be mailed to laboratories. Samples can be archived for comparison and used as a source of DNA material for cloning and sequencing.

Key Words: virus detection, filter paper, PCR


A feather-degrading keratinase was discovered at NC State University. The enzyme, produced by Bacillus licheniformis strain PWD-1 is able to hydrolyze feather keratin and all other proteins tested. The infectious isoform of prion protein (PrPSc) causes transmissible spongiform encephalopathies (TSE), including bovine spongiform encephalopathy (BSE), ovine scrapie and human Creutzfeldt-Jakob disease (CJD). PrPSc and feather keratin share a similar protein structure and stability and resistance toward common proteolytic enzymes. PWD-1 keratinase was tested for its activity against PrPSc in the brain stem tissues of BSE and scrapie. Tissue samples were homogenized and digested by proteinase K (PK). PrPSc that is resistant to PK was detected by Western blot and immunochemical reaction with specific monoclonal antibodies. The same method was used to test PWD-1 keratinase. A pre-cooking step was used to treat the tissue homogenate and PWD-1 keratinase was used to replace PK in enzymatic digestion. It was found that both crude and purified preparations of the keratinase can completely hydrolyze PrPSc in BSE and scrapie tissues. These experiments demonstrated the degradability of PrPSc by PWD-1 keratinase and the potential of development of an enzymatic process capable of disinfecting medical equipment and animal products. (Supported by a USDA-IFAES grant)

Key Words: Prion, Keratinase, BSE, Scrapie


Little has been published on causes of mortality in older laying hens. Two small (2,400) layer flocks housed on a research farm were monitored for mortality over a one year period. Birds that died were collected and quickly chilled in an ice bath to minimize post-mortem autolysis. Chilled birds were refrigerated and collected once per week for necropsy. All birds were examined by necropsy. With few exceptions, all mortality was due to non-infectious causes and a significant percentage can be prevented by proper management. A major cause of mortality in these flocks was vent persecution. Adjustments in lightening reduced this problem significantly. Osteoperosis was present in greater than 50 percent of the hens and was the cause of mortality in many birds due to broken bones followed by dehydration and/or vent persecution. Entrapment resulting in dehydration and/or vent persecution was also a significant cause of mortality. Other causes of mortality included salpingitis with or without peritonitis, fatty liver hemorrhagic syndrome, visceral gout, tumors of the reproductive tract, tumors of the digestive tract, proventricular in-

Key Words: Layers, Mortality, Management

259 Skeletal muscle cation contents and effects of genetic selection for production traits: a relationship with spontaneous myopathy? D. A. Sandercbock*, M. A. Mitchell, and Z. E. Barker, Roslin Institute, Roslin, Midlothian, UK.

Recent studies have indicated that genetic selection for useful production traits in meat type poultry is associated with an increased incidence and degree of idiopathic (spontaneous) and stress-induced myopathies. The mechanisms mediating these pathologies await full elucidation but alterations in intracellular calcium homeostasis have been strongly implicated. Whilst a direct role for altered function in calcium regulatory systems is well recognized, disruptions in the balance of the intracellular active concentrations of other cations may also contribute to these conditions. The present study examined the effects of genetic selection on muscle cation contents and related these to myopathic status. Pectoral muscle samples were prepared from 4 birds (49d) from each of 34 chicken pure-lines from three categories: broiler (B), traditional (T) and layers (L) selected for the relevant production traits. The contents of sodium (Na), potassium (K), calcium (Ca) and magnesium (Mg) were determined by atomic absorption spectrophotometry in muscle homogenates and expressed per unit tissue dry and ash weights. Plasma cation contents were also measured in each bird and the degree of spontaneous myopathy was assessed by plasma creatine kinase (CK) activity. Plasma Ca was 40% (p<0.001) higher in B lines whilst Mg was lower in traditional breeds. Plasma Na and K were higher in B lines than in the other categories (p<0.05). Muscle Ca content did not differ between categories. Muscle Mg content was higher in B lines than in the other lines. Dry and ash weight elemental analysis, however, revealed a 30% (p<0.01) elevation muscle Na content in B lines and a corresponding 14% increase in muscle K. These observations are consistent with myopathic and dystrophic conditions in other species. It is proposed that selection associated alterations in cation (other than Ca) regulation mechanisms play a significant role in the aetiology of spontaneous myopathy in modern poultry.

