incubation of egg pools at an elevated temperature (42° C) could further increase the rate of multiplication of *S. enteritidis* in order to allow the detection of contamination by a rapid method within a single day. Pools of 10 eggs were contaminated with approximately 10 cfu of *S. enteritidis*, supplemented with concentrated broth enrichment medium, and incubated at either 37° C or 42° C. Incubation of contaminated egg pools at 42° C resulted in significantly higher *S. enteritidis* levels after 6, 8, 10, and 12 hours. However, incubation at 42° C could only generate a mean *S. enteritidis* concentration of 1.6×10^4 cfu/ml within a single working day (8 hours), inadequate to support efficient detection by most rapid assays. Accordingly, detection of *S. enteritidis* contamination in egg pools by a rapid lateral flow immunodiffusion test was not achieved at a high frequency until 12 hours of incubation at 42° C.

Key Words: Salmonella enteritidis, Eggs

226 Multiple rinses of eggshells for recovery of aerobes and enterobacteriaceae. M. T. Musgrove*[1], D. R. Jones[1], J. K. Northcutt[1], and M. A. Harrison[2], [1]*USDA-ARS,* [2]*University of Georgia.*

It has been demonstrated that when broiler carcasses are rinsed repeatedly, specific bacterial species can be continuously recovered from multiple rinses. Though eggshells are less convoluted than chicken skin, they do have numerous pores that are large enough to harbor bacteria. In order to test the ability of rinsing to remove bacteria from shell surfaces, the following experiments were designed. In each of three experiments, eggs were rinsed eight times with 10 mL of phosphate buffered saline. Aliquots of the first, second, fourth, and eighth rinses were plated onto plate count agar and violet red bile agar to enumerate aerobic and *Enterobacteriaceae* populations. In each of the experiments, half the eggs (five) had been washed and the other half had not been washed (five). *Enterobacteriaceae* were only recovered from rinses of unwashed eggs with visible feces in the first experiment. All four rinses (1, 2, 4, 8) were positive though average levels (log10 cfu/ml rinse) decreased from 2.8 to 1.2 from the first to the eighth rinse. In all three experiments, recovery rate for aerobic populations decreased with subsequent rinsing. Aerobic organisms were recovered from washed eggs at the following rates: 93, 47, 33, and 20 % for rinses 1, 2, 4, and 8, respectively. Unwashed egg recovery rates were 100, 87, 67 and 53 %. Average aerobic population levels (log10 cfu/ml rinse) for all 3 experiments were 1.4, 0.7, 0.9, and 0.8 for washed eggs and 4.2, 2.9, 2.5, and 2.5 for unwashed eggs. These data indicate that though larger rates of recovery and greater bacterial numbers are observed for the first rinse, subsequent rinses continue to remove organisms from egg shells. This may indicate that shell rinse methodology could be improved.

Key Words: Eggs, Eggshells, Aerobes, Enterobacteriaceae, Methodology

227 Airborne microorganisms in shell egg processing facilities. J. K. Northcutt*, D. R. Jones, K. D. Ingram, and A. Hinton, Jr., *USDA-ARS, Russell Research Center, Athens, GA.*

Total aerobic bacteria, molds/yeasts, coliforms and pseudomonads were determined in the air of three shell egg processing facilities (in-line, off-line and mixed operations) using MicroBio MB2 Air Samplers. Sites were sampled from each facility on three different days (replication) during the same week. Four air samples (1000 L each) were drawn from each sampling site on a given day. Sampling sites, if applicable, included areas in or near the following locations: layer house (in-line and mixed operations), farm transition room (in-line and mixed operations), washers, dryer, packer heads, post-processing cooler, nest-run cooler (off-line and mixed operations), loading dock and dry storage. Type of facility (in-line, off-line or mixed), sampling site and the interaction between facility and site had a significant effect on the number of total aerobic bacteria, molds/yeasts, coliforms and pseudomonads recovered ($P < 0.05$). Highest counts for total aerobic bacteria (5.9 $\log_{10}$ cfu/mL air), molds/yeasts (4.0 $\log_{10}$ cfu/mL air), coliforms (2.5 $\log_{10}$ cfu/mL air) and pseudomonads (3.2 $\log_{10}$ cfu/mL air) were found in the layer house. Lowest counts for total aerobic bacteria (1.25 $\log_{10}$ cfu/mL air) and molds/yeast (2.5 $\log_{10}$ cfu/mL air) were found in the coolers and off-line coolers, respectively. Few samples in the post-processing coolers, nest-run coolers, loading docks and dry storage areas tested positive for coliforms (0/36, 2/24, 1/36, and 0/36, respectively) and pseudomonads (1/36, 2/24, 5/36, and 6/36, respectively). Data gathered during this study may be useful in identifying the sources and levels of airborne contaminates in commercial shell egg processing facilities.

