[a]
Torque	0.05[c]	0.01	NS	0.05	0.01	NS
Angle of Twist	NS	NS	NS	NS	NS	NS
Shear Stress	NS	NS	NS	0.05	0.05	NS
Shear Modulus	0.05	0.05	NS	NS	0.05	NS

[a] See Table 3 and Figure 12, respectively, for protein sequence and cage profile.

[b] Body size categorized as small, medium, or large and body weight is the actual weight of the hens at 72 weeks of age.

[c] Level of significance (NS - not significant, $P > 0.05$).

lists the shear modulus of the tibia. The bones of the medium size birds tended to have a higher shear moduli than the small or larger birds while cage profile did not influence the shear modulus. Protein sequence and body size affected the shear modulus of the tibia ($P < 0.05$ level, Table 15). Juvenile protein sequences two and three showed differences using orthogonal contrasts. A quadratic polynomial described the relationship of body size and the shear modulus. The influence of juvenile protein sequence was removed in the analysis of covariance, however, body weight ($P < 0.05$) still influenced the modulus.

Table C5 contains the results of the torsional testing of the radius. The bones were divided into control or reversed protein sequence because of small sample sizes. Overall, there were no differences noted in the torsional mechanical properties of the radius. There was a 25 percent difference between the ultimate shear stress of the radii from birds fed the two types of protein regime.

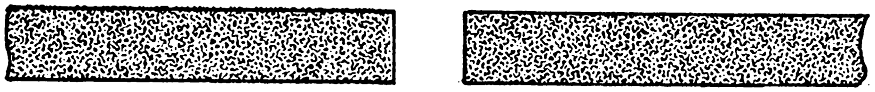
In summary, significant differences in the torsional properties were observed when using the analysis of covariance. The protein sequence affected the shear stress of the tibia using both types of analysis. Body weight rather than body size also tended to influence ($P < 0.05$) the torsional properties of the tibia while cage profile showed no affect.

5.5 TYPE OF FRACTURE

The type of bone fracture was determined by visually inspecting the bones loaded to failure in bending and torsion. All six bones were examined in bending where the fracture occurred at the center load. Torsional loading permitted the tibia to fail at any location between the proximal and distal ends and the radius to fail along the diaphysis. Data were analyzed using the hypothesis of marginal homogenity. This procedure involved a chi-square test on the probability of a certain fracture pattern occurring.

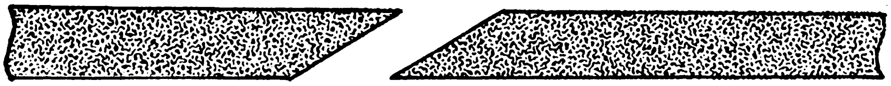
5.5.1 Bending Fractures

Three fracture patterns occurred in the bones loaded to failure using the three point bending test. Figure 19 schematically illustrates the three different fractures. A type 1 fracture produced two surfaces perpendicular to the long axis of the bones and was related to pure tensile stresses. The type 3 fracture resulted in two surfaces along a diagonal plane rather than a vertical plane. A type 2 fracture was a combination of tensile and shear stresses affected the fracture on the upper and lower side of the neutral axis, respectively. The third mode was a type 2 fracture which is a combination of types 1 and 3. Above the neutral axis, the failure was due to tensile stresses and below the axis there



a) Type 1

b) Type 2



c) Type 3

Figure 19. The types of fractures which occurred in the three point bending test.

was a combination of stresses affecting the fracture mode. Figure 20 shows the type 1, 2, and 3 fractures, respectively, occurring in the femur. The three fractures were observed in all six limb bones.

There were no differences in fracture pattern of the bones from the birds fed the three reversed protein sequences. Therefore, the data were analyzed according to control diet or reversed protein diet. Body weight also had no influence on the type of fracture but cage profile appeared to influence the fracture. Table 16 lists the percent occurrence of the fractures based only on the protein regime and bone type while Table 17 includes the effects of cage profile on the fracture.

The analysis indicated the frequency of the fracture modes in the radius, humerus, femur, and tibia were influenced ($P < 0.05$) by protein sequence (Table 16). The influence of cage profile was masked by an interaction between protein sequence and cage profile ($P < 0.05$) in all types of bones except the radius (Table 17). However, a brief discussion of the mode of fracture is provided.

Cage profile had no effect on the fracture of the radius, however, the type 3 fracture occurred only in the radii from birds fed the reversed diet. The reversal in juvenile protein sequence did not influence the fracture of the ulna

Figure 20. Photograph of the bending fractures occurring in the femur.

Table 16. Frequency of bending fractures for the bones from 72 week old caged layers as a function of protein sequence.

Bone Type	Fracture Type[a] 1	2	3	Juvenile Protein Sequence[b]
Radius	30[c]	70	0	Control
	20	55	25	Reversed[d]
Ulna	60	40	0	Control
	59	38	3	Reversed
Humerus	50	20	30	Control
	31	31	38	Reversed[d]
Femur	40	0	60	Control
	43	23	33	Reversed[d]
Tibia	70	20	10	Control
	25	44	31	Reversed[d]
Shank	33	33	33	Control
	26	35	39	Reversed

[a] See Figure 19 for fracture types.

[b] Reversed protein sequence includes juvenile protein sequences one, two, and three, see Table 3.

[c] Percentage occurrence.

[d] Statistical difference in frequency of fracture at $P < 0.05$ level.

Table 17. Frequency of the bending fractures in the bones as a function of both protein sequence and cage profile.[a]

		Juvenile Protein Sequence			
		Control		Reversed[b]	
Bone Type	Fracture Type[c]	Low	High	Low	High
Radius	1	25[d]	33	22	10
	2	75	66	52	50
	3	0	0	26	40
Ulna[e]	1	75	50	56	77
	2	25	50	39	23
	3	0	0	4	0
Humerus[e]	1	75	33	26	33
	2	0	33	30	33
	3	25	33	43	33
Femur[e]	1	50	33	38	77
	2	0	0	14	22
	3	50	66	48	0
Tibia[e]	1	100	50	22	44
	2	0	33	52	11
	3	0	17	26	44
Shank[e]	1	75	0	26	33
	2	25	40	30	44
	3	0	60	43	22

[a] See Figure 12 and Table 3, respectively, for protein sequence and cage profile.

[b] Reversed protein sequence includes juvenile protein sequences one, two, and three, see Table 3.

[c] See Figure 19 for fracture types.

[d] Percent occurrence.

[e] Interaction occurred by protein sequence and cage profile at $P < 0.05$ level.

(Table 16). Table 17 contains the data relative to the influence of cage profile on the failure. For the ulnae of birds receiving the control feeding regime, a higher percentage of type 1 fractures occurred in those birds housed in low profile cages. Ulnae from birds fed the reversed protein diets, displayed the opposite trend where the high profile cages resulted in more type 1 fractures. The type of breakage in the humerus was not affected by protein sequence or cage profile. The three different fractures were equally distributed in the humeri from high profile caged birds fed the reversed protein sequence and those birds fed the control protein sequence. The humeri from birds which received the control diet and housed in low cages predominantly had a type 1 fracture. Except for the humerus, the type 3 fracture was not predominant in the other wing bones.

The femora from the control birds had no type 2 breaks and types 1 and 3 were equally distributed based on cage profile. There was an increase in types 1 and 2 fractures in the femur from birds on the reversed protein feeding regime. The reversed protein sequence shifted the fracture of the tibiae to types 2 or 3. In the control protein diet, 70 percent of the fractures were type 1 and in the reversed group, 75 percent of the breaks were types 2 and 3 (Table 16). Based on diet, the fracture types of the tarsometatarsus were equally distributed. The tarsometatarsus of the control and low profile group had breaks of types 1 and 2. Cage profile did not affect the type of break occurring in the tarsometatarsus of birds fed the reversed diet.

The humerus, femur, and tarsometatarsus had L/D ratios of approximately seven. This indicated shear affected the structural characteristics. For these three bones, greater than 30 percent of fractures were type 3. This particular fracture had a combination of shear and tensile stresses influencing the mode of ultimate deformation. The L/D ratios of the radius, ulna, and tibia were greater than 10 and less than 20 percent of their fractures were type 3. With the radius, the predominant fracture was type 2 while the predominant fracture mode of the ulna and tibia was type 1.

5.5.2 Torsional Fracture

Torsional fractures formed an inclined 45° helix around the tibia (Figure 21) and radius. Fractures occurring in the proximal epiphysis were perpendicular to the long axis of the bone (Figure 22). The different reversed protein regimes and body weight did not influence the type or location of fracture. The majority of the fractures formed a helix around the shaft with differences observed in the location of the fractures. The percent occurrence of fracture based

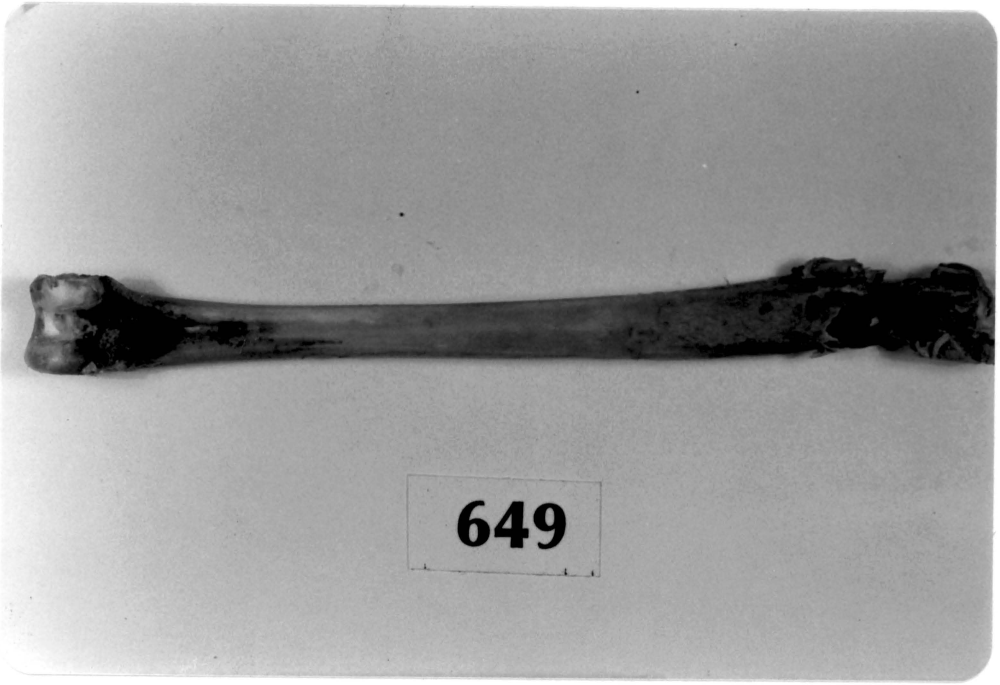


Figure 21. Photograph of a torsional fracture occurring in the proximal epiphysis of the tibia.

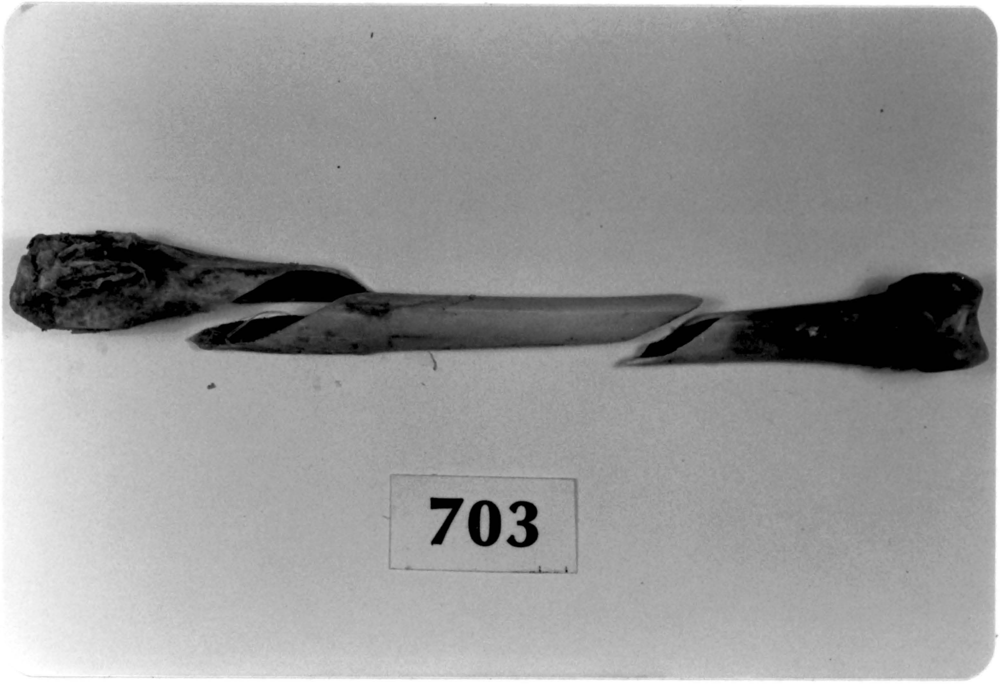


Figure 22. Photograph of a torsional fracture occurring in the diaphysis of the tibia.

on position along the bone is given in Table 18. Cage profile affected the location of the break in the tibiae from birds fed the control diet. The fracture of the tibiae from the control birds held in low profile cages was in the proximal end. However, the tibiae from control birds housed in high profile cages had fractures occurring along the diaphysis. Cage profile did not affect the position of the fracture in the tibiae from birds fed the reversed protein diets where the fractures were predominantly located in the center of the bone (Table 18). The frequency patterns indicated an interaction between cage profile and protein sequence existed ($P < 0.05$).

