

the inclusion levels of CNM in the diet significantly reduced cholesterol content and percentage of some fatty acids but there was no significant effect due to enzyme supplementation or to the interaction (CNM x Enzyme). Levels of cholesterol, palmitic acid and linoleic acid in abdominal broiler fat decreased oleic acid increased as the inclusion level of CNM increased in the diet.

Key Words: Cashew nut meal, Enzyme, Abdominal fat, Cholesterol

335 Lipid oxidation decreases metabolizable energy value of dietary poultry fat for growing broilers. A. M. C. Racanici, J. F. M. Menten*, M. A. B. Regitano-D'Arce, J. B. Gaiotto, A. A. Pedroso, F. A. Longo, and J. O. B. Sorbara, *Escola Superior de Agricultura Luiz de Queiroz - USP - Piracicaba - SP - Brasil.*

In order to determine the apparent metabolizable energy (AME) and nitrogen corrected apparent metabolizable energy (AMEn) of fresh and oxidized poultry fat a metabolic assay with forty-eight AgRoss male broilers from 31 to 34 days of age was conducted. The birds were fed a basal

diet or this diet replaced by 10% of fresh or oxidized fat and the total excreta collection method was applied. The birds were housed in metabolic cages and each diet was supplied for four replications of four birds. Fresh poultry fat was supplied by a local rendering and then stored frozen (-18 OC). The oxidized fat was obtained by heating and specific absorbances were measured frequently to control fat quality. Specific absorbances at 232 and 270 nm were, respectively, 4.64 and 0.47 for fresh fat and 18.54 and 3.76 for oxidized fat, which suggest higher levels of conjugated dienes in the oxidized poultry fat. The results of AME and AMEn were 9,240 and 9,150 kcal/kg (as feed-basis) when fed as fresh poultry fat and 7,770 and 7,595 kcal/kg (as feed-basis) when fed as oxidized poultry fat. AME and AMEn values were statistically different ($p < 0.0001$) and indicate a decrease from the fresh poultry fat to the oxidized fat due to the oxidation

Key Words: Metabolic assay, Oxidized dietary fat, Lipid oxidation, Peroxidation, Broilers

Pathology

336 Immortalisation of avian cells by ectopic expression of telomerase. G. Michailidis*, C.J. Nile, C. Townes, B. Brown, and J. Hall, *The University of Newcastle Upon Tyne, UK.*

Primary cultures of eukaryotic cells have a finite life span due to the process termed replicative senescence. This phenomenon is linked to progressive telomere shortening. Telomeres are found at the chromosome ends of most species and consist of tandem repeats of the sequence TTAGGG. Telomerase is a ribonucleoprotein that adds telomeric DNA repeats onto the 3' ends of chromosomes. It consists of a template RNA (TR) and the telomerase reverse transcriptase (TERT). Recent studies have shown that ectopic expression of (human) hTERT extends the life-span of human and other mammalian cells without causing a loss of differentiation. We attempted to investigate whether primary cultures of avian cells could be immortalised by ectopic expression of hTERT. Primary cultures of chicken embryonic epithelial (kidney and pancreatic acinar) cells, and fibroblast cells were established in vitro. Senescence of the cells was observed within 15 population doublings. Data from telomere length and TRAP (telomerase repeat amplification protocol) assays indicated that cell division was associated with telomere shortening and reduced telomerase activity. In addition RT-PCR analyses revealed reduced expression of the TR gene. To immortalise the cells the hTERT and avian TR genes were stably introduced into the cells by retroviral transfection. The resultant cell lines were analysed for telomere length, TERT activity, TR expression and cell survival. This system provides a novel approach to develop lines of immortalised avian cells.

Key Words: Immortalised Cells, Transfection, TERT

337 Prevalence, distribution and diversity of pathogenic *E. coli* in commercial turkey poul production. S. Banach*, F. Lago, and T. Rehberger, *Agtech Products, Inc.*

Avian colibacillosis is a systemic infection caused by *Escherichia coli* and occurs most commonly in young broilers and poults. Previous research has identified virulence factors commonly associated with avian colibacillosis: *iss*, *iucC*, *tsh*, and *cvaC* and a multiplex PCR method to detect these factors. In this study, *E. coli* isolated from intestinal samples of poults were analyzed by multiplex PCR and randomly amplified polymorphic DNA (RAPD) PCR to determine the diversity of pathogenic *E. coli*. Three poults ranging from 17-36 days old were collected from each of 22 sites of an integrated turkey operation in Virginia. Intestinal scrapings of poults were plated onto CHROMagar for the enumeration of *E. coli*. Of the 22 sites, 19 contained one or more poults with *E. coli*

ranging from 1.0×10^3 to 7.7×10^5 cfu/g. The level of *E. coli* was not related to the age of the poul. *E. coli* levels varied between birds within a site and between sites. *E. coli* colonies from each poul were isolated for PCR analyses. Multiplex PCR analysis of the 147 *E. coli* indicated that 7.5% of the isolates had all four virulence genes present, while 32.0% had three of the genes, 24.5% had two of the genes, 30.6% had one of the genes and 5.4% had none of the four genes present. The *tsh* gene was the most common at 67.3% followed by the *iucC* gene at 64.6%, *cvaC* gene at 51.7%, and *iss* gene at 21.1%. RAPD PCR analysis using two primers indicated that the 147 isolates belonged to 75 clusters at a similarity coefficient of 80%. *E. coli* strains within a cluster did not contain the same pattern of virulence factors. Most sites contained pathogenic isolates with a variety of RAPD DNA fingerprints. These results indicate that the pathogenic *E. coli* at these sites were a heterogeneous population. Overall, the use of multiplex PCR combined with RAPD PCR was useful for studying the distribution and diversity of pathogenic *E. coli*.