Key Words: Airborne, Bioaerosols, Microorganisms, Shell eggs

Avian Osteoporosis: Measurement and Ethical Considerations

228 Welfare implications of avian osteoporosis. A. B. Webster*, *The University of Georgia, Athens, GA.*

The modern laying hen has a high demand for calcium to support egg shell formation. If dietary calcium is insufficient, structural bone may be demineralized, resulting in osteoporosis. Osteoporosis is not readily reversible while egg laying continues. The problem can be exacerbated incrementally by periods of deficient intake of calcium, phosphorous, or vitamin D due to depressed feed intake or errors in feeding management. Hens may experience some behavioral disturbance during episodes of calcium deficiency because heightened activity and stereotypic pecking have been observed in chickens fed calcium-deficient diets. Light-hybrid laying hens, having small skeletal frames, are the most prone to problems due to osteoporosis, especially if housed in cages. Osteoporosis increases risk of cage layer fatigue and bone fracture. A hen with serious cage layer fatigue cannot stand and may eventually die. Hen mortality due to cage layer fatigue appears to have become less prevalent. However, even in apparently healthy hens, folds in the sternum and at the junctions of sternal and vertebral rib segments indicating past fractures due to bone weakness are not uncommon. It is reasonable to believe that bone breakage is painful to hens. Some pain or discomfort may occur when bones such as the sternum or ribs deform due to weakness. Osteoporosis appears to have its most serious welfare implications in regard to spent hen catching and transport. In 2002, roughly 110 million spent laying hens in the U.S. were sent to processing plants. Catching of spent hens can result in high percentages of birds with broken bones, and the rate of breakage is negatively associated with bone strength. Transport for several hundred kilometers and transit times of 12-24 h are not unusual, so pain associated with vibration and jostling of an injury can last a considerable time. Many dead-on-arrival hens have at least one freshly broken bone. At present, care in husbandry is the key to minimize problems due to osteoporosis because, while the hen is in lay, the condition seems not amenable to a cure.

Key Words: Avian osteoporosis, Animal welfare

229 Overview of bone biology in the egg-laying hen. C. C. Whitehead*, *Roslin Institute, Edinburgh, Midlothian, UK.*

In young pullets, long bones elongate by endochondral growth. Growth plate chondrocytes proliferate, then hypertrophy and are replaced by osteoblasts that form a network of trabecular bone. This bone is gradually resorbed by osteoclasts as the bone lengthens. Long bones widen, and flat bones are formed, by intramembranous ossification in which cortical bone formation by osteoblasts in the periosteal layer is accompanied by osteoclastic resorption at the inner endosteal surface. Growth of structural trabecular and cortical bone types continues up to the onset of sexual maturity in pullets. At this point, the large surge in oestrogen changes the function of osteoblasts to forming medullary bone rather than structural bone. Medullary bone is a woven bone that acts as a labile source of calcium for eggshell formation. It lines structural bone and also occurs as spicules within the marrow cavity. It has little inherent strength, though can contribute to fracture resistance. Osteoclasts resorb both medullary and structural bone so that during the period the hen remains in reproductive condition there is a progressive loss of structural bone throughout the skeleton characteristic of osteoporosis. The increasing fragility of the bones makes them more susceptible to fractures. The dynamics of bone loss can be affected by a number of nutritional, environmental and genetic factors. If the hen goes out of reproductive condition, oestrogen levels fall, osteoblasts resume structural bone formation and skeletal regeneration can take place.