5.6 SUMMARY OF RESULTS

In summary, the shear stresses, bending stresses, and moduli of elasticity of the limb bones from 72 week old caged layers were not influenced by protein sequence, body size, and cage profile. The resistance of the molecular structure of the bones to external shear, bending, or torsional forces was the same regardless of the effects of juvenile diet, body size, and housing. These results indicate that living tissue responds to the loading demands placed upon it and adjusts the geometrical properties in accordance with the forces exerted on the external surface.

Table 18. Percent occurrence of the tibia fracture location during the torsional testing.

Location of Fracture	Juvenile Protein Sequence[a]			
	Control		Reversed[b]	
	Cage Profile		Cage Profile[c]	
	Low	High	Low	High
Diaphysis[d]	29[e]	64	76	68
Proximal End[f]	71	36	24	32

[a] Interaction between protein sequence and cage profile at $P < 0.01$.

[b] Reversed protein sequence includes juvenile protein sequences one, two, and three, see Table 3.

[c] See Figure 12 for cage profiles.

[d] Fracture occurs strictly between the distal and proximal epiphyses.

[e] Percent occurrence.

[f] Fracture occurs either in the epiphysis or spirals into the epiphysis.

Chapter 6

DISCUSSION OF RESULTS

Data analyses showed body size and cage profile influencing the structural properties more than protein feeding regime when using a three way analysis of variance, however, these effects were minimized when adjustments were made for body weight. The reversed juvenile protein sequence affected ($P < 0.05$) the fracture mechanism and the torsional properties of the tibia. The discussion presented herein looks at the validity of the data, comparison in structural properties between bones, fracture mode, the effects of protein sequence, body size, cage profile, and methods of testing.

6.1 VALIDITY OF RESULTS

The testing procedures were performed carefully to insure that conditions were as identical as possible. The bones were tested randomly to prevent any bias in the results and were placed on the supports in the same orientation. Procedures were also developed to determine if any of the data or results were outlying values.

Exact comparisons between experimental results and previous studies involving layers (Table 1) was difficult because only the ultimate bending forces have been presented. The force required for permanent deformation would have been different for each study, regardless of the main effects investigated, since the length between the fulcra points were different. If the ultimate bending force had been normalized, (i.e. bone geometry considered) then a comparative analysis between studies would have been possible.

The experimental results were also compared to Yamada (1973) who presented a summary of the mechanical properties of bones from domestic fowls (Table 19). A domestic fowl was considered as birds ranging from ducks to pigeons to hawks by Yamada (1973). The moduli of elasticity between studies were within 15 percent for all bones except the tibia where there was a 39 percent difference. Similar patterns were observed in the ultimate bending forces where a 40 percent difference between experimental and Yamada's value was noted only in the bending force of the ulna. While the other values were within 16 percent of each other, there was some disagreement in the ultimate bending stress where a 50 percent difference was observed in the values of ultimate bending stresses of the radius, ulna, and tibia. Since the ultimate bending forces were in agreement, the differences in bending stress would be related to geometrical properties. Yamada (1973) reported that for small animals the ultimate bending stress was highest in the radius, and in

Table 19. Comparison of flexural properties of 72 week old caged layers to flexural properties of domestic fowls. Domestic fowls included crows, kites, hawks, pigeons, etc. (Yamada, 1973).

Mechanical Property	Investigator	Type of Bone				
		Radius	Ulna	Humerus	Femur	Tibia
Modulus of Elasticity (GPa)	Yamada[1]	13.4	15.3	5.4	6.3	10.9
	Experimental Results[2]	12.9	18.4	6.4	7.5	17.8
Ultimate Bending Strength (MPa)	Yamada	101.0	102.9	97.1	93.2	99.0
	Experimental Results	201.2	190.0	98.9	109.4	190.3
Ultimate Bending Force (N)	Yamada	32.3	141.2	176.0	217.2	188.7
	Experimental Results	38.4	100.9	154.3	200.0	171.5

[1] Domestic fowls.

[2] 72 week old caged layers.

descending order, the ulna, tibia, humerus, and femur. Experimental results showed the highest stress occurred in the radius, and then in descending order, the ulna, tibia, femur, and humerus. Therefore, the rank of the bending stresses was in reasonable agreement with Yamada (1973).

The mechanical properties of the tarsometatarsus have not been reported in past literature. The comparative analysis of the different studies was based on the results of the three point bending test. The pure shear and torsional tests have not been used in prior studies involving chickens. Overall, the results of the three point bending test were in agreement with past studies involving layers or domestic fowl.

6.2 DISCUSSION OF MECHANICAL PROPERTIES

Figures 23 and 24 may be used to compare the overall least square means, regardless of main effects, of the ultimate shear forces and stresses, respectively, of the limb bones. Ultimate shear forces of the leg bones were within 15 percent of one another and approximately 30 percent higher than that of the wing bones. Figure 23 shows that the ultimate shear forces for the ulna and humerus were 20 percent higher than the shear force for the radius. However, figure 24 shows the ultimate shear stress of the radius was

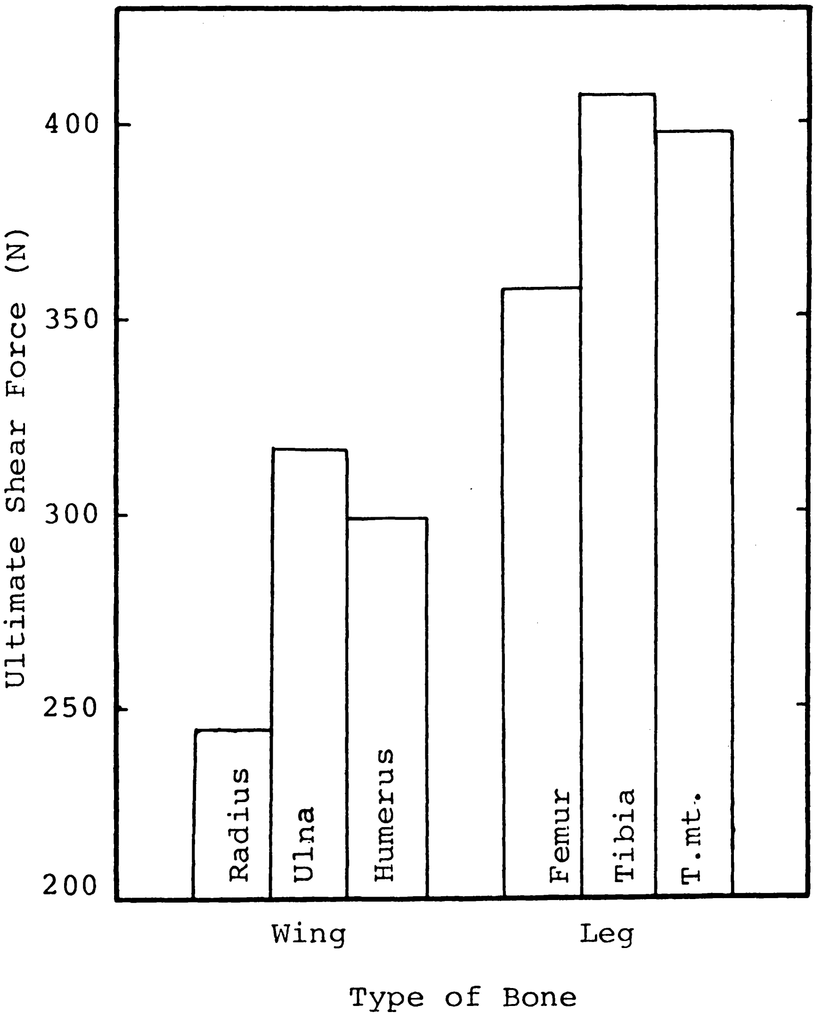


Figure 23. Comparison of the ultimate shear force of the different bones from 72 week old caged layers.

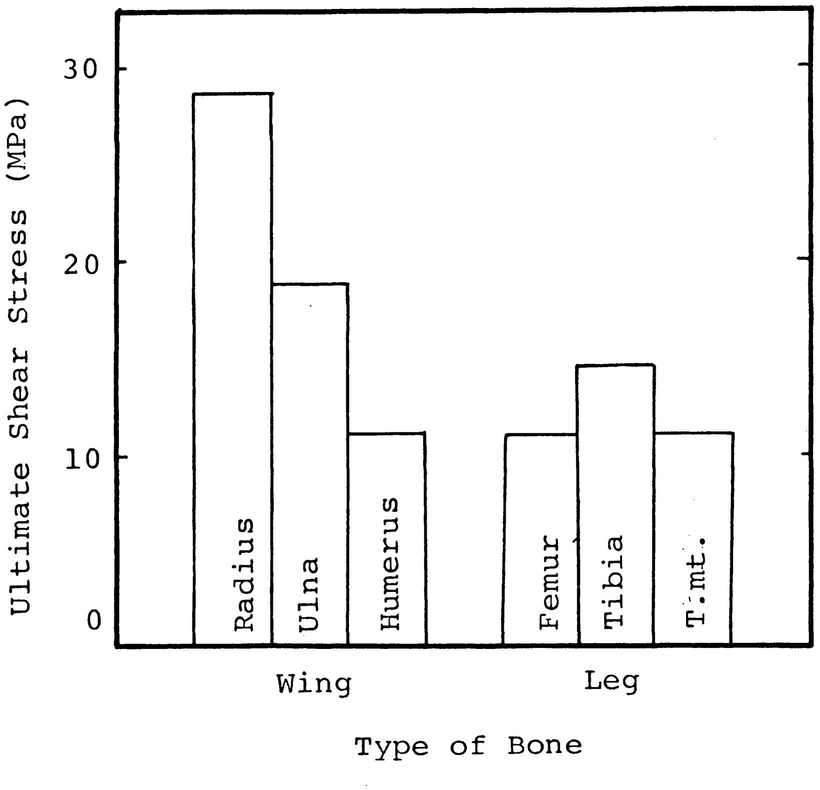


Figure 24. Comparison of the ultimate shear stress of the different bones from 72 week old caged layers.

30 percent higher than the ulna, 50 percent higher than the tibia, and 60 percent higher than the other bones. The shear stresses of the leg bones were similar to shear stress of the humerus.

Stress is the ability of the intermolecular bonds of a material to resist deformation caused by an external load. The results indicate that the molecular structure of the radius and ulna were different than those of the humerus. Figure 25 illustrates the relationship in size between the calcium and phosphate atoms as pictured by Posner and Betts (1981). These molecules make up the apatite crystal and chain growth of the bone. The smaller particles represent calcium atoms and the larger ones are phosphate atoms. Pure shear is the result of two planes of atoms being able to slide across one another. The phosphate and calcium atoms are depleted from the bones of layers during the egg production cycle. Therefore, the mineral content is decreasing at varying rates from each of the wing bones. Assuming the mineral content of each of the wing bones was similar prior to the egg production cycle, then a reduction in the shear stress of the humerus would be caused by minerals being depleted from the bone at a faster rate. The shearing of the radius and ulna was potentially higher because of increases phosphate and calcium atoms. It was noted early that the

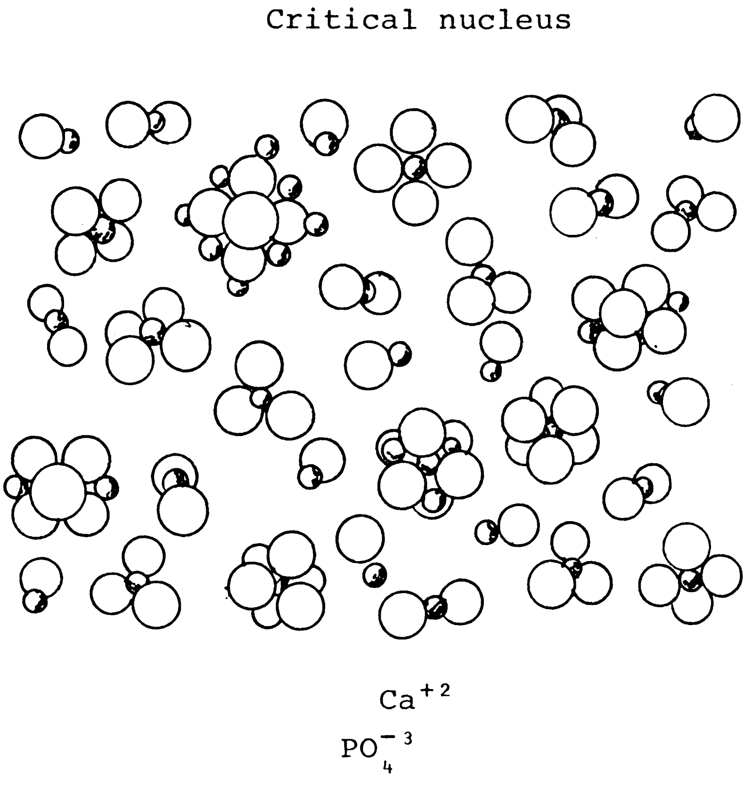


Figure 25. Illustration of the molecular structure of the calcium-phosphate atoms (taken from Posner and Betts, 1981).

leg bones and humerus had similar shear stresses. However, the molecular structures of the leg bones may be considerably different than the structure of the humerus. If the crystal lattice was not as dense in leg bones, then the shear stress would decrease. Two planes of atoms which are densely packed will have more resistance to slippage than planes which are loosely packed.