Key Words: Poults, *E. coli*, Virulence

338 Co-infection of hens with *Salmonella typhimurium* and *S. enteritidis* reduces *S. enteritidis* infection severity during induced molt. P. S. Holt* and R. K. Gast, *Southeast Poultry Research Laboratory, Athens, GA USA.*

It has been shown previously in the field that multiple *Salmonella* serovars can infect laying flocks simultaneously. Such co-infections can have dramatic effects on the survival and persistence of other salmonellae, including *S. enteritidis*. Prior studies in our laboratory demonstrated that *S. enteritidis* infections in hens undergoing molt via feed withdrawal were substantially more severe than in their unmolted counterparts and we have been investigating various situations which may ameliorate this problem. In the current study, hens were infected with *S. typhimurium* 7 days prior to feed withdrawal and then challenged with *S. enteritidis* four days into the molt. Hens receiving the *S. typhimurium* shed significantly less *S. enteritidis* at day 10 post challenge in Trial 1 and days 3 and 10 post challenge in Trial 2. Significantly fewer *S. enteritidis* organisms were detected in livers and spleens in hens receiving the *S. typhimurium* prior to *S. enteritidis* challenge in Trial 2. These results indicate that the presence of other serovars of *Salmonella* can reduce potential *S. enteritidis* problems that may occur in hens during molt.

Key Words: Induced molting, Salmonella, Food safety

Physiology

339 Development of a cannulation procedure for broiler breeder hens. H.-K. Liu*, J. A. Anderson, and W. L. Bacon, *The Ohio State University.*

Restricted feeding of broiler breeder hen candidates during growth and reproductive periods is a standard industry practice to achieve increased reproductive efficiency. High resolution patterns of concentration change

of hormones associated with reproduction in restricted-fed in comparison to full-fed hens are poorly documented. To monitor the concentration change patterns of these reproductive hormones associated with oviposition and ovulation, we have developed a jugular vein cannulation and serial bleeding procedure. After cannulation, the hens were returned to individual cages equipped with a tether and swivel system for serial

bleeding and long-term maintenance. With the procedure, a short-term study with hens bled every 12 min for 36 hr, and 4 long-term studies with hens bled hourly for 10 d have been completed. For both studies, 1.5 mL blood samples were collected using sodium citrate as anticoagulant, and after removal of plasma, the red blood cells were reconstituted to original volume in saline and returned to the hen of origin to avoid hemodilution. Successful collection of serial blood samples was 75 to 100% for 2 short term studies, and 53 to 100% for 4 long-term studies. Egg production was not affected ($P \geq 0.05$) by cannulation for up to 6 weeks. Also, egg production for 10 d prior to cannulation and serial bleeding was not different ($P \geq 0.05$) from egg production for 10 d during serial bleeding in the 4 long-term trials. We conclude that these procedures can be used to study short-term or long-term reproductive hormone concentration change patterns associated with oviposition and ovulation in laying broiler breeder hens with no effect on egg production.

Key Words: Broiler breeder hen, Cannulation, Serial bleeding, Hormones, Egg production

340 Precocious semen production by turkey breeders. B. Koyeri* and W. L. Bacon, *The Ohio State University*.

Artificial Insemination (AI) is used in all commercial turkey breeding operations. In the present study, the effects of lighting treatments on age and duration of sexual maturity, rate of sperm production during sexual maturity, and fertilizing ability of sperm were studied. The three lighting treatments studied were continuous (Continuous) lighting from 4 weeks of age (WOA), early long-day (ELD) lighting (16L:8D) from 4 WOA, and conventional lighting (Conventional) of 16L:8D to 16 WOA, then 10L:14D to 27 WOA, and then 16L:8D. Treatments were at 25 lux intensity. Semen volume was estimated by weight and sperm concentration by a spectrophotometric procedure. Sperm production rate was then calculated weekly. Age at sexual maturity was earlier ($P \leq 0.10$) for the ELD treatment than for the Continuous and Conventional treatments. The males in all groups were still sexually mature when the trial was terminated. Sperm production rate was higher in the Conventional than the Continuous treatment ($P = 0.07$) and ELD treatment ($P \leq 0.01$). Testes weights at termination of the study followed the same pattern as sperm production rate. Fertilizing ability of sperm was tested three times during the trial (early, middle and late insemination periods) by inseminating test hens with 125 million sperm weekly for two wk and allowing fertility to drop to 0% before inseminating the hens again. Fertility was constant and high for the first 3 wk following each insemination period. Duration of fertility or percentage fertility was not affected by lighting treatment or age of the males/females. Percent fertility of the initial three weeks was highest during the middle insemination period, intermediate during the early insemination period, and lowest during the late insemination period. The results of this experiment lead to the following conclusions: 1) semen production of long duration can be effectively induced at an early age with early long day lighting. However, sperm production rate was lowest with this treatment. 2) semen production with a higher sperm production rate can be effectively induced using a conventional lighting treatment.