Key Words: Muscle, Cations, Myopathy

260 In vitro production of Interleukin-6 by macrophages with an extract of Aspergillus meal prebiotic. G. Tellez1,2, D. Horlick1, G. Nava2, A. Donoghue3, and N. Rath3, 1 Departamento de Produccion Animal: Aves, FMVZ/UNAM, Mexico, 2 Dept. of Poultry Science, University of Arkansas, Fayetteville, AR, 3 PPPSR, ARS, USDA, Fayetteville, AR.

Previous studies conducted in our laboratories have shown that dietary use of 0.2% of the prebiotic Aspergillus meal (AM) reduces the level of Salmonella enteritidis in neonate broilers and pouls. In an effort to understand how AM protects the neonatal poult, we wanted to determine if AM has immunomodulatory effects. The objective of this study was to evaluate the effects of water-soluble extracts of AM on macrophages using an Interleukin 6 (IL-6) production bioassay. Fifty mg of AM was rinsed briefly with distilled ultra-filtered H2O (diUFH2O) before extraction in 1.5ml diUFH2O by rocking overnight at 4C. A blank of diUFH2O was also used throughout the remaining steps. Samples were centrifuged at 21,000xg for 4 minutes. An aliquot of supernatant (0.5 ml) was then dried, re-suspended in 250 ul of RPMI-1640 medium and filtered through a 0.2 um filter. For testing immune responsiveness, 20 ul of the AM H2O soluble extracts, the blank and a positive control, lipopolysaccharide (LPS, 25ug/ml), were added to each well of a 48-well plate containing 0.5 ml of a transformed chicken macrophage cell line (HTC) at a concentration of 1 million cells/ml in RPMI-1640 with 1.5% chicken serum. The plate was then incubated for 24 h at 37C in a CO2 incubator. The conditioned medium (CM) was then harvested, centrifuged at 21,000xg and the supernatants stored at 4C. The presence of IL-6 in the CM supernatants was then quantified using the B9 cell proliferation bioassay (an IL-6 dependent cell line, using the reduction of 3-(4,5-di-methylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide to measure proliferation). IL-6 concentrations were then determined by comparison to a recombiant IL-6 standard. The AM extract responded similarly to the LPS positive control (1.423 and 1.533 U/ml respectively). The results of this study show that a water-soluble extract of AM is able to induce IL-6 production by HTC macrophages. It is not known whether the compounds in this extract could be related to an endotoxin or an AM-polysaccharide that may stimulate the immune system, thereby providing protection to the birds. Studies are in progress to characterize compounds present in the extract.

Key Words: Aspergillus meal, IL-6, bioassay, prebiotic
261 Long term hatching egg storage alters the metabolism of broiler embryos. G. M. Fasenko\textsuperscript{a}, F. E. Robinson, J. C. Segura, J. J. R. Feddes, and C. A. Ouellette, \textsuperscript{1}Dept. of AFNS, University of Alberta, Edmonton, Alberta/Canada.

It is known that long term hatching egg storage has negative effects on embryonic growth. The present study examined the effects of broiler egg storage for 4 and 18 d on embryonic CO\textsubscript{2} output from eggs stored for 0 to 18 d of incubation. Freshly laid eggs (n=80) were collected on two days and stored for 4 or 15 d. Sixteen eggs per each storage group were selected for incubation based on similar fresh egg weights and weight losses during storage. A sub-sample of eggs (n=40) from each storage group were set aside to establish the embryonic development of 20 embryos. Ten eggs per each storage group were replaced with one of the six spare eggs from the same storage treatment.

262 Myostatin gene expression in early developing chicken embryos from commercial broiler and layer strains. C.N. Schauermann\textsuperscript{a}, S.F. Bilgili\textsuperscript{a}, S. Yuzun\textsuperscript{a}, and D.R. Mulvaney\textsuperscript{b,\textsuperscript{c}}, \textsuperscript{1}Dept. Poultry Science, Auburn University, Auburn, AL, \textsuperscript{2}EMBRAPA, Brazil, \textsuperscript{3}Cell and Molecular Biology, Auburn University, Auburn, AL, \textsuperscript{4}Dept. Animal Sciences, Auburn University, Auburn, AL.