The comparison of the bending results was similar to the shear results. Figure 26 shows the least square means of the ultimate bending force of bones without considering the main effects. The breaking force of the humerus was 33 and 66 percent higher than that of the ulna and radius, respectively. The bending forces of the leg bones were approximately equal to 175 N. The ultimate bending stress varied for the six limb bones (Figure 27). The radius, ulna, and tibia displayed similar bending stresses. The ultimate bending stress in the humerus was at least 50 percent lower than the other wing bones. The femur displayed a stress 50 percent lower than the tibia and approximately equal to the stress in the humerus. The bending stresses in the tarsometatarsus were 60 percent lower than the stress of tibia.

The failure observed during bending was tensile related or the molecular bonds broke rather than shear planes slipping. The bones from birds fed the control diet ($P < 0.05$)

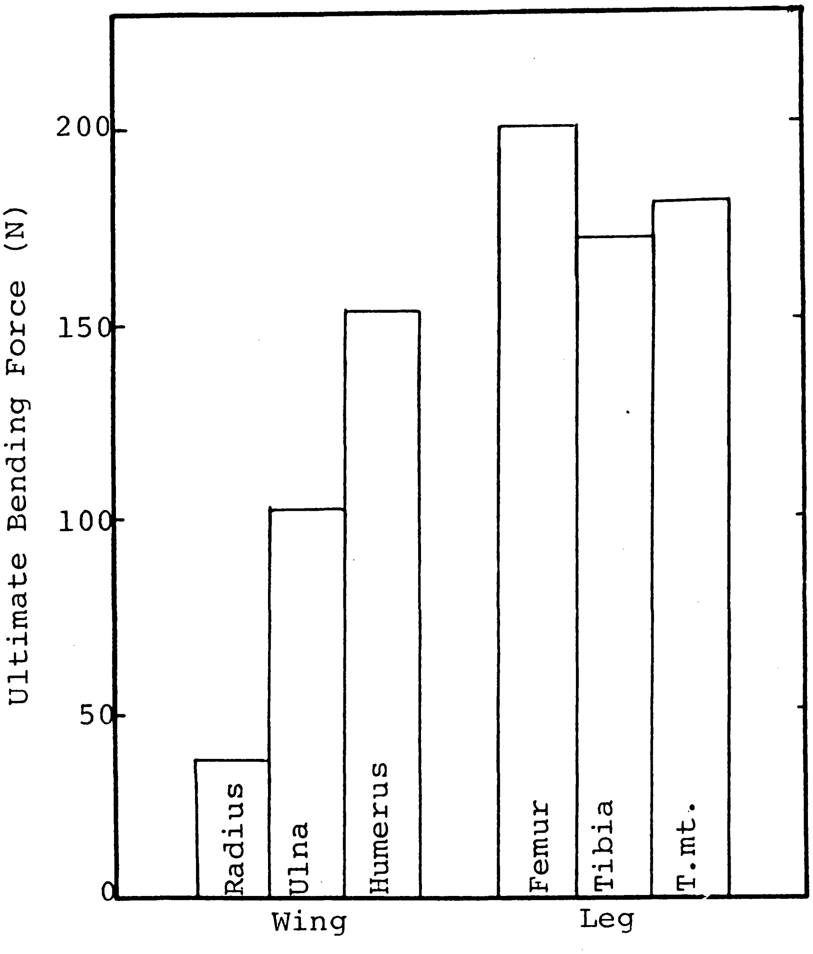


Figure 26. Comparison of the ultimate bending force of the different bones from 72 week old caged layers.

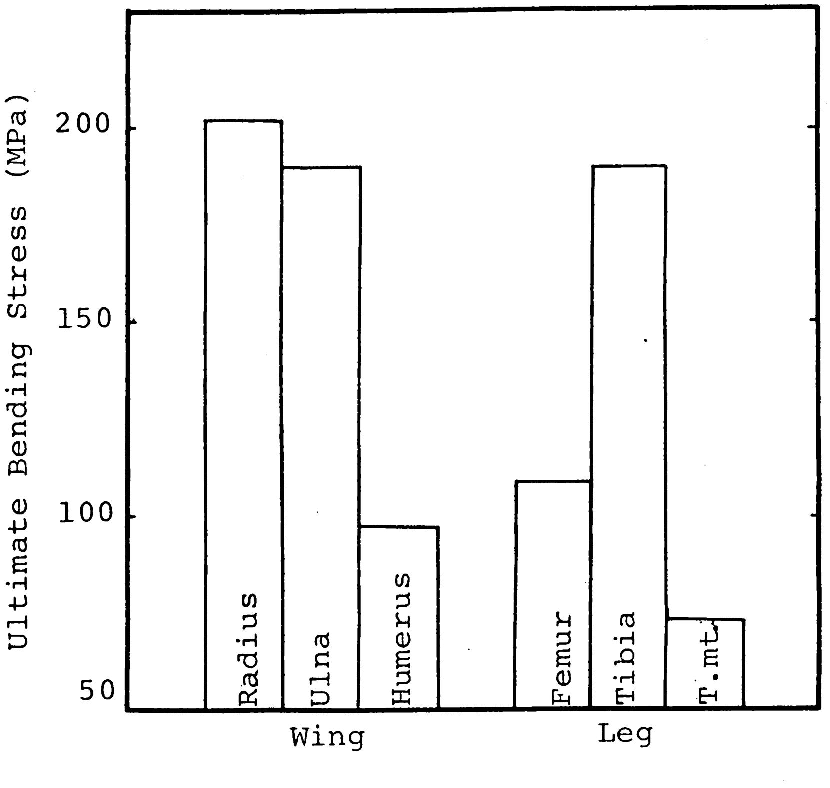


Figure 27. Comparison of the ultimate bending stress for the different bones from 72 week old caged layers.

broke due to pure tensile stresses. Bones from bird fed the reversed protein diet had fractures related to a combination of tensile and shear stresses. Glimcher (1981) stated that protein molecules provide a nucleation site between the collegan fibers and the calcium phosphate molecules during early stages of bone formation. Without a nucleation site, the calcium phosphate molecules cannot bond molecularly together to form the crystal structure for bone growth. Eventually, the protein and water molecules are replaced by the crystal lattice. Using Glimcher's (1981) theory, it may be hypothesized that reversing the protein during the first weeks of bone growth decreases the nucleation sites along the collegan fibers. This would change the lattice structure of the diaphysis, particularly near the center. Using the assumption that protein is needed for bone growth, more calcium-phosphate molecules or an increase in mineral content would exist at the center of the diaphysis of the bones from birds fed the control diet. The increase in minerals would act as interstitial atoms and increase the shear stress causing the bone to fail due to pure tensile stresses. In bones from birds fed the reversed protein regimes, the mineral content would not be as abundant at the center of the diaphysis (point of fracture). Therefore, the number of interstitial atoms may decrease causing a reduction in the shear stress.

The depletion of molecules also causes a decrease in the tensile strength since the molecules only have to separate from one another rather than other molecules.

Figure 28 contains a comparison of the flexural moduli of elasticity for the six bones. The overall modulus of elasticity of the ulna was higher than the moduli for the humerus and radius by 60 and 20 percent, respectively. The modulus of elasticity of the tibia was 66 percent higher than the moduli of the femur or tarsometatarsus.

Figure 7 illustrated the effects of not incorporating shear into the determination of the modulus of elasticity if the length to diameter ratio was less than 10. According to Figure 7, the actual modulus of elasticity may be 10 to 15 percent higher than the experimental value for the humerus, femur, and tarsometatarsus. Wilson and Baker (1978) reported the modulus of elasticity was 38 percent lower when shear was neglected in evaluating bones from broilers with L/D ratio near 5. Statistically the modulus of elasticity was not influenced by diet, body size, or cage profile. This follows the pattern of other materials such as steel where regardless of the alloys added to steel during processing, the modulus of elasticity will remain near 207 GPa.

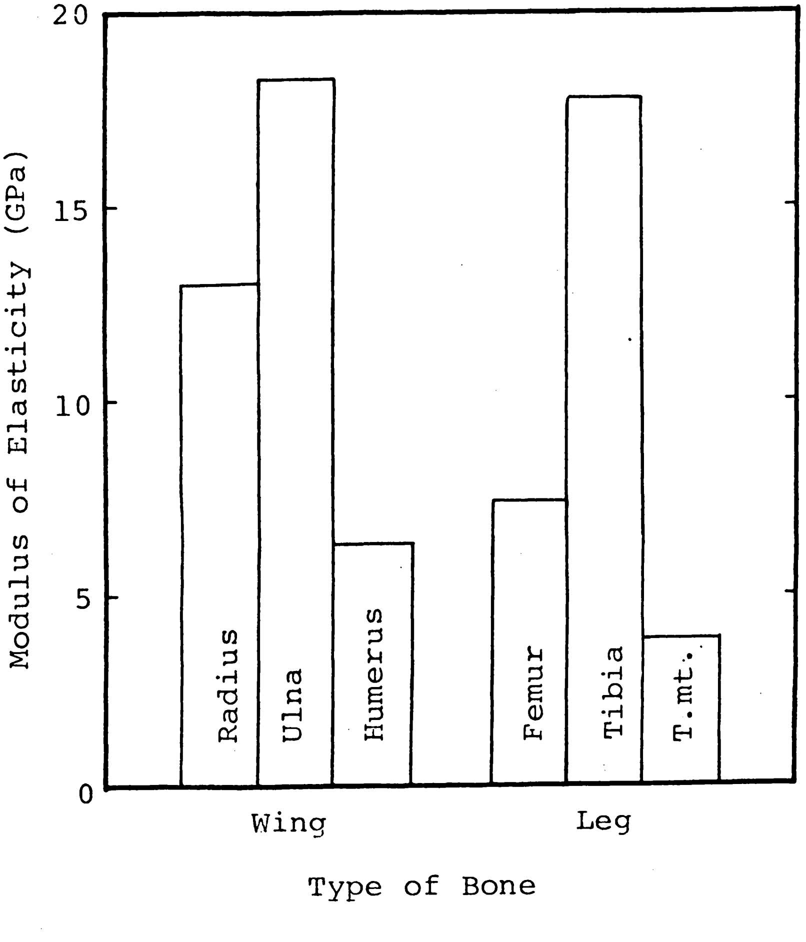


Figure 28. Comparison of the modulus of elasticity for the different bones from 72 week old caged layers.

6.3 DISCUSSION OF FRACTURE MODE

The frequency of certain fractures was influenced ($P < 0.05$) by protein feeding regime rather than body size or cage profile. In both the bending and torsional tests, the tibia had different fractures based on diet. Bending fractures have been discussed in a previous section. This discussion is focused on the fracture of the tibia using the torsional test.

The difference in fracture and location may be related to the protein sequence. The possible effects of reversing the protein in the juvenile sequence has been discussed. Since the protein complexes may provide a possible nucleation site between the calcium and collegan fibers, this could also influence the location of the fracture during torsional testing. In the control diet, the fracture tended to occur near the proximal end. Assuming that protein is needed for nucleation site, there would be less mineral content in epipyhses of bones from the control birds since the amount of protein in the control diet decreased with time. Therefore, the calcium-phosphate chemical structure (mineral content) would be changed as the bone grew longitudinally. This could be a possible explanation for the bones of the control birds being weaker in the proximal end. Since bone is anistropic, it would not be expected for the stress to be equally distributed in the longitudinal direction in the torsional test. The combination of potentially less nucleation sites and different calcium-phosphate crystals could possibly reduce the tensile stresses in the proximal ends of the tibia. In the reversed diet, there would be less nucleation sites during the first weeks of growth and possibly an increase in number as the birds age. With this feeding schedule there was an increase in protein in the diet up to 20 weeks of age. This could result in an increase of mineral content in the proximal end and lesser amounts in the center of the diaphysis. Table 16 showed 73 percent of the fractures from the tibiae of birds fed the reversed diet occurred in the diaphysis. This could be an indication that tensile stresses in the proximal and distal ends were stronger than those in the diaphysis, whereas, the opposite relationship was true in the control group.

The concept of protein being needed for nucleation sites and bone formation was endorsed by Glimcher (1981). This concept has been applied, in part, to the fracture mechanism of the tibia. The radius was also tested in torsion, however, because the epiphysis of the bones had to be placed in molds, the fracture was forced to occur along or in the diaphysis of the bone. Effective evaluations of the torsional fracture mode of the other bones was not possible because their irregular geometrical characteristics prevented the torsion test from being used.

6.4 EFFECTS OF DIET, BODY SIZE, AND CAGE PROFILE ON PROPERTIES

Analyses of data showed the juvenile protein sequence did not affect any of the shear or bending properties of the bones from 72 week old caged layers. Body size affected ($P < 0.05$) the properties more than diet or cage profile when using an analysis of variance. However, considering body weight as a covariant removed any influence of body size on the shear and bending properties. The effects of categorized body size were predominantly seen in the leg bones rather than the wing bones. The analysis of variance indicated that cage profile also influenced the mechanical properties of the bones. As compared to the body weight, this main effect influenced the properties of wing bones more than those of the leg bones. The effects of cage profile were minimized when using the analysis of covariance. This tends to indicate that cage profile may have influenced nutrition intake which would tend to affect the final body weight.

6.5 DISCUSSION OF TEST PROCEDURES

Four different test procedures were used in evaluating bones as a structural material. The ultimate forces resulted in the most significant differences in the analysis of variance. Even though the breaking forces were different, the stress of the bones were not influenced by protein feeding sequence, body size, or cage profile. This was an indication that the geometrical properties changed proportionally to changes in forces.