Key Words: Turkey, Semen, Fertility, Lighting, Sexual maturity

341 Reproductive characteristics and immune responses of cockerels under different thyroid status rendered after puberty. M. A. Abaza, S. A. Elnagar*, and A. El-Sebai, *Alexandria University*.

To study the effects of different thyroid status on male reproductive characteristics and immune responses, 45 Alexandria cockerels (an Egyptian local strain) at 7 months of age were randomly assigned to 3 treatments each of 3 replicates of 5 birds each. Hypothyroid and hyperthyroid status was induced by feeding 0.1% thiouracil and DL-thyroxin at the rate of 0.5ppm in the diet, and the third group maintained an euthyroid status and served as control. Semen was collected weekly to evaluate its quality. At the end of the 4 week experimental period 3 birds from each treatment were slaughtered and testes, thyroid gland, lymphoid organs and the comb were weighed. Also, blood was collected for hematological, T3 and T4 analysis. The induction of hypo and hyperthyroidism were evidenced by the increase and decrease in thyroid gland weights, and by plasma T3 and T4 concentrations. Semen volume was not affected by the different thyroid status induced in this study, but overall, hyperthyroidism enhanced semen quality by increasing concentration and motility and decreasing dead sperm percent. The histological examination of tests,

which showed an increase in the seminiferous tubules, spermatogenesis, and Leydig cells of males under hyperthyroidism status supported the present data. Also, increased comb relative weight indicated enhanced androgen production. Thymus and spleen relative weights and white blood cell count, mainly lymphocytes were higher than the controls' in hyperthyroid males. These results were accompanied with better immune responses to sheep red blood cells (SRBC) and higher immunoglobulins (IgG and IgM) under hyperthyroidism. It can be concluded that hyperthyroidism induced after sexual maturity can enhance reproduction and hypothyroidism had no effect on semen quality and immune responses in adult Alexandria cockerels.

Key Words: Semen, Immune, Thyroid, Cockerels

342 Age influences the response to progesterone injection in laying turkey hens. Wayne Bacon* and Han-Ken Liu, *The Ohio State University*.

An arrest in laying associated with a polycystic ovarian follicle syndrome (PCOF) has been reported in laying turkey hens exposed to constant light early in the egg production (EP) period (*Poultry Sci.* (2001) 80:1509). In hens with the PCOF syndrome, the ovary contained an increased number of follicles and the plasma concentration of progesterone (P) was relatively high and constant. We hypothesized that the PCOF syndrome may be due to the relatively high plasma P concentration which may block preovulatory surges of luteinizing hormone. Six studies were conducted with laying turkey hens of the Egg line. The hens were photostimulated with 14L:10D or constant lighting and injected daily for up to 14 d with P (up to 1.50 mg/kg/d) in canola oil. Duration of EP before the start of injections ranged from 1 wk to 38 wk. Measurements included EP, oviductal weight, and number and weight of ovarian follicles subdivided into hierarchical follicles (Type I), mature size follicles (Type II), cystic follicles (Type III), and atretic follicles. The following results were obtained: 1) in most hens at all ages, EP was reduced with injection of P at a dosage of 0.17 mg/kg/d and ceased within about 2d with injection of P at 0.33 mg/kg/d or greater, 2) for hens with less than 15 wk EP before injected, oviductal weight and Type I ovarian follicle number were the same as controls, but Type II, Type III, and atretic follicle number were all increased, 3) for hens with 38 wk EP before injected, oviductal weight and Type I follicle number both decreased but atretic follicle number increased with injection of 0.33 mg/kg/d of P, and 4) there was no effect of lighting treatment on EP, oviductal weight, or follicle number. From these studies we conclude: 1) ovulation and egg production are blocked by daily injection of P at 0.33 mg/kg/d or greater at all ages studied, 2) when hens are injected with P at a dosage of 0.33 mg/kg/d or greater relatively early in the EP cycle, new follicles continue to enter the hierarchy and reach maturity and may then be maintained as Type II or Type III follicles or become atretic, and 3) when hens are injected with P at a dosage of 0.33 mg/kg/d or greater relatively late in the EP cycle, additional follicles cease to enter the hierarchy.

Key Words: Turkey, Progesterone, Egg production, Polycystic ovary

343 Localization of photostimulated fos-like and VIP immunoreactivity in the turkey hen tuberal hypothalamus. L. L. Hurley*¹, T. D. Siopes², and J. R. Millam¹, ¹*University of California, Davis, CA*, ²*North Carolina State University, Raleigh, NC*.