Key Words: Laying hens, Bone, Osteoporosis

230 Role of estrogen in avian osteoporosis. M. M. Beck*[1] and K. K. Hansen[2], [1]*University of Nebraska-Lincoln, Lincoln, NE,* [2]*University of Nebraska Medical Center, Omaha, NE.*

One of the difficulties associated with commercial layer production is the development of osteoporosis in hens late in the production cycle. In light of this fact and because of the hen's unique requirement for calcium (Ca), many studies have focused on the regulation of Ca and the role(s) of estrogen (E2) in this process. The time course of E2 synthesis over the productive life of the hen has been well documented; increased circulating E2 accompanies the onset of sexual maturity while decreases signal the declines in egg production prior to a molt. E2 receptor populations decrease with age in numerous tissues; the parallel changes in calcium-regulating proteins, primarily Calbindin D28k, and in the ability of duodenal cells to transport Ca, are thought to occur as a result of the changes in estrogen, and are also reversible by the molt process. In addition to the traditional model of E2 action, evidence now exists for a possible "non-genomic" action of E2 via membrane-bound receptors, demonstrated by extremely rapid surges of ionized Ca in chicken granulosa cells in response to 17b-E2. E2 receptors have also been discovered in duodenal tissue; and tamoxifen, which is known to bind to the estrogen receptor, has been shown to cause a rapid increase in calcium transport in duodenum. In addition, recent evidence also suggests that mineralization of bone per se may not explain entirely the etiology of osteoporosis in the hen but that changes in the collagen matrix may contribute through decreases in bone elasticity. Taken together, these studies suggest that changes in E2 synthesis and E2 receptor populations may underlie the age-related changes in bone; as with postmenopausal women, dietary Ca and/or Vitamin D are of limited benefit as remedies for osteoporosis in the hen.

Key Words: osteoporosis, estrogen, calcium

231 Assessing bone mineral density in vivo: Digitised fluoroscopy and ultrasound. R. H. Fleming*[1], D. Korver[2], H. A. McCormack[1], and C. C. Whitehead[1], [1]*Roslin Institute, Scotland,* [2]*University of Alberta, Canada.*

Osteoporosis in caged laying hens can lead to fracture incidences of up to 30% by end-of-lay. The genetic component of osteoporosis is large, and a methodology to detect hens susceptible to fracture could eliminate poor birds in breeding programmes. We applied 2 methodologies to this problem. A radiographic absorptiometry (RA) film method was modified by video digitization from an image intensifier and computer analysis. This we termed digitized fluoroscopy (DF). In a longitudinal study humeral and ulnar DF values were measured in 165 laying hens. Significant relationships ($P<0.001$) were seen between DF assessments made from 25 weeks onwards and *post mortem* skeletal measures at 70 weeks. We conclude that DF detects hens with poor bones from 25 weeks and could be useful in diagnosis. However, DF is problematic; soft tissue density is included, equipment is bulky, radiography hazardous. Quantitative ultrasound is an established method in bone assessment. The DBM Sonic 1200 (IGEA,Italy) measures Amplitude dependent Speed-of-Sound (Ad-SoS). A satisfactory Ad-SoS signal is produced at the 1st joint of the 3rd toe in hens. Preliminary studies at this site revealed Ad-SoS correlated weakly but significantly with radiographic density (RD),($r=0.24$,$P<0.001$) and peripheral Quantitative Computerized Tomography (pQCT), ($r=0.14$,$P=0.04$). Longitudinal Ad-SoS measurements were then made in hens from a genetic selection study. Weight restricted divergent selection has produced high and low Bone Index (BI) lines with 92% difference in tibia strength in generation 8 (G8) at 30 weeks (303.7 v.157.9N, $P<0.001$). Ad-SoS in high BI hens was significantly higher than low BI hens at 32($P<0.001$), 42($P<0.001$), 52($P<0.05$) and 62weeks($P<0.001$)in G8. In a study of cage v. free-range hens, 3rd toe Ad-SoS correlated with shear strength ($r=0.31$, $P<0.001$)and RD values ($r=0.5$,$P<0.001$) measured *post mortem*. Free-range hens had higher Ad-SoS values than cage hens (1904 v 1850m/s, $P<0.001$). We conclude that rapid detection of poor bone quality in hens is possible with Ad-SoS ultrasound.