The shear test was the simplest of the tests to perform and the data could be quickly analyzed. This test procedure has not been used frequently in past research. However, the analysis of variance showed difference existed in the main effects based on shear properties, whereas, no difference existed when using the analysis of covariance. Examining the shear force, the bird's size and weight affected ($P < 0.01$ and $P < 0.05$ levels, respectively) the ultimate force of the leg bones. An interaction between protein level and body size occurred in the shear force of the humerus, and the stress of the humerus and tarsometatarsus. Otherwise, the shear stresses of the bones were not influenced by protein regime, body size, or cage profile.

The four point bending test did not accurately predict the deflection at the center of the bone because of the length of the bone and the distance between the force couple. The main hinderance was the geometrical configuration of whole bones. These geometrical differences resulted in unsymmetrical loads being applied to the bones. If the L/D ratio is significantly less than 10 ($L/D < 7$), the shear properties would be examined rather than the flexural properties. The four point bending tests should be used with short thick bones with straight shafts and uniform cross sectional areas.

The three point bending test was easy to apply, however, data reduction took longer than the shear test data. This procedure had been used in the past and the results (or similar results) were comparable to other studies. The main effects were noted to cause differences ($P < 0.05$) in the bending forces when using an analysis of variance. Five of the six bones showed significant differences existed in bending force based on body size. Three of the bones were also influenced by cage profile. However, the analysis of covariance indicated these differences were caused by differences in body weight.

Table 20 shows the correlation coefficients between ultimate shear and bending force and ultimate shear and bending stress for the different bones. In all cases, the coefficient was less than 0.6 which indicated that a bending test could not be used to predict the results of a shear test.

Table 20. Correlation coefficient between bending and shear properties of the bones from 72 week old caged layers.

Bone	Forces	Stresses
Radius	0.422	0.471
Ulna	0.0325	0.127
Humerus	0.388	0.168
Femur	0.593	0.288
Tibia	0.523	0.176
T.mt.	0.428	0.412

Similar coeffients occurred when comparing the torsion properties to the shear and bending properties of the tibia.

Use of the torsion test allowed detection of possible molecular structural differences based on protein sequence, whereas, with the shear and bending tests did not. This procedure took considerably more time for testing because of the special preparation needed for each bone and time required to mount the bones into the test fixture. The test fixture performed well, however, there were several limitations in its usage. With the Instron and present test procedures, the angle of twist was limited to 40° after which the force exerted on the load cell was questionable. Another difficulty with the design was lack of adaptability to bones with different size epiphyses, for example, radii versus tibiae. While there was some difficulty with the procedures, torsion provided a different and unique view of the structure of bone which was not seen with shear or bending tests. The torsional test requires whole bones to have a relatively uniform cross-section perpendicular to the longitudinal axis and a straight diaphysis. This was probably the biggest drawback since it prevented torsion tests from being performed on the humerus or femur.

6.6 DISCUSSION OF SELECTION OF BONES

Six different bones were used for the various test procedures. The radius, femur, and tarsometatarsus were well suited for the bending test. These bones have cross sections perpendicular to the long axes which are symmetrical about the neutral axes, therefore, miminizing the eccentricity of loading. Standard geometrical shapes could be used to model the cross section of the radius, femur, humerus, and tarsometatarsus. The tibia was not desireable for bending test because of a tendency for the bone to rotate causing a moment to be exerted about the neutral axis. This change resulted in an increase in the moment of inertia, therefore, the bending stress and modulus of elasticity would be different. Along with the humerus and tibia, these same bones could be used in shear test. The bone geometry permitted the cross-sectional area to be estimated using known geometries. The shear test was more desirable than bending for the humerus because the changing cross sectional area along the bone shaft would not influence the shear properties as much as those of bending. Because of their straight and uniform diaphysis, the radius and tibia were the only bones which could be used in torsional testing. The ulna was not a good selection because of its curvature in the diaphysis and the oblique cross sectional area which was difficult to model. Summarizing, the radius was the only bone selected in which shear, bending, and torsional tests could accurately be performed.

6.7 SUMMARY OF RESULTS

The testing procedures provided the neccessary data for determining mechanical properties of bones from chickens. The restriction of protein during the initial phase of bone growth influenced the mode of failure of the bones tested in bending. Results of this study showed that overall there were no differences in the shear, and bending properties of the different bones after adjustments were made for body weight.

Chapter 7

CONCLUSIONS

7.1 OBJECTIVES

This study had four primary objectives:

1. Develop test procedures for determining mechanical properties of bones from spent hens,
2. Determine mechanical properties of bones from layers raised on a reversed protein sequence during the early stages of life (0 to 20 weeks),
3. Investigate whether bone fractures are related to shear or tensile stresses, and
4. Determine the effect of protein sequence and body weight on the mechanical properties of bone.

7.2 CONCLUSIONS

7.2.1 Objective 1

Four different loading procedures were used to evaluate the mechanical properties of bones from spent hens. The three point bending test would be recommmended for use with the radius, femur, and tarsometatarsus. Along with the humerus and tibia, these bones could be used with the shear test. This test is also recommended if the length to diameter ratio is less than ten. Bending or shear test was not desireable with the ulna because of bone geometrical characteristics. The four point bending test did not work with whole bones because cross sectional shapes prevented symmetrical loading of the diaphysis. The torsional test is recommended for evaluating the fracture mode and shear modulus of the tibia and radius.

7.2.2 Objective 2

The ultimate shear force and stress were determined for the different bones from the caged layers. The ultimate bending force, bending stress, and modulus of elasticity were determined using the three point bending test. While the humerus had higher shear and bending forces at failure, the ulna and radius had higher stresses and thus, more resistance to permanent deformation. The properties of the leg bones were similar except for the bending stress in the tibia which was much higher than for the femur or tarsometatarsus. This could be related to the bending moment which was produced about the nuetral axis due to a rotation of the shaft during the bending test. Mechanical properties were determined based on protein sequence, body size, and cage profile. Because of limitations in the torsional test apparatus, only the shear modulus, ultimate shear stress, torque at failure, and maximum angle of twist of the tibia and radius were determined.

7.2.3 Objective 3

The bending and torsion fractures of the radius and tibia from the caged layer were related to tensile stresses. The torsion loading produced a 45° helix around the diaphysis of the bones which is typical of a tensile mode of failure. The atoms tended to debond rather than slide across another plane of atoms. The bending test also produced fractures predominantly related to a tensile weakness.

7.2.4 Objective 4

Juvenile protein sequence did not influence any of the bending or shear forces of the different bones of 72 week old spent hens at the $P < 0.05$ level. Body size influenced ($P < 0.05$) the shear ultimate forces of the leg bones but not the forces of the wing bones. However, cage profile significantly influenced the ultimate forces of the wing bones and not those of the leg bones. The effects of body size and cage profile were miminized when body weight was used as a covariant and the data normalized. The shear and bending stresses were not influenced by protein sequence, body size, or cage profile. Similarly, the modulus of elasticity and shear modulus were not significantly affected by the main effects. Torsional shear stress and torque were influenced ($P < 0.05$) by protein level and body size after adjustments were made for body weight. Based on the results of the study, juvenile protein sequence, body size, and cage profile did not influence the mechanical properties of the limb bones from 72 week old spent hens.

Chapter 8

SUMMARY AND RECOMMENDATIONS

8.1 SUMMARY

The effects of reversed juvenile protein levels, body size, and cage profile on the mechanical properties of limb bones from spent hens were investigated. The following provides a summary of the results:

1. The ultimate shear force of the femur, tibia, and tarsometatarsus was influenced by body weight ($P < 0.05$),
2. The ultimate bending force of the tibia was affected by body weight ($P < 0.05$),
3. The ultimate torque of the tibia was influenced by juvenile protein sequence and body weight ($P < 0.05$),
4. The torsional shear stress of the tibia was influenced by juvenile protein sequence and body weight ($P < 0.05$),
5. The shear modulus of the tibia was affected by body weight ($P < 0.05$),
6. The frequency pattern of the bending fractures of the radius, ulna, femur, and tibia were influenced by juvenile protein sequence ($P < 0.05$), and

7. The location of the torsional fracture was affected by juvenile protein sequence ($P < 0.05$).

The three point bending would be recommended if the L/D ratio is equal to or greater than 10. The shear test is recommended if the ratio is less than 10. Torsional tests provided an opportunity to load the bone with both tensile and shear stresses simultaneously to examine the fracture mode. The four point bending test procedure was not desireable in evaluating whole bones because of the unsymmetrical loading due to the irregular geometrical configuration of the diaphysis.

8.2 RECOMMENDATIONS

The basic premise of this work was that the ultimate breaking force does not accurately reflect the structural characteristics of bones. The results of this study have shown that while statistical differences existed in ultimate forces, the bones from different size layers which were fed different protein sequences and reared in different cages had similar stress values or equal resistance to failure. The fracture of bones from spent hens needs to be further investigated from the molecular structure and a histological perspective. This need is based on the ideal that the strength may be changed if the molecules are oriented differently in the crystal lattice. Effective evaluations of body size and cage profile were difficult because of the number of different test procedures and main effects being examined. Other areas where research is needed is in that of the fracture of wet versus dry versus frozen bones, the error introduced by modelling the bones as known geometrical shapes, and the effect of long term storage of bones on the mechanical properties. A final recommendation involves doing an indepth study at a processing plant with an aim of determining exactly what type of forces are applied during the processing of spent hens or broilers. It may be determined that prevention of fractures during the processing of chickens should be approached with the idea of redesigning the processing equipment rather than genetic and dietary changes in the growth of layers or broilers.

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APPENDIX A

Least square means of the shear properties of the radius, ulna, humerus, femur, tibia, and tarsometatarsus of 72 week old caged layers.

Table A1. Least square means of the ultimate shear force (N) of the radius in a 72 week old caged layer.

Cage Profile[a]	Juvenile Protein Sequence[a]	Body Size			Mean of Diet
		Small	Medium	Large	
Low	Control	235.8 ± 27.7	245.4 ± 27.7	307.7 ± 27.7	262.9 ± 16.0
	One	173.5 ± 27.7	194.3 ± 24.0	223.5 ± 24.0	197.1 ± 16.0
	Two	222.4 ± 27.7	272.5 ± 34.0	206.1 ± 27.7	233.7 ± 14.6
	Three	224.6 ± 34.0	264.7 ± 48.1	215.7 ± 34.0	235.0 ± 18.5
	Mean of Body Size	214.1 ± 14.7	244.2 ± 17.3	238.3 ± 14.3	232.0 ± 9.0
High	Control	224.6 ± 27.7	188.3 ± 27.7	298.8 ± 27.7	237.2 ± 17.3
	One	226.1 ± 27.7	250.2 ± 34.0	236.9 ± 34.0	237.7 ± 15.3
	Two	275.8 ± 27.7	275.2 ± 24.0	254.1 ± 24.0	280.3 ± 27.7
	Three	231.3 ± 24.0	285.1 ± 21.5	254.1 ± 24.0	256.8 ± 13.4
	Mean of Body Size	239.5 ± 13.4	249.7 ± 13.6	269.9 ± 14.3	253.0 ± 8.0
Overall Mean of Body Size		226.7 ± 9.9	246.9 ± 11.0	254.1 ± 10.1	242.6 ± 3.6

[a] See Figure 12 and Table 3, respectively, for cage profile and protein sequence.

Table A2. Least square means of the ultimate shear force (N) of the ulna in a 72 week old caged layer.

Cage Profile[a]	Juvenile Protein Sequence[a]	Body Size			Mean of Diet
		Small	Medium	Large	
Low	Control	328.3 ± 44.2	290.5 ± 40.3	332.3 ± 49.4	317.0 ± 25.8
	One	261.3 ± 40.3	257.3 ± 40.3	311.4 ± 57.0	276.7 ± 26.9
	Two	279.8 ± 44.1	338.3 ± 32.9	285.4 ± 40.3	301.2 ± 22.7
	Three	284.3 ± 40.3	237.1 ± 44.1	299.9 ± 37.3	273.8 ± 23.5
	Mean of Body Size	298.4 ± 21.1	280.8 ± 19.3	307.3 ± 23.3	292.1 ± 12.4[b]
High	Control	404.8 ± 37.3	334.4 ± 40.3	315.3 ± 34.9	351.5 ± 21.7
	One	348.4 ± 40.3	336.2 ± 40.3	308.4 ± 32.9	331.0 ± 21.9
	Two	331.7 ± 37.3	327.7 ± 57.0	412.6 ± 40.3	345.6 ± 44.1
	Three	372.9 ± 40.3	248.5 ± 37.3	345.6 ± 44.1	322.3 ± 23.5
	Mean of Body Size	364.5 ± 19.4	311.7 ± 22.2	345.5 ± 19.1	340.5 ± 11.7
Overall Mean of Body Size		326.4 ± 14.4	296.2 ± 14.9	326.4 ± 15.1	316.3 ± 5.0

[a] See Figure 12 and Table 3, respectively, for cage profile and protein sequence.

[b] Cage profile effect (P < 0.01).

Table A3. Least square means of the ultimate shear force (N) of the humerus in a 72 week old caged layer.