Exposing photosensitive turkey hens to long days induces fos-like immunoreactivity (FLI) in neurons and glial cells in the tuberal hypothalamus. These cells are implicated as a neural locus of photorefractoriness in turkey hens because the FLI cells are essentially absent in photorefractory hens photostimulated after a two week period of short days, an insufficient amount of time to reverse photorefractoriness. The phenotypic identity of FLI neurons is not established, although vasoactive intestinal polypeptide is a candidate based on its previously reported occurrence in the tuber and its association with seasonality in several species. We therefore conducted detailed single-label studies of FLI and vasoactive intestinal polypeptide immunoreactivity (VIP-ir) throughout the tuberal hypothalamus to determine the likelihood that FLI neurons contain VIP.

We found FLI and VIP-ir had both overlapping and non-overlapping distributions, with the distribution of FLI cells being more extensive than VIP-ir. We conclude that while some tuberal FLI neurons may contain

VIP, the neurons activated by photostimulation in photosensitive hens probably represent more than one phenotype.

Key Words: Photostimulation, Photorefractoriness, Vasoactive intestinal polypeptide, c-fos, Turkey

344 Immunohistochemical visualization of the vasotocin VT2 receptor in the pituitary gland of the domestic chicken. A. Jurkevich^{*1}, L. R. Berghman², L. E. Cornett³, and W. J. Kuenzel¹, ¹Department of Poultry Science, University of Arkansas, Fayetteville, AR, ²Texas A&M University, TX, ³University of Arkansas for Medical Sciences, Little Rock, AR.

Arginine vasotocin (VT), besides its well-known osmoregulatory function, plays a role in the neuroendocrine control of reproduction and stress response of the domestic chickens. These multiple central and peripheral functions of VT could be mediated by different target cells and/or by different subtypes of the VT receptors. Recently the gene encoding a new subtype of VT receptor denoted as VT2 was cloned from the chicken. The aim of this study was to determine by immunohistochemistry the distribution of the VT2 receptor protein in the pituitary gland of male broiler chickens. The mouse monoclonal antibody was produced against the synthetic fragment of the aminoterminal of the chicken VT2 receptor protein. Our data confirmed earlier findings showing the expression of the VT2 receptor gene in the pituitary gland. Immunoreactive (ir) cells were revealed throughout the entire cephalic lobe and in the posterior and ventral aspects of the caudal lobe of adenohypophysis. One of the prime candidates for containing VT2 are corticotrophs, however, labeled cells containing receptor ir differed in shape and grouping suggesting that cells of more than one endocrine phenotype express this receptor. Ir nerve terminals were found in the intermediate fiber layer of the anterior median eminence. Very dense ir terminals were further observed in the most caudal aspect of the external zone of the posterior median eminence and throughout the neurohypophysis. These last findings indicate that ir VT2 receptor protein in the neurohypophysis is likely to be originating from the sites of synthesis within the brain. Studies aiming to identify specific endocrine phenotypes of adenohypophyseal cells containing the VT2 receptor are currently underway. [Supported in part by NSF grant # IBN-01111006].

Key Words: Vasotocin, Pituitary gland, Vasotocin receptor

345 Expression of insulin-like growth factor (IGF) genes in liver and brain tissue during embryonic and post-hatch development of the turkey. M. Richards^{*}, S. Poch, S. Clarke, and J. McMurtry, USDA, ARS, Growth Biology Laboratory, Beltsville, MD.

Because of their multiple effects on cellular differentiation and metabolism, IGF-I, IGF-II and IGF-I receptor (IGF-IR) are thought to play important roles in regulating the growth and development of avian species. A molecular cloning strategy using primer-directed reverse transcription polymerase chain reaction (RT-PCR) was developed to sequence 1300 bp of a turkey liver-derived cDNA corresponding to the complete coding region and the 5'- and 3'-untranslated regions of the IGF-II mRNA transcript. The turkey IGF-II gene codes for a 187 amino acid pre-protein that includes a signal peptide, the mature hormone, and a C-terminal extension peptide comprised of 24, 67 and 96 amino acids, respectively. Turkey IGF-II showed greater than 95% sequence identity at both the nucleotide and amino acid level with chicken IGF-II. The expression of IGF-I, IGF-II and IGF-IR genes was quantified relative to an internal 18S rRNA standard by RT-PCR in liver and whole brain tissue on days 14, 16, 18, 20, 22, 24 and 26 of embryonic development, as well as at hatch (H, day 28) and at 3 wk post-hatching (PH). Expression of liver IGF-I was low throughout embryonic development, but increased more than 10-fold by 3 wk PH. In contrast, IGF-I was expressed in brain tissue at much higher levels than liver throughout development and this level of expression in brain increased gradually, reaching a peak at 3 wk PH. IGF-II was expressed at comparable levels in brain and liver tissue, except for transient increases in liver just prior to H (days 24 and 26) and at 3 wk PH. Expression of IGF-IR declined in brain throughout development reaching its lowest level at 3 wk PH. In liver, IGF-IR expression was lower than that of brain throughout development. An inverse relationship was observed for the expression of IGF-I and IGF-IR genes in brain, but not in liver, through 3 wk PH. Our data suggests differential

regulation of IGF gene expression during growth and development of the turkey.