Because myostatin inhibits proliferation of precursor cells, our objective was to compare its expression in a commercial broiler strain to that of a layer strain of chicken. Myostatin, a member of the TGF-\beta family of growth factors, has been shown to be a negative regulator of muscle mass through involvement in the myogenic regulatory gene pathway. Published work has indicated expression may be down-regulated during developmental windows prior to embryonic day 10. Consequently, embryos were obtained daily from fertile eggs of each strain incubated 3 through 10 days. Prior to day five, whole embryo pools were made to represent each day, day 6 to 8 pools were from decapitated embryos, and for days 9-10, samples of the pectoralis were taken. An RT-PCR procedure (Qiagen) was utilized to amplify expression of myostatin normalized to an S18 ribosomal PCR cDNA product. Primers for both myostatin and S18 amplified single electrophoretic bands of predicted size. Densitometric procedures were used to determine relative expression and data were subjected to ANOVA using GLM procedures of SAS accounting for strain and day as main effects. Myostatin mRNA expression increased by 1.13-fold (P < 0.01) from day 3 to 10. While layers tended to have lower expression (P < 0.05) compared to broilers, no significant strain by day interaction was detected. These data represent a first comparison of myostatin expression between broilers and layers. We cautiously suggest the lines may differ but additional investigations with staged embryos prior to sampling for RNA to standardize the stage of development, are needed to show differences in the timing of expression during this window.

263 A Study Of Casts From Epoxy Embedded Eggshell Of White Leghorn Chickens. S. L. Westmoreland\textsuperscript{a,\textsuperscript{b}}, \textsuperscript{1}Dept. of Biology, The University of Texas-Arlington, Arlington, TX.

The avian eggshell has evolved to provide physical protection, a reservoir of calcium, and gas exchange for the embryo that it encloses. High-pressure epoxy casts of eggshell samples from eggs of White Leghorn chickens were prepared to study the pore system, through which gas exchange occurs during embryogenesis. In addition to the details of pore structure that were revealed, an exceptional three-dimensional view of the interior of the mammillary cone, made possible by plastic replicas, has provided a clearer picture of this important eggshell region where nucleation for biomineralization of the eggshell occurs. Unfertilized eggs of Hy-line W98 White Leghorn chickens, obtained from the Poultry Science Department of Texas A & M University, were emptied and the shell was treated with Clorox bleach to remove the organic shell cuticle and shell membranes. Small shell fragments were placed in holders, covered in CIDA 506 resin epoxy with Polycon hardener, and placed in a pressure vessel in a closed vacuum at 1,200 psi until set. Epoxy-embedded shell blocks were cut in half to expose radial shell surfaces and were then placed in concentrated hydrochloric acid to dissolve the eggshell. Shell casts viewed on the JOEL 35C scanning electron microscope were observed to contain a replica of the upper shell surface, the mammillary cone region, and the pores. The pores in the plastic casts were seen as solid columns of plastic that were continuous connecting the upper and lower shell surface replicas. The replica of the mammillary cone surface, when viewed from the side, contained a wave-like pattern, which indicated the impression of the spaces of the adjacent mammillary cone junctions. When the mammillary cone casts were viewed from directly above, replicas of individual mammillary cones could be seen. The basin-like structures contained replicas of shell membranes, which were seen in an interwoven pattern. The cast of a single spherical body could be seen in many of the mammillary cone replicas. These bodies are proposed to be the mammillary cores, sites of shell nucleation and of calcium translocation during embryogenesis.

Key Words: Avian Eggshell, Embryogenesis, Biomineralization, Eggshell Pores, Mammillary Cones

264 The effect of age on bone mineral density and content of White Pekin ducks as assessed through densitometry. P. Y. Hester\textsuperscript{a}, Z. Kounov, and M. A. Schreier, \textsuperscript{a}Purdue University, W. Lafayette, IN.

The objective of this study was to determine the effect of age on bone traits of White Pekins as assessed through densitometry. The femur and tibia of female breeder ducks were collected at 19 different ages between 2 and 48 wk of age. Excised bones (n = 70) were scanned using a Norland pDEXA X-ray densitometer, providing data on bone mineral density (BMD, g/cm\textsuperscript{2}), total bone mineral content (BMC, g), and length of individual bones. Using the MIXED model of SAS, an ANOVA was conducted using age of the duck as the whole plot with the type of bone (femur vs tibia) as the sub-plot. Correlations among bone traits were calculated. The BMD was significantly higher for the femur as compared to the tibia (P < 0.0001), but because the tibia was a longer bone than the femur, total BMC was greater for the tibia than the femur (P < 0.0001). A large increase in BMD (P < 0.0001) and total BMC (P < 0.0001) occurred after 22 wk of age, which was sustained through 48 wk of age. There was a significant interaction between the age of the duck and the type of bone (femur vs tibia) for BMD (P < 0.0001), but not for BMC. The BMD between the femur and the tibia was similar between 2 and 22 wk of age. From 24 to 48 wk of age, the femur had significantly greater BMD than the tibia at the bone area (P < 0.0001) and length (P < 0.0001) increased significantly up to 6 wk of age, and thereafter remained relatively constant. Strong correlations were evident between BMD and BMC for both the femur (r = 0.99; P < 0.0001) and the tibia (r = 0.98; P < 0.0001). The BMC was moderately correlated with bone length (r = 0.33 and 0.43 for the femur and tibia, respectively, P < 0.05). The sustained increase in BMD and BMC observed from 24 to 48 wk of age in female breeder ducks could have been influenced by the calcium enriched breeder diet and endogenous sex steroids. Research supported by Maple Leaf Farms, Milford, IN.