Cage Profile[a]	Juvenile Protein Sequence[a]	Body Size			Mean of Diet
		Small	Medium	Large	
Low	Control	294.8 ± 28.8	271.3 ± 28.8	284.2 ± 31.5	284.7 ± 17.2[b,d]
	One	255.8 ± 28.8	237.2 ± 28.8	244.7 ± 78.8	245.9 ± 16.6
	Two	195.7 ± 31.5	364.2 ± 24.9	292.8 ± 78.8	284.3 ± 16.5
	Three	243.2 ± 28.8	248.2 ± 31.5	299.5 ± 28.8	263.6 ± 17.2
	Mean of Body Size	248.3 ± 14.7	280.2 ± 14.3	280.3 ± 14.7	269.6 ± 8.4[c]
High	Control	323.2 ± 28.8	369.6 ± 28.8	364.1 ± 26.6	352.3 ± 16.2
	One	292.8 ± 28.8	334.0 ± 29.8	291.0 ± 28.8	305.9 ± 16.6
	Two	309.8 ± 26.6	361.4 ± 35.3	425.9 ± 28.8	365.7 ± 17.6
	Three	283.6 ± 28.8	263.4 ± 26.6	316.9 ± 28.8	287.9 ± 16.2
	Mean of Body Size	302.4 ± 14.1	332.1 ± 15.0	349.5 ± 14.1	328.0 ± 8.4
Overall Mean of Body Size		275.3 ± 10.2[d]	306.2 ± 10.4	314.9 ± 10.2	299.8 ± 3.8

[a] See Figure 12 and Table 3, respectively, for cage profile and protein sequence.

[b] Protein sequence effect ($P < 0.01$).

[c] Cage profile effect ($P < 0.01$).

[d] Protein sequence - body size interaction ($P < 0.05$).

Table A4. Least square means of the ultimate shear force (N) of the femur in a 72 week old caged layer.

Cage Profile[a]	Juvenile Protein Sequence[a]	Body Size			Mean of Diet
		Small	Medium	Large	
Low	Control	311.4 ± 53.7	361.0 ± 53.7	479.3 ± 65.8	383.9 ± 33.5
	One	316.7 ± 58.8	307.3 ± 53.7	327.7 ± 53.7	317.2 ± 32.0
	Two	268.7 ± 58.8	376.7 ± 46.5	335.8 ± 53.7	327.0 ± 30.8
	Three	274.7 ± 53.7	348.7 ± 58.8	587.7 ± 53.7	403.7 ± 32.0
	Mean of Body Size	292.8 ± 28.1	348.4 ± 26.6	432.6 ± 28.4	358.0 ± 16.0
High	Control	316.2 ± 53.7	362.5 ± 53.7	379.9 ± 46.5	349.9 ± 29.7
	One	362.6 ± 49.7	306.2 ± 53.7	433.3 ± 53.7	367.4 ± 30.3
	Two	356.5 ± 49.7	378.7 ± 65.8	429.6 ± 53.7	388.3 ± 32.8
	Three	286.5 ± 53.7	264.0 ± 49.7	404.8 ± 53.7	318.0 ± 30.3
	Mean of Body Size	330.4 ± 25.8	327.8 ± 28.0	409.7 ± 26.0	356.0 ± 15.4
Overall Mean of Body Size		311.6 ± 19.1[b]	338.1 ± 19.4	421.1 ± 19.3	357.0 ± 6.5

[a] See Figure 12 and Table 3, respectively, for cage profile and protein sequence.

[b] Body size effect ($P < 0.01$).

Table A5. Least square means of the ultimate shear force (N) of the tibia in a 72 week old caged layer.

Cage Profile[a]	Juvenile Protein Sequence[a]	Body Size			Mean of Diet
		Small	Medium	Large	
Low	Control	396.6 ± 55.4	296.5 ± 55.4	506.0 ± 67.9	399.7 ± 34.6
	One	352.9 ± 55.4	431.5 ± 48.0	356.4 ± 48.0	380.3 ± 29.2
	Two	327.7 ± 55.4	452.6 ± 67.9	469.3 ± 55.4	416.5 ± 34.6
	Three	288.0 ± 67.9	491.5 ± 96.0	488.2 ± 67.9	472.6 ± 45.3
	Mean of Body Size	341.3 ± 29.4	418.0 ± 34.6	454.9 ± 30.2	404.8 ± 18.2
High	Control	384.8 ± 55.4	374.4 ± 55.4	354.2 ± 48.0	371.1 ± 30.7
	One	430.7 ± 55.4	361.4 ± 67.9	569.3 ± 67.9	453.8 ± 36.9
	Two	341.3 ± 29.4	418.0 ± 34.6	454.9 ± 30.2	404.8 ± 18.2
	Three	274.1 ± 48.0	470.2 ± 42.9	474.8 ± 48.0	406.4 ± 26.8
	Mean of Body Size	351.0 ± 26.8	413.0 ± 27.2	466.4 ± 27.7	410.1 ± 15.7
Overall Mean of Body Size		346.2 ± 19.9[b]	415.5 ± 22.0	460.6 ± 20.5	407.5 ± 7.3

[a] See Figure 12 and Table 3, respectively, for cage profile and protein sequence.

[b] Body size effect ($P < 0.01$).

Table A6. Least square means of the ultimate shear force (N) of the tarsometatarsus in a 72 week old caged layer.

Cage Profile[a]	Juvenile Protein Sequence[a]	Body Size			Mean of Diet
		Small	Medium	Large	
Low	Control	351.4 ± 39.1	409.2 ± 42.9	383.1 ± 47.9	381.2 ± 25.1
	One	389.6 ± 39.1	335.8 ± 36.2	409.0 ± 39.1	378.1 ± 22.1
	Two	354.9 ± 36.2	419.2 ± 33.9	418.9 ± 39.1	397.7 ± 21.1
	Three	339.9 ± 39.1	321.1 ± 42.9	506.4 ± 39.1	386.5 ± 23.4
	Mean of Body Size	358.9 ± 19.2	371.4 ± 19.6	429.3 ± 20.7	386.5 ± 11.5
High	Control	359.9 ± 39.1	368.3 ± 36.2	490.7 ± 33.9	439.7 ± 21.1
	One	401.5 ± 39.1	378.5 ± 42.9	373.6 ± 39.1	384.5 ± 23.4
	Two	404.3 ± 42.9	407.0 ± 47.99	521.6 ± 39.1	444.3 ± 25.1
	Three	350.5 ± 42.9	367.0 ± 36.2	413.3 ± 39.1	376.9 ± 22.8
	Mean of Body Size	379.1 ± 20.5	405.2 ± 20.5	449.8 ± 18.9	376.9 ± 22.8
Overall Mean of Body Size		369.0 ± 14.1[b]	388.3 ± 14.2	439.6 ± 14.1	389.9 ± 4.7

[a] See Figure 12 and Table 3, respectively, for cage profile and protein sequence.

[b] Body size effect ($P < 0.05$).

Table A7. Least square means of the ultimate shear stress (MPa) of the radius in a 72 week old caged layer.

Cage Profile[a]	Juvenile Protein Sequence[a]	Body Size			
		Small	Medium	Large	Mean of Diet
Low	Control	29.5 ± 3.1	28.2 ± 3.1	35.4 ± 3.1	31.0 ± 1.8[b]
	One	31.2 ± 3.1	27.6 ± 2.6	25.0 ± 2.6	27.9 ± 1.6
	Two	29.6 ± 3.1	30.2 ± 3.7	22.5 ± 3.1	27.5 ± 1.9
	Three	25.3 ± 3.7	37.6 ± 5.3	23.4 ± 3.7	28.0 ± 2.5
	Mean of Body Size	28.9 ± 1.6	30.9 ± 1.9	26.6 ± 1.5	28.8 ± 1.0
High	Control	31.5 ± 3.1	19.6 ± 3.1	35.6 ± 3.1	28.9 ± 1.8
	One	34.6 ± 3.1	26.0 ± 3.7	24.5 ± 3.7	28.4 ± 2.1
	Two	31.9 ± 3.1	30.9 ± 2.6	33.9 ± 3.1	32.3 ± 1.7
	Three	28.3 ± 2.6	33.2 ± 2.4	24.2 ± 2.6	28.6 ± 1.5
	Mean of Body Size	31.5 ± 1.5	27.5 ± 1.5	29.5 ± 1.6	29.5 ± 0.9
Overall Mean of Body Size		30.2 ± 1.1[b]	29.2 ± 1.2	28.1 ± 1.1	29.2 ± 0.4

[a] See Figure 12 and Table 3, respectively, for cage profile and protein sequence.

[b] Protein sequence - body size interaction ($P < 0.01$).

Table A8. Least square means of the ultimate shear stress (MPa) of the ulna in a 72 week old caged layer.

Cage Profile[a]	Juvenile Protein Sequence[a]	Body Size			Mean of Diet
		Small	Medium	Large	
Low	Control	20.0 ± 3.3	17.8 ± 3.0	20.5 ± 3.6	19.4 ± 1.9
	One	19.1 ± 3.0	14.9 ± 3.0	15.9 ± 4.2	16.6 ± 2.0
	Two	16.1 ± 3.3	18.1 ± 2.4	18.2 ± 3.0	17.4 ± 1.7
	Three	20.3 ± 3.0	16.5 ± 3.3	25.6 ± 2.7	20.8 ± 1.8
	Mean of Body Size	18.6 ± 1.5	16.8 ± 1.4	20.0 ± 1.7	18.6 ± 0.9
High	Control	21.8 ± 2.7	20.7 ± 3.0	22.1 ± 2.6	21.5 ± 1.6
	One	23.6 ± 3.0	18.0 ± 3.0	16.2 ± 2.4	19.3 ± 1.6
	Two	18.4 ± 2.7	18.0 ± 4.2	21.0 ± 3.0	19.1 ± 2.0
	Three	18.4 ± 3.0	16.1 ± 2.7	19.8 ± 3.3	18.1 ± 1.8
	Mean of Body Size	20.6 ± 1.4	18.2 ± 1.6	19.8 ± 1.4	19.5 ± 0.9
Overall Mean of Body Size		19.7 ± 1.1	17.5 ± 1.1	19.9 ± 1.1	19.1 ± 0.4

[a] See Figure 12 and Table 3, respectively, for cage profile and protein sequence.

Table A9. Least square means of the ultimate shear stress (MPa) of the humerus in a 72 week old caged layer.

Cage Profile[a]	Juvenile Protein Sequence[a]	Body Size			
		Small	Medium	Large	Mean of Diet
Low	Control	11.7 ± 1.1	8.6 ± 1.1	14.0 ± 1.2	11.4 ± 0.7[b]
	One	10.7 ± 1.1	10.5 ± 1.1	9.9 ± 1.1	10.3 ± 0.6
	Two	7.5 ± 1.2	13.0 ± 0.9	12.2 ± 1.1	10.9 ± 0.6
	Three	11.3 ± 1.1	9.0 ± 1.2	8.9 ± 1.1	9.7 ± 0.7
	Mean of Body Size	10.3 ± 0.5	10.3 ± 0.5	11.2 ± 0.5	10.6 ± 0.3
High	Control	13.2 ± 1.1	13.1 ± 1.1	12.9 ± 1.0	13.1 ± 0.6
	One	12.0 ± 1.1	11.9 ± 1.1	11.7 ± 1.1	11.9 ± 0.6
	Two	9.8 ± 1.0	15.2 ± 1.3	16.4 ± 1.1	13.8 ± 0.7
	Three	9.5 ± 1.3	9.8 ± 1.0	10.8 ± 1.1	10.0 ± 0.6
	Mean of Body Size	11.1 ± 0.5	12.5 ± 0.5	12.9 ± 0.5	12.2 ± 0.3
Overall Mean of Body Size		10.7 ± 0.4[b]	11.4 ± 0.4	12.1 ± 0.4	11.4 ± 0.2

[a] See Figure 12 and Table 3, respectively, for cage profile and protein sequence.

[b] Protein sequence - body size interaction ($P < 0.01$).

Table A10. Least square means of the ultimate shear stress (MPa) of the femur in a 72 week old caged layer.

Cage Profile[a]	Juvenile Protein Sequence[a]	Body Size			Mean of Diet
		Small	Medium	Large	
Low	Control	11.5 ± 1.5	12.2 ± 1.5	14.8 ± 1.8	13.2 ± 0.9
	One	14.7 ± 1.6	8.8 ± 1.5	10.4 ± 1.5	11.3 ± 0.9
	Two	10.1 ± 1.6	10.1 ± 1.3	11.0 ± 1.5	10.4 ± 0.9
	Three	9.8 ± 1.5	13.0 ± 1.6	15.0 ± 1.5	12.6 ± 0.9
	Mean of Body Size	11.5 ± 0.7	11.0 ± 0.7	13.0 ± 0.7	11.9 ± 0.4
High	Control	11.6 ± 1.5	12.1 ± 1.5	10.5 ± 1.3	11.4 ± 0.8
	One	11.9 ± 1.3	9.4 ± 1.5	10.6 ± 1.5	10.6 ± 0.8
	Two	12.5 ± 1.3	9.0 ± 1.8	12.4 ± 1.5	11.3 ± 0.9
	Three	10.0 ± 1.5	8.7 ± 1.3	10.1 ± 1.5	9.6 ± 0.8
	Mean of Body Size	11.5 ± 0.7	9.8 ± 0.7	10.9 ± 0.7	10.7 ± 0.4
Overall Mean of Body Size		11.5 ± 0.5	10.4 ± 0.5	12.0 ± 0.5	11.3 ± 0.3

[a] See Figure 12 and Table 3, respectively, for cage profile and protein sequence.

Table A11. Least square means of the ultimate shear stress (MPa) of the tibia in a 72 week old caged layer.