Key Words: Insulin-like growth factor, Turkey, Gene expression, Development, Growth

346 Identification of nuclear and cytoplasmic peptides from the chicken bursa of Fabricius associated with inhibition of mitogen-stimulated B-cell DNA-synthesis. G. Garcia-Espinosa^{*1}, S. Clerens², L. Arckens², G. F. Erf¹, and B. M. Hargis¹, ¹University of Arkansas, Fayetteville, AR/USA, ²Katholieke Universiteit Leuven, Leuven/Belgium.

The bursa of Fabricius (BF) is responsible for B-lymphocyte ontogeny in avian species. However, substantial experimental *in vitro* and *in vivo* observations have postulated this organ to be potentially related to endocrine activities. Previously, we extracted, purified and identified a ~32kDa protein from the BF with remarkable anti-DNA synthesis and anti-steroidogenic activities in avian and mammal cells. This protein, named bursal anti-steroidogenic peptide (BASP), has been recently identified to be indistinguishable from the histone H1 (HH1) family. However, during the reverse-phase HPLC (rpHPLC) purification of bursal extract, a moderate anti-DNA synthesis bioactivity was found involving proteins determined to be between 3-10kDa smaller than intact histone H1. Presently, we concentrated and subjected those proteins to mass spectrophotometric (MS) Q-TOF and MS/MS analysis. The partial amino acid sequence identified several nuclear and cytoplasmic proteins with a 100% match to the following chicken proteins: 1) thymosin β 4, 2) nonhistone chromosomal protein high mobility group 17 (HMG-17), 3) HMG subtype 14A, and 4) histone H1. Additionally, two different fragments were identified to be the homologous 60S ribosomal protein and thymosin/interferon-inducible multigene family member protein described in mammals. The molecule responsible for the BASP bioactivity was not identified in the present study. The known hormone-like bioactivity of nuclear HH1 and cytoplasmic thymosin β 4, in relationship to BASP, are currently under investigation.

Key Words: Bursa of Fabricius, Anti-DNA synthesis, Peptides

347 Effect of selected feed or water acidifiers on enteric pH of chicks. R. L. Jarquin^{*1}, G. M. Nava¹, A. D. Drake¹, S. E. Higgins¹, L. A. Newberry¹, D. J. Donoghue¹, A. M. Donoghue², and B. M. Hargis, ¹Department of Poultry Science, University of Arkansas, Fayetteville AR 72701, ²USDA-ARS-PPPSRU University of Arkansas, Fayetteville AR. 72701.

There is some commercial use of feed/drinking water (DW) acidifiers for putative modification of gut flora. We evaluated the effect of commercially-available DW (PerformaxTM), DW (PWT[®]) and/or feed (Biotronic[®]USACID) acidifiers, according to label directions, on the lower ileal and cecal pH of chicks in 5 Exps. For each Exp, chicks received balanced feed/water ad libitum, age-appropriate temp., in 1.8 m² pens, with clean shavings. In Exp 1, each DW acidifier, or untreated control, were individually administered on day 8 (n=55/pen). After 8 h treatment (TRT), the cecal pH of PerformaxTM (5.6) or PWT[®] (5.8) groups were lower (p<.05) than controls (6.2). After 25 h, the cecal pH of the PerformaxTM (5.6) group was lower (p<.05) than controls (6.5) or PWT[®]-TRT chicks (6.2). After 48 h, no TRT-associated changes in pH were observed. TRT-associated ileal pH changes were not observed (p>.05) at 8, 25, or 48 h. In Exp 2, control, PerformaxTM, neomycin(.4g/l) +penicillin (2.5g/l) , or PerformaxTM + neomycin+penicillin were evaluated beginning on day 8 (n=60/pen). No difference (p>.05) in ileal (range: 6.1-7.2) or cecal (range: 5.7-6.7) pH was observed at 6, 25 or 48 h. In Exp 3, one pen received PerformaxTM in DW for 48 h, with 4 doses PerformaxTM(0.25 ml) by oral gavage each h during the last 4 h. Controls received water alone by sham gavage and DW. At 48 h, no difference (p>.05) in ileal (range: 6.6-6.7) or cecal (range: 5.6-5.8) pH was observed between TRTs. In Exp 4, control, PerformaxTM, Biotronic[®]USACID, or PerformaxTM+Biotronic[®]USACID were compared (n=15 per pen). TRTs were initiated on day 1, and PerformaxTM was additionally administered by gavage 4x as per Exp. 3 during the last 4 h of the 72h TRT. Ileal pH was reduced (p<.05) by PerformaxTM (5.3) and Biotronic[®]USACID (5.2) alone, but not in combination, as compared to controls (5.9). No TRT-associated change (p>.05) in cecal pH was observed. In Exp 5, TRTs described in Exp 4 were repeated and no TRT-associated change in ileal or cecal pH was observed at 6, 12, 24, 36,

52, 74, or 120 h. These Exps indicate that these water or feed acidifiers do not consistently alter lower ileal or cecal pH in young chicks.