Key Words: Bone mineral density, Densitometer, Ducks
265 Calcium mobilization in the aging hen: II. Effect of the anti-estrogen, tamoxifen, on duodenal calcium absorption. K.K. Franzen1, M.M. Beck1, and L.G. Robeson1, 1University of Nebraska-Lincoln, Lincoln, NE.

Numerous studies have been conducted in an attempt to understand the estrogen/calcium relationship and its effect on optimizing egg production and shell quality. Calcium homeostasis in the laying hen is intimately tied to plasma estrogen profiles. Increases in plasma estrogen concentrations, either endogenous or exogenous, leads to increased calcium absorption and plasma Ca2+ (Sommerville et al., 1989; Qin & Klandorf, 1995; Elaroussi et al.,1993; Hansen, 1998). Two studies were conducted using a potent anti-estrogen, tamoxifen, in an effort to further elucidate the estrogen/calcium relationship in the hen and to determine whether estrogen’s effect on calcium metabolism is mediated through its receptor.

Hyline W36 laying hens at peak production (PP; 33 wks of age, 93% production) and late stage production (LS; 43 wks of age, 83% production) were used in this study. Tamoxifen was dissolved in propylene glycol and administered i.m. 2-3 hours after oviposition and again 8 hours later. Hens were then euthanized by cervical dislocation and the duodenal loop was excised for in vitro calcium transport (CaT) determination. Tamoxifen treatment had no effect on plasma E2 or LH concentrations in either study. However, tamoxifen treatment resulted in a significant decrease in plasma P4 concentrations in PP hens (P=0.0455). There was a significant (P<0.05) increase in CaT in tamoxifen treated birds as compared to control birds in both studies. This observation suggests two possibilities: First, that tamoxifen may not be a “pure” antagonist in the chicken and second, that estrogen may act in a rapid, nongenomic fashion at the intestine. In the latter case, this finding would explain earlier findings that E2 implants caused a rapid, transient increase in CaT that occurs more rapidly than would be expected from an effect via the kidney.

Key Words: tamoxifen, calcium transport, age

266 Global gene expression profiling in liver of thyroid manipulated and/or growth hormone (GH) injected broiler chickens. W. Wang1, W. Carre, L. Rejto, and L. A. Cogburn, University of Delaware, Newark, DE.

DNA microarray analysis is a powerful tool for profiling gene expression on a genomic scale. We have developed a chicken liver cDNA microarray containing 3,100 unique cDNA clones which contain estimated 1,459 novel exons (ESTs) that were sequenced from a normalized chicken liver cDNA library. The unique cDNA clones were PCR amplified and printed onto 8 x 12 cm nylon membranes. We have used 24 liver DNA microarrays for global gene expression profiling of hepatic genes in broiler chickens in response to thyroid manipulation and/or daily growth hormone injection. For control birds broiler chickens were fed 1.25 ppm T3, 0.6% propylthiouracil (PTU) or control feed (CF) for three weeks. Three additional treatment groups (4 birds/group) were included [buffer controls (BC), cGH (250 µg/kg) or T3+cGH]. All total RNA sample, prepared from liver, was reverse transcribed with 3P-dCTP and hybridized to each microarray. A large number of known genes (and unknown ESTs) were up regulated by T3 treatment [C/EBP (CCAAT/enhancer binding protein), CCAAT displacement protein, SPOT 14 (thyroid hormone responsive hepatic protein), hydroxymethylglutaryl-CoA synthase, carboxyptidase E (CPE), retinal synthase (Glu) and zinc finger protein, etc.]. Another group of genes (named and unknown ESTs) were down regulated by T3 [adipophilin, glutathione S-transferase (GST), cytochrome, acetyl-Coa acetyltransferase 2 (ACAT2), 90kDa heat shock protein, etc.]. The expression of a small number of genes was affected by cGH (endolase-α, carboxyptidase E, PPAR-γ, Vit.D binding protein, etc.). One unknown EST (pg11c.pk002.m22) was highly induced in PTU treated (slightly obese) chickens. This unknown liver-specific gene was previously identified by one of us (W.C.) as a fat-specific EST that has two single nucleotide polymorphism sites (SNPs), which respond to genetic selection for fat content. This initial study demonstrates the power of DNA microarray-based gene expression profiling to discover key regulatory genes in important metabolic pathways that regulate growth and body composition.