Cage Profile[a]	Juvenile Protein Sequence[a]	Body Size			Mean of Diet
		Small	Medium	Large	
Low	Control	14.3 ± 2.6	11.8 ± 2.6	15.7 ± 3.2	13.9 ± 1.7
	One	13.4 ± 2.6	17.8 ± 2.3	13.1 ± 2.3	14.8 ± 1.4
	Two	12.0 ± 2.6	15.3 ± 3.2	17.1 ± 2.6	14.8 ± 1.7
	Three	12.1 ± 3.2	14.5 ± 4.6	17.9 ± 3.2	14.8 ± 2.1
	Mean of Body Size	12.9 ± 1.4	14.8 ± 1.6	15.9 ± 1.4	14.6 ± 0.9
High	Control	11.5 ± 2.6	12.9 ± 2.6	16.1 ± 2.3	13.5 ± 1.5
	One	17.9 ± 2.6	13.9 ± 3.2	13.8 ± 3.2	15.2 ± 1.8
	Two	10.8 ± 2.6	13.7 ± 2.3	16.1 ± 2.6	13.5 ± 1.5
	Three	10.8 ± 2.3	14.3 ± 2.0	14.6 ± 2.3	13.2 ± 1.3
	Mean of Body Size	12.7 ± 1.2	13.7 ± 1.3	15.2 ± 1.3	13.9 ± 0.8
Overall Mean of Body Size		12.8 ± 0.9	14.3 ± 1.1	15.5 ± 1.0	14.4 ± 0.4

[a] See Figure 12 and Table 3, respectively, for cage profile and protein sequence.

Table A12. Least square means of the ultimate shear stress (MPa) of the tarsometatarsus in a 72 week old caged layer.

Cage Profile[a]	Juvenile Protein Sequence[a]	Body Size			Mean of Diet
		Small	Medium	Large	
Low	Control	11.9 ± 1.2	11.1 ± 1.3	12.4 ± 1.4	11.8 ± 0.8
	One	11.0 ± 1.2	8.9 ± 1.1	12.7 ± 1.2	10.8 ± 0.7
	Two	11.6 ± 1.1	11.2 ± 1.0	12.3 ± 1.2	11.7 ± 0.6
	Three	11.7 ± 1.2	9.0 ± 1.3	13.8 ± 1.2	11.5 ± 0.7
	Mean of Body Size	11.5 ± 0.5	10.1 ± 0.6	12.8 ± 1.0	11.5 ± 0.4
High	Control	11.5 ± 1.2	11.8 ± 1.1	12.8 ± 1.0	12.0 ± 0.6
	One	14.1 ± 1.2	9.7 ± 1.3	12.2 ± 1.2	12.0 ± 0.7
	Two	11.3 ± 1.3	9.3 ± 1.4	12.3 ± 1.2	10.9 ± 0.8
	Three	10.8 ± 1.3	10.3 ± 1.1	9.4 ± 1.2	10.1 ± 0.7
	Mean of Body Size	11.9 ± 0.6	10.3 ± 0.6	11.7 ± 0.5	11.3 ± 0.4
Overall Mean of Body Size		11.7 ± 0.4	10.2 ± 0.4	12.2 ± 0.4	11.4 ± 0.1

[a] See Figure 12 and Table 3, respectively, for cage profile and protein sequence.

APPENDIX B

Least square means of the bending properties of the radius, ulna, humerus, femur, tibia, and tarsometatarsus of 72 week old caged layers.

Table B1. Least square means of the ultimate bending force (N) of the radius in a 72 week old caged layer.

Cage Profile[a]	Juvenile Protein Sequence[a]	Body Size			Mean of Diet
		Small	Medium	Large	
Low	Control	34.8 ± 3.4	32.9 ± 2.9	41.1 ± 3.4	36.3 ± 1.9
	One	29.1 ± 3.4	39.6 ± 4.2	37.6 ± 2.6	35.4 ± 2.0
	Two	37.2 ± 3.4	44.5 ± 4.2	39.3 ± 3.4	40.3 ± 2.1
	Three	32.3 ± 4.2	32.5 ± 5.9	37.8 ± 4.2	34.2 ± 2.8
	Mean of Body Size	33.3 ± 1.8	37.4 ± 2.2	39.0 ± 1.7	36.6 ± 1.1[b]
High	Control	36.8 ± 3.4	40.5 ± 4.2	39.0 ± 3.4	38.7 ± 2.1
	One	32.5 ± 3.4	42.5 ± 2.9	46.3 ± 5.9	40.4 ± 2.5
	Two	39.6 ± 3.4	42.1 ± 2.9	38.7 ± 3.4	40.1 ± 1.9
	Three	41.3 ± 2.9	38.7 ± 2.6	45.2 ± 2.9	41.7 ± 1.7
	Mean of Body Size	37.5 ± 1.6	41.0 ± 1.6	42.3 ± 2.0	40.2 ± 1.0
Overall Mean of Body Size		35.4 ± 1.2[c]	39.2 ± 1.4	40.6 ± 1.3	38.4 ± 0.3

[a] See Figure 12 and Table 3, respectively, for cage profile and protein sequence.

[b] Cage profile effect ($P < 0.05$).

[c] Body size effect ($P < 0.05$).

Table B2. Least square means of the ultimate bending force (N) of the ulna in a 72 week old caged layer.

Cage Profile[a]	Juvenile Protein Sequence[a]	Body Size Small	Body Size Medium	Body Size Large	Mean of Diet
Low	Control	88.5 ± 8.7	87.9 ± 12.4	112.7 ± 10.1	96.4 ± 6.1
	One	87.0 ± 10.1	87.5 ± 10.1	97.5 ± 8.7	90.7 ± 5.6
	Two	107.5 ± 8.7	112.7 ± 10.1	89.0 ± 10.1	103.1 ± 5.6
	Three	93.9 ± 12.4	75.6 ± 17.5	114.5 ± 10.1	94.6 ± 7.9
	Mean of Body Size	94.2 ± 5.0	90.9 ± 6.4	103.4 ± 4.9	96.2 ± 3.2[b]
High	Control	94.5 ± 12.4	103.6 ± 8.7	109.4 ± 10.1	102.5 ± 6.1
	One	89.7 ± 10.1	99.2 ± 10.1	114.5 ± 12.4	101.1 ± 6.3
	Two	108.3 ± 12.4	102.3 ± 10.1	121.3 ± 10.1	110.6 ± 6.3
	Three	106.2 ± 8.7	200.1 ± 7.8	117.1 ± 10.1	107.8 ± 5.2
	Mean of Body Size	99.7 ± 5.5	101.3 ± 4.6	115.6 ± 5.3	105.5 ± 3.0
Overall Mean of Body Size		97.0 ± 3.7[c]	96.1 ± 4.0	109.5 ± 3.6	100.9 ± 1.2

[a] See Figure 12 and Table 3, respectively, for cage profile and protein sequence.

[b] Cage profile effect ($P < 0.05$).

[c] Body size effect ($P < 0.05$).

Table B3. Least square means of the ultimate bending force (N) of the humerus in a 72 week old caged layer.

Cage Profile[a]	Juvenile Protein Sequence[a]	Body Size Small	Body Size Medium	Body Size Large	Mean of Diet
Low	Control	121.1 ± 18.0	124.5 ± 18.0	142.3 ± 18.0	129.3 ± 10.4
	One	125.3 ± 18.0	114.9 ± 18.0	148.2 ± 15.6	129.5 ± 10.0
	Two	147.0 ± 22.1	153.9 ± 22.1	183.5 ± 22.1	161.5 ± 12.0
	Three	140.7 ± 22.1	125.2 ± 22.1	184.0 ± 15.6	149.9 ± 11.7
	Mean of Body Size	133.5 ± 10.0	129.6 ± 10.0	164.5 ± 9.0	142.6 ± 5.6[b]
High	Control	153.5 ± 18.0	146.8 ± 18.0	190.9 ± 18.0	160.1 ± 10.4
	One	117.9 ± 18.0	194.9 ± 18.0	164.6 ± 22.1	159.1 ± 11.2
	Two	157.4 ± 15.6	176.3 ± 15.6	197.9 ± 15.6	177.2 ± 9.0
	Three	169.6 ± 15.6	162.4 ± 15.6	170.1 ± 22.1	167.3 ± 10.4
	Mean of Body Size	149.6 ± 8.4	169.6 ± 8.4	178.4 ± 9.8	166.0 ± 5.2
Overall Mean of Body Size		141.5 ± 6.6[c]	149.8 ± 6.6	171.5 ± 6.7	154.3 ± 2.1

[a] See Figure 12 and Table 3, respectively, for cage profile and protein sequence.

[b] Cage profile effect ($P < 0.05$).

[c] Body size effect ($P < 0.05$).

Table B4. Least square means of the ultimate bending force (N) of the femur in a 72 week old caged layer.

Cage Profile[a]	Juvenile Protein Sequence[a]	Body Size			Mean of Diet
		Small	Medium	Large	
Low	Control	176.8 ± 19.3	186.8 ± 27.3	265.9 ± 22.3	209.8 ± 13.4
	One	173.5 ± 19.3	177.2 ± 22.3	172.4 ± 19.3	174.3 ± 11.8
	Two	163.1 ± 22.3	199.3 ± 22.3	205.4 ± 22.3	189.2 ± 12.9
	Three	155.7 ± 38.6	227.9 ± 27.3	226.9 ± 22.3	203.5 ± 17.5
	Mean of Body Size	167.3 ± 13.0	197.8 ± 12.4	217.6 ± 10.8	194.2 ± 7.0
High	Control	179.0 ± 27.3	188.5 ± 19.3	199.4 ± 22.3	189.0 ± 13.4
	One	145.7 ± 27.3	190.0 ± 22.3	263.6 ± 27.3	199.8 ± 14.9
	Two	216.5 ± 22.3	209.8 ± 22.3	231.3 ± 22.3	219.2 ± 12.9
	Three	217.9 ± 17.2	211.8 ± 19.3	214.3 ± 22.3	214.7 ± 11.4
	Mean of Body Size	189.8 ± 11.9	200.1 ± 10.4	227.1 ± 11.8	205.7 ± 6.6
Overall Mean of Body Size		178.5 ± 8.9[b]	198.9 ± 8.1	222.3 ± 8.0	200.0 ± 2.5

[a] See Figure 12 and Table 3, respectively, for cage profile and protein sequence.

[b] Body size effect ($P < 0.01$).

Table B5. Least square means of the ultimate bending force (N) of the tibia in a 72 week old caged layer.

Cage Profile[a]	Juvenile Protein Sequence[a]	Body Size			Mean of Diet
		Small	Medium	Large	
Low	Control	136.8 ± 24.7	167.5 ± 20.2	234.3 ± 20.2	179.5 ± 12.6
	One	149.0 ± 20.2	166.8 ± 20.2	165.9 ± 15.6	160.6 ± 10.9
	Two	146.2 ± 17.5	186.8 ± 24.7	167.6 ± 20.2	166.9 ± 12.1
	Three	147.9 ± 24.7	173.5 ± 24.7	189.1 ± 24.7	170.1 ± 14.3
	Mean of Body Size	145.0 ± 11.0	173.7 ± 11.2	139.2 ± 10.2	169.3 ± 6.3
High	Control	154.0 ± 17.5	160.1 ± 20.2	180.6 ± 20.2	164.9 ± 11.2
	One	158.2 ± 20.2	167.6 ± 20.2	182.4 ± 35.0	169.4 ± 15.1
	Two	185.7 ± 24.7	185.7 ± 17.5	195.7 ± 20.2	189.1 ± 12.1
	Three	170.7 ± 17.5	173.5 ± 17.5	169.6 ± 17.5	171.3 ± 10.1
	Mean of Body Size	167.2 ± 10.1	171.7 ± 9.4	182.1 ± 12.1	173.7 ± 6.1
Overall Mean of Body Size		156.0 ± 7.5[b]	172.7 ± 7.4	185.6 ± 7.9	171.5 ± 2.2

[a] See Figure 12 and Table 3, respectively, for cage profile and protein sequence.

[b] Body size effect ($P < 0.05$).

Table B6. Least square means of the ultimate bending force (N) of the tarsometatarsus in a 72 week old caged layer.

Cage Profile[a]	Juvenile Protein Sequence[a]	Body Size			Mean of Diet
		Small	Medium	Large	
Low	Control	148.1 ± 16.1	154.6 ± 19.8	192.8 ± 19.8	165.1 ± 10.8[b]
	One	158.6 ± 16.1	221.3 ± 19.8	147.3 ± 14.0	175.8 ± 9.7
	Two	172.0 ± 16.1	196.5 ± 16.1	167.6 ± 16.1	178.6 ± 9.3
	Three	155.4 ± 16.1	169.0 ± 28.0	182.4 ± 19.8	168.9 ± 12.6
	Mean of Body Size	158.5 ± 8.0	185.3 ± 10.7	172.5 ± 8.8	172.1 ± 5.3
High	Control	183.9 ± 16.1	180.4 ± 14.0	195.6 ± 14.0	186.6 ± 8.5
	One	142.0 ± 16.1	208.8 ± 14.0	166.8 ± 19.8	172.5 ± 9.7
	Two	210.6 ± 16.1	229.1 ± 16.1	185.3 ± 16.1	208.3 ± 9.3
	Three	201.0 ± 16.1	181.9 ± 12.5	188.5 ± 14.0	189.5 ± 8.3
	Mean of Body Size	184.4 ± 8.0[b]	200.0 ± 7.1	184.1 ± 8.0	189.5 ± 4.5
Overall Mean of Body Size		171.5 ± 5.7	192.7 ± 6.4	178.3 ± 6.0	180.8 ± 2.0

[a] See Figure 12 and Table 3, respectively, for cage profile and protein sequence.

[b] Protein sequence - body size interaction (P < 0.05).

Table B7. Least square means of the ultimate bending stress (MPa) of the radius in a 72 week old caged layer.