Key Words: Intestinal pH, Acidifiers, Chicks

348 Primordial germ cells as possible somatic cell nuclear recipients in the domestic chicken. T. Minematsu*, A. Tajima, and Y. Kanai, *Institute of Agriculture and Forestry, University of Tsukuba.*

Somatic nuclear transfer technique is an attractive means for conserving avian genetic resources as well as for producing transgenic birds. In this regard, primordial germ cells (PGCs) could be potential recipients of the somatic cell nuclei. In the present study, the effect of UV irradiation for inactivating the PGC nucleus as well as condition of electrofusion with embryonic blood cells (EBCs) was investigated. In Experiment 1 PGCs collected from 2-day-old White Leghorn embryos were irradiated with UV (254 nm, $0.9 \pm 0.1 \mu\text{W}/\text{cm}^2$) for 0, 10, 30, 60, or 120 seconds. The DNA fragmentation of PGC nuclei was analyzed by comet assay, and the damages on cell membrane and cytoplasm were examined by double staining with PI and FDA, respectively. UV treated PGCs were then transplanted into 2-day-old embryos after labeling with fluorescent dye (PKH-26). The number of fluorescent-labeled germ cells in the gonad was counted at 5 days after transfer. In Experiment 2, in order to examine the condition of electrofusion between fluorescent labeled PGCs and EBCs, the effects of AC field, DC pulses, cell density and saccharose concentration of fusion fluid were investigated. After electrofusion, fluorescent-labeled PGCs were stained with Hoechst 33342 and observed under fluorescent microscope. Experiment 1 revealed that the UV irradiation induced dose dependent DNA specific damage on PGCs nuclei. The transplantation experiment showed that the number of fluorescent labeled germ cells decreased significantly when PGCs were exposed to UV for more than 30 seconds compared with control. Electrofusion (Experiment 2) resulted in approximately 10 % of 20 PGCs on average, being fused with EBC under the following conditions; AC field of 350 V/cm for 60 seconds, three DC pulses of 4 kV/cm, 2×10^4 EBCs were dispersed to 0.25 M saccharose solution. The combination of these techniques could lead to the production of nuclear transferred birds.

Key Words: PGCs, Somatic nuclear transfer, Chicken

349 The effect of a feed removal molting program on the skeletal integrity of White Leghorns. H. Mazzuco*^{1,2}, I. Grader¹, and P. Y. Hester¹, ¹*Purdue University, W. Lafayette, IN,* ²*Embrapa Swine and Poultry, Brazil.*

A molting program using feed withdrawal and light restriction is a common industry practice to induce an ovarian arrest leading to a second cycle of egg production. The objective of the current study was to determine the effect of an induced molt on the skeletal integrity of a pedigree line of Hy-Line White Leghorns. Molt was induced at 76 wk of age through light restriction (8 hr/d) and feed removal for 10 days followed by ad libitum consumption of cracked corn for 7 days and a pullet developer diet for 10 days. Molted hens were returned to a photoperiod of 16 hr and an egg laying diet at 27 d post-molt. The control birds consumed the egg laying diet throughout the experiment and were exposed to an unchanging daily photoperiod of 16 h. Bone mineral densities (BMD, g/cm^2) of the left leg (tibia and fibula) and wing (humerus) were determined in 6 live, unanaesthetized hens subjected to the molting regimen and 6 control non-molting hens using a Norland pDexa X-ray bone densitometer (Model No. 476D014). Using the mixed model procedure of SAS, an analysis of variance with repeated measurements (-1, 1, 3, 5, 7, 9, 11, 18, 25, 32, 39, and 67 days post-molt) was conducted using the molting treatment as the whole plot with the type of bone (tibia and humerus) within a bird as a sub-plot. The treatment by age interaction was significant for both BW and BMD ($P < 0.0001$). Molted hens lost 28% of their BW by 9 days post-molt with a gradual return to pre-molt values by day 67 post-molt. Molted hens as compared to controls experienced a precipitous drop in BMD during the molt. By 25 days post-molt, molted hens experienced a 28% loss in BMD when compared to their pre-molt values. A gradual recovery in BMD occurred in the molted hens with the return of the higher calcium laying hen diet on day 27. These results suggest that skeletal integrity is compromised during the molt period (days 1 through 25) with evidence towards recovery in

BMD by 67 days post-molt. H. Mazzuco was supported by a scholarship from CNPq, Brazil.