Key Words: Hens, Osteoporosis, Digitised fluoroscopy, Ultrasound

232 Assessing bone mineral density in vivo: Dual emission X-ray absorptiometry. P. Y. Hester*[1], M. A. Schreiweis[1], H. Mazzuco[1], M. N. Kopka[1], J. I. Orban[2], M. C. Ledur[3], and D. E. Moody[1], [1]*Purdue University, W. Lafayette, IN,* [2]*Southern University, Shreveport, LA,* [3]*Embrapa Swine and Poultry Research Center, Concordia, Brazil.*

Bone densitometry or dual-energy X-ray absorptiometry is being used as a noninvasive tool to monitor skeletal integrity of live birds for osteoporosis. A Norland pDexa X-ray bone densitometer (Model No. 476D014) was used to determine bone mineral densities (BMD) of the left tibia and fibula and the humerus of live, unanesthetized birds. Densitometry effectively detected changes in bone integrity of live birds fed varying levels of dietary calcium. Hens consuming 1.8, 3.6, and 5.4% levels of dietary calcium had BMD of 0.147, 0.157, and 0.176 g/cm^2 (SEM = 0.005), respectively (linear effect, $P < 0.001$). Likewise, bone ash weight, breaking force, stress, modulus of elasticity as well as eggshell traits also increased linearly in response to increased calcium level in the diet ($P < 0.05$). Densitometric live scans for BMD were highly correlated ($P < 0.001$) with bone breaking force ($r = 0.65$) and bone ash ($r = 0.77$). We have also monitored BMD in live Leghorn and broiler females during their life cycle. The tibial BMD of White Leghorns and broilers increased as the birds aged from 15 to 65 wk of age with the BMD of the broiler tibia increasing at a greater rate than the Leghorn tibia (line x age interaction, $P < 0.0001$). A precipitous drop in BMD occurred during an induced molt of Leghorns subjected to 10-d of feed withdrawal. Our long-term goal is to reduce the incidence of osteoporosis in egg-type chickens by genetic selection for improved BMD. By crossing a broiler with an egg line, an F2 resource population of birds has been developed to identify quantitative trait loci influencing BMD in chickens. Research supported by SCRP-USDA No. PL95-113 and NRI Competitive Grants Program No. 2001-02426 and 2002-03394.

Key Words: Bone Mineral Density, Osteoporosis, Densitometry

233 Assessing bone mineral density in vivo: Quantitative Computed Tomography. D. R. Korver*, *University of Alberta, Edmonton, AB, Canada.*

Laying hens deposit labile calcium stores in the medullary region of long bones that can be mobilized to support eggshell formation. Over time, repletion of the medullary bone stores can decrease, leading to osteoporosis. Quantitative Computed Tomography (QCT) is a non-destructive technique used to measure bone mineral density (BMD). QCT is used diagnostically in humans to assess osteoporosis and experimentally in other mammals. BMD determined by QCT is highly correlated with other, more invasive methods of bone mineral determinations, such as ashing. QCT uses an X-ray sent through a bone at multiple angles to generate a two dimensional image and a three dimensional calculation of volume and BMD. The technique allows resolution of total, trabecular and cortical BMD and cross-sectional areas. The separation of bone types allows very precise measurements of the bone compartments most important in calcium supply for eggshell formation and bone strength. QCT has been adapted in our lab to measure BMD in vivo and ex vivo in poultry. QCT values obtained for poultry bones correlate well with other, destructive means of assessing bone quality such as breaking strength, ashing and chemical bone mineral determinations. In vivo, birds are anaesthetized to ensure they remain motionless during the scan. Repeated anaesthesia does not affect BMD, feed intake or egg production in laying hens. Thus, changes in BMD of individual birds can be measured over time; BMD at specific time points can be correlated with production parameters and eggshell quality traits. For ex vivo scans, the bone of interest can be removed from the carcass and frozen for subsequent BMD analysis, without having to remove the soft tissue. The limitations of QCT include the initial cost of the equipment, the need to anaesthetize live birds, and the amount of time needed for each scan (approximately 20 minutes per bone for acceptable resolution). QCT is an effective technique to measure BMD in laying hens, allowing resolution of not only total BMD, but cortical and trabecular BMD as well.

Key Words: Laying hen, Quantitative Computed Tomography, bone mineral density, Osteoporosis, Trabecular bone