Key Words: DNA Microarrays, Gene Expression Profiling, Hormonal Manipulation, Single Nucleotide Polymorphism (SNP)

Processing & Products

Meat Quality

267 Induced red discoloration in fully-cooked chicken or turkey breast meat. J.K. Northcutt1 and D.P. Smith1, 1USDA, ARS, Russell Research Center.

The present study was conducted to determine if red discoloration could be induced in fully-cooked chicken or turkey breast meat patties using bone marrow (fragments or water-soluble extracts). Five, 6 or 7 week old broilers (2 replications each) were slaughtered by cervical dislocation and femurs (n=20 femurs per replication and bird age) were removed. Primary spongosia from each end of the bone was pooled and blended (1:1 wt/vol) with 50 mM Tris (pH 7.5) for 30 s. This mixture was centrifuged (20,000 x g, 45 min at 4 C), and three distinct layers were noted: a supernatant, an intermediate paste layer, and bone pellet. Zero, 1, or 2 g of supernatant, an intermediate paste layer, and bone pellet were mixed with 50 g of ground breast meat. Meat was formed into patties, vacuum-sealed in cooking bags, and cooked to internal temperature of 82.2 C (n=3 per treatment). Triplicate readings of internal and external color (CIE Lab) were measured on each patty. One g of chicken supernatant, paste or bone had little effect on color of either patty. Two g of supernatant extracted from 5 and 6 week old broiler femurs increased redness (a*) by 4.2 to 4.4 units, while extracts from 7 week old broiler femurs increased redness by 7 units. Comparable results were observed for bone and paste, with redness values increasing by 6 to 9 units for both treatments. The experiment was repeated using femurs from 16 week old tom turkeys, where only dialyzed supernatant (molecular weight cut-off of 12,000 dalton (MW12) or 25,000 dalton (MW25)) was used. Dialysis of turkey supernatant gave interior red discoloration may be induced using water soluble bone marrow extracts from chicken or turkey femurs.

Key Words: red discoloration, chicken, turkey

268 Skin Pigmentation Evaluation in Broilers Reared under Light and Dark Conditions and Fed Natural and Synthetic Pigments. M.P. Castañeda* and A.R. Sans, Texas A&M University, College Station, TX.

Xanthophylls are a class of carotenoids and are the most prominent source of pigmentation in poultry feeds. It has been reported that birds reared in outside pens were more orange and darker than birds reared in dark houses. This suggested that changes in pigmentation are influenced by light. Therefore, the purpose of this study was to determine the effect of light exposure in broilers fed natural and synthetic pigments at the levels commonly found in the Mexican market. Six hundred and ninety day-old chicks in two trials were obtained from a commercial hatchery and randomly distributed into six treatments (3 pigments classes x two lighting levels). The light pens were in a window house and were supplied with light from quartz halogen light sources. Dark houses had 0 - 3 foot candles of incandescent light. The feeding treatments were: Control group (without pigment); natural high levels of yellow and red; a blend (natural yellow and synthetic red). Body weight, feed consumption, skin color (Minolta CR-200 Chroma meter), blood and skin pigment levels were measured during growth and after processing. Final body weight and feed consumption were not significantly different among the treatments. No differences due to light level were observed in any parameter except skin yellowness. The effect of darkness was not as previously reported, possibly due to the higher levels of pigments used in this study to stimulate the Mexican industry. The effect of darkness was greater when the natural yellow was combined with natural red than when the synthetic red was used. This may be due to metabolic differences and supports the preference for using the more expensive synthetic red pigment for dark growing situations.

Key Words: Synthetic Pigments, Natural Pigments, Light, Broiler Skin Color, Blood Pigments