Key Words: Molting, Bone mineral density, Feed withdrawal, White Leghorns

350 Comparative assessment of bone parameters among wild-type, restricted ovulator, and out of production hens. W. K. Kim*¹, B. C. Ford¹, A. Mitchell², R. G. Elkin¹, and R. M. Leach Jr.¹, ¹*The Pennsylvania State University, University Park, PA,* ²*USDA-BARC, Beltsville, MD.*

Restricted ovulator (RO) hens are generally unable to ovulate due to a point mutation in the oocyte VLDL receptor gene whose protein product mediates the uptake of yolk precursors. Since RO hens should not have cyclic calcium metabolism associated with egg shell formation, they could be a useful model for studying bone metabolism. Blood was collected to measure plasma Ca from 17 wild-type (WT), 12 RO, and 6 out of production (OP) hens. Tibia, femurs, and humeri were collected from each bird to evaluate various bone parameters using mineral assays and dual-energy X-ray absorptiometry (DEXA). Plasma Ca levels of RO hens (40 mg/dL) were significantly higher than WT (24 mg/dL) and OP hens (11 mg/dL). RO hens had significantly higher humerus, femur, and tibia ash contents, femur and tibia percent ash, and humerus, femur, and tibia ash concentrations than WT and OP hens. Bone mineral content and density obtained with DEXA closely mirrored the results of mineral analysis. Moreover, differences of mineral content and density among the treatments in femur and tibia, which contain medullary bone, were much greater than humerus which lacks medullary bone. Bone parameters of femur and tibia were highly correlated each other whereas those of humerus were less highly correlated to femur and tibia. Correlation coefficients of humerus, femur, and tibia mineral content between mineral assay and DEXA values were 0.942, 0.996, and 0.989, respectively. Mineral densities using DEXA were also highly correlated to their ash concentrations (0.997). These results indicated that 1) greater serum Ca concentration in RO hens is most likely a reflection of elevated levels of circulating Ca-binding yolk precursor macromolecules, 2) better bone status in RO hens reflects increased serum estrogen and lack of cyclic Ca metabolism associated with egg shell formation, and 3) DEXA is a good tool for endpoint bone measurements.

Key Words: Restricted ovulator, Bone parameters, Calcium

351 BrdU administration to avian embryos. D. T. Moore*, P. R. Ferket, and P. E. Mozdziaik, *North Carolina State University.*

5-bromo-2#-deoxyuridine (BrdU) is a thymidine analog that labels cells entering the S-phase of the cell cycle. The eggshell makes it technically difficult to deliver BrdU to avian embryos. Therefore, a new technique was developed to administer BrdU to turkey embryos in ovo. The point of entrance for the intra-abdominal injection of BrdU in ovo was determined by candling. An entrance into the eggshell was accomplished by incising a 5mm diameter hole in the shell. After the intra-abdominal injection of BrdU (10mg/ml; 1ml/100grams of egg weight), the holes were sealed with scotch tape, and the eggs were placed in the incubator for two hours. Success of the technique was determined by labeling sections of Pectoralis thoracicus with a primary monoclonal (anti-BrdU) antibody detected with a goat anti-mouse IgG conjugated to fluorescein-5-isothiocyanate (FITC). The tissues were counterstained with propidium iodide (PI) to label all nuclei. After labeling, a Leica DMR microscope equipped with epifluorescence illumination was used to observe the tissue sections. All nuclei were observed with a propidium iodide (PI) filter set, and the BrdU labeled nuclei were observed with a FITC filter set. A Spot-RT CCD camera was used to capture the images of nuclei visualized by the FITC and PI filter sets. The number of BrdU labeled nuclei per 1000 PI labeled nuclei was used as an index of myoblast mitotic activity. The BrdU injection technique was deemed successful because 13 out of 13 injected embryos successfully stained for BrdU. The technique was used to determine developmental differences in myoblast proliferation between 25 days of incubation (25E) to day of hatch in the turkey. Myoblast mitotic activity was significantly lower ($P < 0.05$) at 25E (0.014 0.0018) when compared to day of hatch (0.078 0.016). The low level of myoblast mitotic activity at 25E may represent a target for embryonic manipulations aimed at increasing meat production.

Key Words: Myoblast, Turkey, Embryonic development, Skeletal muscle

352 Feed withdrawal alters intestinal morphology and attachment of *Salmonella enteritidis* in broilers. K. M. Burkholder*, K. L. Thompson, K. M. Banks, T. J. Applegate, and J. A. Patterson, *Purdue University, West Lafayette, IN 47907.*

Feed withdrawal during molting or transportation may cause increased food-borne pathogen colonization in broilers. A trial was conducted to determine the effects of feed withdrawal on the intestinal morphology and microbial ecology of the broiler. Male broilers were raised on industry diets to 44 d of age in floor pens. Feed was withdrawn (0 hr) and birds were kept on litter and given access to water for 4 hr before being placed in transport crates. For morphology measurements, five birds were sampled at 0, 8, 12, and 24 hr. Distal ileal sections were collected, fixed in neutral buffered formalin, routinely processed, sectioned, and stained with hematoxylin and eosin for determination of morphological characteristics. Although no significant effects were noted for villi height, villi width was significantly reduced in birds at the 8 (141.01 μ m), 12 (139.75 μ m) and 24 (121.81 μ m) hr feed withdrawal times when compared to the 0 hr feed withdrawal time (175.75 μ m; $P < 0.05$). Crypt depth at the 24 hr feed withdrawal time (105.73 μ m) was significantly less than the 0 (162.69 μ m), 8 (137.52 μ m), and 12 hr (139.00 μ m) feed withdrawal times ($P < 0.05$). Pathogen attachment was determined using an in vitro ileal loop assay. Ten replicate, 10 cm ileal samples from 0 and 24 hr time points were rinsed and inoculated with 7ml of 5×10^9 cfu *S. enteritidis* culture. Samples were incubated in DMEM for one hr with 10% CO₂ at 37°C. After incubation, tissues were washed, homogenized and *Salmonella* were enumerated on LB plates. Birds withheld from feed for 24 hr had increased attachment of *S. enteritidis* by 1.5 logs (7.59 vs 9.05 cfu/g ileum for control and fasted birds, respectively; $P < 0.0001$). Replicates within time periods were not different ($P > 0.1$). The observed changes in intestinal morphology and increase in *S. enteritidis* attachment may contribute to increased bacterial translocation and contamination during feed withdrawal.