Cage Profile[a]	Juvenile Protein Sequence[a]	Body Size			Mean of Diet
		Small	Medium	Large	
Low	Control	188.2 ± 15.7	181.7 ± 13.6	232.4 ± 15.7	200.7 ± 8.7
	One	221.1 ± 15.7	193.9 ± 19.3	186.4 ± 12.2	200.5 ± 9.3
	Two	199.1 ± 15.7	212.5 ± 19.3	199.9 ± 15.7	203.9 ± 9.8
	Three	159.3 ± 19.3	185.3 ± 27.3	181.0 ± 19.3	195.0 ± 12.9
	Mean of Body Size	191.9 ± 8.3	193.4 ± 10.2	199.9 ± 7.9	195.0 ± 5.1
High	Control	206.1 ± 15.7	172.9 ± 19.3	187.7 ± 15.7	188.9 ± 9.8
	One	224.5 ± 15.7	226.8 ± 13.6	223.4 ± 27.3	224.9 ± 11.5
	Two	222.3 ± 15.7	222.8 ± 13.6	199.1 ± 15.7	214.7 ± 8.7
	Three	208.8 ± 13.6	206.4 ± 12.2	188.3 ± 13.6	201.2 ± 7.6
	Mean of Body Size	215.4 ± 7.6	207.2 ± 7.4	199.6 ± 9.4	207.4 ± 4.8
Overall Mean of Body Size		203.7 ± 5.7	200.3 ± 6.3	199.8 ± 6.2	201.2 ± 2.2

[a] See Figure 12 and Table 3, respectively, for cage profile and protein sequence.

Table B8. Least square means of the ultimate bending stress (MPa) of the ulna in a 72 week old caged layer.

Cage Profile[a]	Juvenile Protein Sequence[a]	Body Size			Mean of Diet
		Small	Medium	Large	
Low	Control	206.2 ± 16.3	170.6 ± 23.1	173.7 ± 18.9	183.5 ± 11.4
	One	182.4 ± 18.9	170.3 ± 18.9	180.9 ± 16.3	177.9 ± 10.5
	Two	217.5 ± 16.3	214.3 ± 18.9	177.9 ± 18.9	203.2 ± 10.5
	Three	235.1 ± 23.1	149.5 ± 32.7	171.3 ± 18.9	185.3 ± 14.8
	Mean of Body Size	210.3 ± 9.4	176.2 ± 12.0	175.9 ± 9.1	187.5 ± 6.0
High	Control	181.8 ± 23.1	167.0 ± 16.3	168.8 ± 18.9	172.5 ± 11.4
	One	204.9 ± 18.9	206.6 ± 18.9	182.5 ± 23.1	193.0 ± 11.8
	Two	196.7 ± 23.1	187.9 ± 18.9	230.8 ± 18.9	205.1 ± 11.8
	Three	200.4 ± 16.3	194.4 ± 14.6	187.8 ± 18.9	194.2 ± 9.7
	Mean of Body Size	195.9 ± 10.3	188.9 ± 8.6	192.5 ± 10.0	192.4 ± 5.6
Overall Mean of Body Size		203.9 ± 7.0	182.6 ± 7.4	184.2 ± 6.8	190.0 ± 2.3

[a] See Figure 12 and Table 3, respectively, for cage profile and protein sequence.

Table B9. Least square means of the ultimate shear stress (MPa) of the humerus in a 72 week old caged layer.

Cage Profile[a]	Juvenile Protein Sequence[a]	Body Size			Mean of Diet
		Small	Medium	Large	
Low	Control	90.1 ± 12.1	88.0 ± 12.1	81.5 ± 12.1	86.6 ± 7.0
	One	78.4 ± 12.1	78.5 ± 12.1	86.7 ± 10.5	81.2 ± 6.7
	Two	114.5 ± 14.9	93.3 ± 14.9	110.9 ± 14.9	106.2 ± 8.6
	Three	91.9 ± 14.9	85.4 ± 14.9	116.5 ± 10.5	97.9 ± 7.8
	Mean of Body Size	93.7 ± 6.8	86.3 ± 6.8	98.9 ± 6.0	93.0 ± 3.8[b]
High	Control	97.2 ± 12.1	113.7 ± 12.1	103.1 ± 12.1	104.7 ± 7.0
	One	99.6 ± 12.1	117.9 ± 12.1	96.3 ± 14.9	104.6 ± 7.6
	Two	103.1 ± 10.5	121.7 ± 10.5	111.9 ± 10.5	112.2 ± 6.0
	Three	106.8 ± 10.5	97.5 ± 10.5	87.6 ± 14.9	97.3 ± 7.0
	Mean of Body Size	101.7 ± 5.6	112.7 ± 5.6	99.3 ± 4.5	104.7 ± 3.5
Overall Mean of Body Size		97.7 ± 4.4	99.5 ± 4.4	99.3 ± 4.5	98.9 ± 1.5

[a] See Figure 12 and Table 3, respectively, for cage profile and protein sequence.

[b] Cage profile effect ($P < 0.05$).

Table B10. Least square means of the ultimate bending stress (MPa) of the femur in a 72 week old caged layer.

Cage Profile[a]	Juvenile Protein Sequence[a]	Body Size			Mean of Diet
		Small	Medium	Large	
Low	Control	107.1 ± 14.6	98.7 ± 20.7	142.7 ± 16.7	116.2 ± 10.2
	One	121.2 ± 14.6	88.5 ± 16.9	91.9 ± 14.6	100.5 ± 8.9
	Two	105.9 ± 16.9	92.4 ± 16.9	104.2 ± 16.9	100.8 ± 9.7
	Three	130.6 ± 29.2	115.2 ± 20.7	97.4 ± 16.9	114.3 ± 13.2
	Mean of Body Size	116.2 ± 9.9	98.7 ± 9.4	109.1 ± 8.1	107.9 ± 5.3
High	Control	105.2 ± 20.7	99.4 ± 14.6	117.6 ± 16.9	107.4 ± 10.2
	One	97.1 ± 20.7	115.2 ± 16.9	116.1 ± 20.7	109.4 ± 11.3
	Two	120.8 ± 16.9	110.9 ± 16.9	114.8 ± 16.9	115.5 ± 9.7
	Three	148.8 ± 13.0	113.2 ± 14.6	72.3 ± 16.9	111.4 ± 8.6
	Mean of Body Size	117.9 ± 7.0	109.7 ± 7.9	105.2 ± 8.9	110.9 ± 5.0
Overall Mean of Body Size		117.0 ± 6.7	104.1 ± 6.2	107.1 ± 6.1	109.4 ± 1.9

[a] See Figure 12 and Table 3, respectively, for cage profile and protein sequence.

Table B11. Least square means of the ultimate bending stress (MPa) of the tibia in a 72 week old caged layer.

Cage Profile[a]	Juvenile Protein Sequence[a]	Body Size			Mean of Diet
		Small	Medium	Large	
Low	Control	156.6 ± 31.9	212.0 ± 26.0	211.2 ± 26.0	193.3 ± 16.2
	One	199.8 ± 26.0	171.2 ± 26.0	156.3 ± 20.1	175.8 ± 14.0
	Two	195.1 ± 22.5	203.7 ± 31.9	180.9 ± 26.0	193.2 ± 15.7
	Three	191.5 ± 31.9	188.1 ± 31.9	196.4 ± 31.9	192.0 ± 18.4
	Mean of Body Size	185.8 ± 14.1	193.8 ± 14.5	186.2 ± 13.1	188.6 ± 8.1
High	Control	175.5 ± 22.5	145.4 ± 26.0	201.4 ± 26.0	174.1 ± 14.4
	One	233.3 ± 26.0	207.0 ± 26.0	142.5 ± 45.1	194.3 ± 19.4
	Two	232.2 ± 31.9	184.7 ± 22.5	232.9 ± 26.0	216.6 ± 15.7
	Three	212.5 ± 22.5	192.2 ± 22.5	144.8 ± 22.5	183.2 ± 13.0
	Mean of Body Size	213.4 ± 13.0	182.3 ± 12.1	180.4 ± 15.6	192.0 ± 7.9
Overall Mean of Body Size		199.6 ± 9.6	188.0 ± 9.5	183.3 ± 10.2	190.3 ± 3.3

[a] See Figure 12 and Table 3, respectively, for cage profile and protein sequence.

Table B12. Least square means of the ultimate bending stress (MPa) of the tarsometatarsus in a 72 week old caged layer.

Cage Profile[a]	Juvenile Protein Sequence[a]	Body Size			Mean of Diet
		Small	Medium	Large	
Low	Control	62.0 ± 7.7	58.0 ± 9.4	71.7 ± 9.4	63.9 ± 5.1[c]
	One	75.8 ± 7.7	76.4 ± 9.4	56.1 ± 6.6	79.5 ± 4.7
	Two	70.7 ± 7.7	72.1 ± 7.7	57.6 ± 7.7	66.7 ± 4.4
	Three	76.8 ± 7.7	90.4 ± 13.3	49.8 ± 9.4	72.3 ± 6.0
	Mean of Body Size	71.3 ± 3.8	74.2 ± 5.0	58.8 ± 4.1	68.1 ± 2.5[b]
High	Control	76.0 ± 7.7	62.9 ± 6.6	65.0 ± 6.6	68.0 ± 4.1
	One	77.5 ± 7.7	87.2 ± 6.6	61.7 ± 9.4	75.5 ± 4.7
	Two	78.9 ± 7.7	71.5 ± 7.7	78.5 ± 7.7	76.3 ± 4.4
	Three	102.3 ± 7.7	77.1 ± 5.9	61.8 ± 6.6	80.4 ± 3.9
	Mean of Body Size	83.7 ± 3.8	74.7 ± 3.3	66.7 ± 3.8	75.0 ± 2.1
Overall Mean of Body Size		77.5 ± 2.7[b,c]	74.4 ± 3.1	62.8 ± 2.8	71.6 ± 1.0

[a] See Figure 12 and Table 3, respectively, for cage profile and protein sequence.

[b] Body size effect ($P < 0.01$).

[c] Protein sequence - body size interaction ($P < 0.05$).

Table B13. Least square means of the flexural modulus of elasticity (GPa) of the radius in a 72 week old caged layer.

Cage Profile[a]	Juvenile Protein Sequence[a]	Body Size			Mean of Diet
		Small	Medium	Large	
Low	Control	12.9 ± 1.1	12.5 ± 1.0	17.3 ± 1.1	14.2 ± 0.6
	One	13.6 ± 1.1	11.5 ± 1.4	13.1 ± 0.8	12.7 ± 0.7
	Two	12.4 ± 1.1	11.9 ± 1.4	11.6 ± 1.1	11.9 ± 0.7
	Three	8.5 ± 1.4	13.3 ± 2.0	12.1 ± 1.4	11.3 ± 0.9
	Mean of Body Size	11.9 ± 0.6	12.3 ± 0.7	13.5 ± 0.5	12.6 ± 0.4
High	Control	13.1 ± 1.1	11.3 ± 1.4	13.6 ± 1.1	12.7 ± 0.7
	One	13.7 ± 1.1	13.8 ± 1.0	14.4 ± 2.0	14.0 ± 0.8
	Two	13.7 ± 1.1	13.8 ± 1.0	13.2 ± 1.1	13.6 ± 0.6
	Three	12.5 ± 1.0	13.0 ± 0.8	12.0 ± 0.6	12.5 ± 0.5
	Mean of Body Size	13.2 ± 0.5	13.0 ± 0.5	13.3 ± 0.6	13.2 ± 0.3
Overall Mean of Body Size		12.5 ± 0.4	12.6 ± 0.5	13.4 ± 0.5	12.9 ± 0.2

[a] See Figure 12 and Table 3, respectively, for cage profile and protein sequence.

Table B14. Least square means of the flexural modulus of elasticity (GPa) of the ulna in a 72 week old caged layer.

Cage Profile[a]	Juvenile Protein Sequence[a]	Body Size			Mean of Diet
		Small	Medium	Large	
Low	Control	21.1 ± 2.0	15.5 ± 2.8	16.5 ± 2.3	17.7 ± 1.4
	One	14.8 ± 2.3	17.1 ± 2.3	17.4 ± 2.0	16.5 ± 1.3
	Two	23.1 ± 2.0	20.0 ± 2.3	17.9 ± 2.3	20.3 ± 1.3
	Three	21.9 ± 2.8	17.0 ± 4.0	16.9 ± 2.3	13.6 ± 1.8
	Mean of Body Size	20.2 ± 1.1	17.4 ± 1.4	17.2 ± 1.1	18.3 ± 0.7
High	Control	17.6 ± 2.8	15.1 ± 2.0	15.8 ± 2.3	16.1 ± 1.4
	One	18.2 ± 2.3	19.0 ± 2.3	17.0 ± 2.8	18.0 ± 1.4
	Two	18.5 ± 2.8	18.0 ± 2.3	24.2 ± 2.3	20.3 ± 1.4
	Three	18.8 ± 2.0	18.6 ± 1.8	20.1 ± 2.3	19.2 ± 1.2
	Mean of Body Size	18.3 ± 1.2	17.7 ± 1.0	19.3 ± 1.2	18.4 ± 0.7
Overall Mean of Body Size		19.2 ± 0.9	17.5 ± 0.9	18.2 ± 0.8	18.4 ± 0.3

[a] See Figure 12 and Table 3, respectively, for cage profile and protein sequence.

Table B15. Least square means of the flexural modulus of elasticity (GPa) of the humerus in a 72 week old caged layer.

Cage Profile[a]	Juvenile Protein Sequence[a]	Body Size: Small	Body Size: Medium	Body Size: Large	Mean of Diet
Low	Control	6.4 ± 0.9	6.4 ± 0.9	5.5 ± 0.9	6.1 ± 0.6[b]
	One	4.8 ± 0.9	7.6 ± 0.9	5.3 ± 0.8	5.9 ± 0.5
	Two	6.7 ± 1.1	5.1 ± 1.1	7.3 ± 1.1	6.3 ± 0.7
	Three	4.6 ± 1.1	4.6 ± 1.1	7.3 ± 0.8	5.5 ± 0.6
	Mean of Body Size	5.6 ± 0.5	5.9 ± 0.5	6.4 ± 0.4	6.0 ± 0.3
High	Control	6.6 ± 0.9	9.5 ± 0.9	4.8 ± 0.9	7.0 ± 0.6
	One	8.0 ± 0.9	7.9 ± 0.9	5.3 ± 0.8	7.1 ± 0.6
	Two	7.3 ± 0.8	6.6 ± 0.8	7.4 ± 0.8	7.1 ± 0.5
	Three	7.4 ± 0.8	6.0 ± 0.8	5.3 ± 1.1	6.2 ± 0.6
	Mean of Body Size	7.4 ± 0.4	0.5 ± 0.4	5.7 ± 0.5	6.8 ± 0.3
Overall Mean of Body Size		6.5 ± 0.4[b]	6.7 ± 0.4	6.0 ± 0.4	6.4 ± 0.1

[a] See Figure 12 and Table 3, respectively, for cage profile and protein sequence.

[b] Protein sequence - body size interaction ($P < 0.05$).

Table B16. Least square means of the flexural modulus of elasticity (GPa) of the femur in a 72 week old caged layer.

Cage Profile[a]	Juvenile Protein Sequence[a]	Body Size			Mean of Diet
		Small	Medium	Large	
Low	Control	8.2 ± 1.2	6.8 ± 1.7	11.1 ± 1.4	8.7 ± 0.9
	One	8.1 ± 1.2	6.9 ± 1.4	5.8 ± 1.2	6.9 ± 0.8
	Two	6.4 ± 1.4	7.3 ± 1.4	7.6 ± 1.4	7.1 ± 0.8
	Three	6.8 ± 2.5	7.0 ± 1.7	7.0 ± 1.4	6.9 ± 1.1
	Mean of Body Size	7.4 ± 0.8	7.0 ± 0.8	7.8 ± 0.7	7.4 ± 0.5
High	Control	8.5 ± 1.7	6.7 ± 1.2	7.0 ± 1.4	7.4 ± 0.9
	One	6.1 ± 1.7	9.4 ± 1.4	7.6 ± 1.7	7.7 ± 1.0
	Two	7.6 ± 1.4	8.5 ± 1.4	6.9 ± 1.4	7.7 ± 0.8
	Three	10.7 ± 1.1	7.8 ± 1.2	4.7 ± 1.4	7.7 ± 0.7
	Mean of Body Size	8.2 ± 0.7	8.1 ± 0.6	6.6 ± 0.7	7.6 ± 0.4
Overall Mean of Body Size		7.8 ± 0.6	7.5 ± 0.5	7.2 ± 0.5	7.5 ± 0.2

[a] See Figure 12 and Table 3, respectively, for cage profile and protein sequence.

Table B17. Least square means of the flexural modulus of elasticity (GPa) of the tibia in a 72 week old caged layer.

Cage Profile[a]	Juvenile Protein Sequence[a]	Body Size			Mean of Diet
		Small	Medium	Large	
Low	Control	15.5 ± 2.2	20.2 ± 1.8	15.0 ± 1.8	16.9 ± 1.1
	One	18.5 ± 1.8	17.3 ± 1.8	16.6 ± 1.4	17.5 ± 1.0
	Two	18.5 ± 1.5	19.8 ± 2.2	17.9 ± 1.8	18.8 ± 1.1
	Three	20.1 ± 2.2	18.5 ± 2.2	18.2 ± 2.2	18.9 ± 1.3
	Mean of Body Size	18.2 ± 1.0	18.9 ± 1.0	16.9 ± 0.9	18.0 ± 0.6
High	Control	17.7 ± 1.5	14.8 ± 1.8	18.2 ± 1.8	16.9 ± 1.0
	One	18.8 ± 1.84	20.0 ± 1.8	14.2 ± 3.1	17.7 ± 1.4
	Two	20.4 ± 2.2	16.5 ± 1.5	17.1 ± 1.8	18.0 ± 1.1
	Three	19.2 ± 1.5	17.4 ± 1.5	15.5 ± 1.5	17.4 ± 0.9
	Mean of Body Size	19.0 ± 0.9	17.2 ± 0.8	16.2 ± 1.1	17.5 ± 0.5
Overall Mean of Body Size		18.6 ± 0.2	18.0 ± 0.7	16.6 ± 0.7	17.8 ± 0.2

[a] See Figure 12 and Table 3, respectively, for cage profile and protein sequence.

Table B18. Least square means of the flexural modulus of elasticity (GPa) of the tarsometatarsus in a 72 week old caged layer.

Cage Profile[a]	Juvenile Protein Sequence[a]	Body Size			Mean of Diet
		Small	Medium	Large	
Low	Control	3.8 ± 0.5	3.0 ± 0.7	3.2 ± 0.7	3.3 ± 0.4
	One	4.7 ± 0.5	3.4 ± 0.7	3.1 ± 0.5	3.7 ± 0.3
	Two	4.0 ± 0.5	3.9 ± 0.5	3.1 ± 0.5	3.7 ± 0.3
	Three	4.8 ± 0.5	4.2 ± 1.0	2.7 ± 0.7	3.9 ± 0.2
	Mean of Body Size	4.3 ± 0.2	3.6 ± 0.3	3.0 ± 0.3	3.7 ± 0.2
High	Control	4.1 ± 0.5	3.2 ± 0.5	3.7 ± 0.5	3.7 ± 0.3
	One	4.8 ± 0.5	4.6 ± 0.5	3.1 ± 0.7	4.1 ± 0.4
	Two	4.0 ± 0.5	3.0 ± 0.5	4.1 ± 0.5	3.7 ± 0.3
	Three	6.3 ± 0.5	4.5 ± 0.4	2.8 ± 0.5	4.6 ± 0.3
	Mean of Body Size	4.8 ± 0.2	3.8 ± 0.2	3.4 ± 0.2	4.0 ± 0.2
Overall Mean of Body Size		4.6 ± 0.2[b]	3.7 ± 0.2	3.2 ± 0.2	3.8 ± 0.1

[a] See Figure 12 and Table 3, respectively, for cage profile and protein sequence.

[b] Body size effect ($P < 0.01$).

APPENDIX C

Least square means of the torsional properties of the radius and tibia of 72 week old caged layers.

Table C1. Least square means of the ultimate bending angle of twist (degrees) of the tibia in a 72 week old caged layer.

Cage Profile[a]	Juvenile Protein Sequence[a]	Body Size			Mean of Diet
		Small	Medium	Large	
Low	Control	31.4 ± 5.7	31.3 ± 8.1	43.0 ± 8.2	35.2 ± 4.3
	One	19.5 ± 6.6	23.4 ± 6.6	29.6 ± 6.6	24.2 ± 3.9
	Two	33.6 ± 6.6	23.7 ± 5.1	25.7 ± 5.7	27.7 ± 3.4
	Three	27.7 ± 6.6	25.5 ± 5.7	28.0 ± 5.7	27.0 ± 3.5
	Mean of Body Size	28.1 ± 2.3	26.0 ± 3.2	31.6 ± 3.3	28.5 ± 1.9
High	Control	22.8 ± 5.7	22.3 ± 6.6	26.1 ± 6.6	23.7 ± 3.7
	One	38.7 ± 8.2	22.9 ± 8.2	28.7 ± 5.7	30.1 ± 4.3
	Two	34.3 ± 5.7	25.8 ± 11.5	28.3 ± 5.7	29.5 ± 4.7
	Three	14.3 ± 8.2	25.6 ± 6.6	32.4 ± 6.6	24.1 ± 4.1
	Mean of Body Size	27.5 ± 3.5	24.2 ± 4.2	28.9 ± 3.1	26.9 ± 2.1
Overall Mean of Body Size		27.8 ± 2.4	25.1 ± 2.7	30.2 ± 2.3	27.7 ± 0.9

[a] See Figure 12 and Table 3, respectively, for cage profile and protein sequence.

Table C2. Least square means of the ultimate torque (N-m) of the tibia in a 72 week old caged layer.

Cage Profile[a]	Juvenile Protein Sequence[a]	Body Size			Mean of Diet
		Small	Medium	Large	
Low	Control	0.065 ± 0.015	0.078 ± 0.017	0.071 ± 0.017	0.077 ± 0.011
	One	0.043 ± 0.017	0.068 ± 0.017	0.071 ± 0.017	0.061 ± 0.010
	Two	0.066 ± 0.017	0.076 ± 0.013	0.071 ± 0.015	0.071 ± 0.009
	Three	0.039 ± 0.017	0.060 ± 0.015	0.067 ± 0.015	0.055 ± 0.009
	Mean of Body Size	0.053 ± 0.008	0.071 ± 0.008	0.073 ± 0.008	0.066 ± 0.005
High	Control	0.058 ± 0.015	0.042 ± 0.017	0.088 ± 0.017	0.063 ± 0.009
	One	0.070 ± 0.021	0.047 ± 0.021	0.086 ± 0.015	0.068 ± 0.011
	Two	0.065 ± 0.015	0.104 ± 0.030	0.082 ± 0.015	0.084 ± 0.012
	Three	0.036 ± 0.021	0.037 ± 0.017	0.107 ± 0.017	0.060 ± 0.011
	Mean of Body Size	0.057 ± 0.009	0.058 ± 0.011	0.091 ± 0.008	0.069 ± 0.005
Overall Mean of Body Size		0.055 ± 0.006[b]	0.064 ± 0.007	0.081 ± 0.006	0.068 ± 0.002

[a] See Figure 12 and Table 3, respectively, for cage profile and protein sequence.

[b] Body size effect ($P < 0.05$).

Table C3. Least square means of the ultimate torsional shear stress (MPa) of the tibia in a 72 week old caged layer.

Cage Profile[a]	Juvenile Protein Sequence[a]	Body Size			Mean of Diet
		Small	Medium	Large	
Low	Control	45.6 ± 8.3	47.2 ± 11.8	37.8 ± 11.8	43.5 ± 6.2
	One	23.0 ± 9.6	50.2 ± 9.6	37.3 ± 9.6	36.8 ± 5.5
	Two	44.5 ± 9.6	49.2 ± 7.4	38.9 ± 8.3	44.2 ± 4.9
	Three	28.3 ± 9.6	36.0 ± 8.3	33.8 ± 8.3	32.7 ± 5.1
	Mean of Body Size	35.3 ± 4.6	45.7 ± 4.7	37.0 ± 4.8	39.3 ± 2.7
High	Control	29.1 ± 8.3	25.4 ± 9.6	39.0 ± 9.6	31.1 ± 5.3
	One	37.6 ± 11.8	32.5 ± 11.8	43.0 ± 8.3	37.7 ± 6.2
	Two	44.2 ± 8.3	68.4 ± 16.6	40.7 ± 8.3	51.1 ± 6.8
	Three	21.7 ± 11.8	25.5 ± 9.6	57.3 ± 9.6	34.8 ± 6.0
	Mean of Body Size	33.1 ± 5.1	38.0 ± 4.8	45.0 ± 4.5	38.7 ± 3.0
Overall Mean of Body Size		34.2 ± 3.5	41.8 ± 3.9	41.0 ± 3.3	39.0 ± 0.9

[a] See Figure 12 and Table 3, respectively, for cage profile and protein sequence.

Table C4. Least square means of the ultimate shear modulus (GPa) of the tibia in a 72 week old caged layer.

Cage Profile[a]	Juvenile Protein Sequence[a]	Body Size			Mean of Diet
		Small	Medium	Large	
Low	Control	5.7 ± 0.6	4.8 ± 0.9	2.9 ± 0.8	4.4 ± 0.5[b]
	One	2.6 ± 0.8	5.6 ± 0.8	5.9 ± 0.8	4.7 ± 0.5
	Two	5.2 ± 0.8	6.4 ± 0.6	5.2 ± 0.6	5.6 ± 0.4
	Three	4.9 ± 0.8	4.6 ± 0.6	5.4 ± 0.6	5.0 ± 0.4
	Mean of Body Size	4.6 ± 0.3	5.3 ± 0.4	4.9 ± 0.4	4.9 ± 0.2
High	Control	3.7 ± 0.6	5.4 ± 0.8	4.1 ± 0.3	4.4 ± 0.4
	One	3.9 ± 0.9	6.1 ± 0.9	5.2 ± 0.6	5.0 ± 0.5
	Two	5.3 ± 0.6	6.9 ± 1.3	5.6 ± 0.6	5.9 ± 0.6
	Three	3.0 ± 0.9	4.3 ± 0.8	5.1 ± 0.8	4.2 ± 0.5
	Mean of Body Size	4.0 ± 0.4	5.7 ± 0.5	5.0 ± 0.3	4.9 ± 0.3
Overall Mean of Body Size		4.3 ± 0.2[c]	5.5 ± 0.3	5.0 ± 0.3	4.9 ± 0.1

[a] See Figure 12 and Table 3, respectively, for cage profile and protein sequence.

[b] Protein sequence effect ($P < 0.05$).

[c] Body size effect ($P < 0.05$).

Table C5. Least square means of the torsional properties of radius from 72 week old caged layers.

	Juvenile Protein Sequence	
Mechanical Property	Reversed	Control
Angle of Twist (degrees)	32.1 ± 3.22	34.2 ± 3.72
Ultimate Torque (N - M)	0.022 ± 0.0027	0.023 ± 0.0031
Ultimate Shear Stress (MPa)	135.9 ± 19.08	101.6 ± 22.02
Shear Modulus (GPa)	3.87 ± 0.376	3.83 ± 0.434

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