Key Words: Broiler, Feed withdrawal, Intestinal morphology, *Salmonella enteritidis*

353 Protection of DNA and cellular membranes from reactive oxygen species mediated damage by uric acid and the effect of dietary induced changes in plasma uric acid on pulmonary hypertension syndrome (ascites) in broilers. B. M. Stinefelt*¹, S. Leonard², X. Shi², J. S. Moritz¹, K. P. Blemings¹, and H. Klandorf¹, ¹*West Virginia University, Morgantown, WV/United States*, ²*National Institute of Occupational Safety and Health, Morgantown, WV/United States.*

Two studies were conducted to investigate the potential antioxidant activity of uric acid (UA). Study one investigated the in vitro quenching effect of UA on specific reactive oxygen species (ROS) and the ability of UA to protect DNA and cellular membranes from ROS mediated damage. Hydroxyl and superoxide radical ESR signals were both reduced by addition of UA in a concentration dependent manner ($P < 0.05$). Moreover, UA prevented lipid peroxidation in RAW 264.7 cells exposed to a silica based stimulant of ROS generation ($P < 0.015$). Uric acid inhibited hydroxyl-mediated damage to Hind III digested λ DNA, as indicated by the presence of intact bands after electrophoresis. The objective of study two was to determine the effect of increased plasma UA (PUA) on growth performance and measures of ascites in broilers. Ninety six 308 x 344 Ross (20 day old) broiler chicks were randomly allotted to 2 rooms of 12 pens each. Chicks were fed a grower diet supplemented with 0.5 moles inosine/kg feed to raise PUA, 15 mg allopurinol/kg body weight/day to lower PUA, or a control diet. Birds were fed these diets until day 32 when inosine and allopurinol supplements were discontinued. From day 21 through 42 birds in one room were maintained at 14 degree C and minimal ventilation to promote ascites, while standard growing conditions were used in the second room. It was hypothesized that inosine treated birds would show resistance to ascites development whereas birds treated with allopurinol would show an increase in ascites expression. Plasma uric acid measured on days 21 and 32 was higher ($P < 0.001$) in inosine treated birds than control or allopurinol treated birds. Although a difference in PUA was observed, this increase had no effect on performance or ascites measured on day 42. Given that ascites was not particularly severe, further investigation into the role of uric acid on performance is warranted.

Key Words: Uric Acid, Ascites, Antioxidant

354 Hatchability of chicken embryos following intra-somite injection. C. Giamario*, J. N. Petitte, and P. E. Mozdziaik, *North Carolina State University.*

Manipulation of the avian embryo in vitro and in ovo during the first few days of incubation is a powerful classical tool for cell lineage analysis and developmental biology research. In most cases, manipulations are performed to examine effects on the embryos for a short period of time. However, a few researchers have attempted to employ manipulations on the chicken embryo and taken their experiments to hatching. The hatchability of manipulated embryos has been extremely poor, which reduces the possibility of examining the posthatch effects of the embryonic manipulation. The objective of the current study was to test the effects of somite injection of the chicken embryo on subsequent hatchability. White Leghorn embryos were incubated to reach stage 10-15. Eggs were positioned under a dissection microscope with the blunt end uppermost. The egg was illuminated with a paired fiber optic light source with blue gelatin filters. A window was made over the air cell and the inner shell membrane was removed exposing the underlying embryo. The embryo was staged based upon the number of somites. Subsequently, 3 to 4 individual somites were injected with cells suspended in culture medium (3 microliters) using a micropipette. The egg was sealed with plastic cling film and placed in an egg incubator. The eggs were rocked hourly until day 18 of incubation, and the eggs were transferred to a hatcher. We achieved between 19 and 60% hatchability following embryonic manipulation of the somites. Therefore, it is possible to manipulate avian somites, and achieve a level of hatchability that will allow for the study of posthatch chickens.

Key Words: Embryonic development, In ovo culture, Microinjection

355 Strain differences in endocrine responses to high temperatures: possible involvement of heat shock protein. D. J. Franco*, L. G. Robeson, and M. M. Beck, *University of Nebraska-Lincoln, Lincoln, Nebraska.*

Although Hyline W36, W98, and Brown hens lay approximately the same number of eggs/hen housed to 80 weeks, fewer eggs and more soft shelled or broken shell eggs are observed during periods of heat stress. Studies evaluated differences in gut calcium uptake and endocrine status in the 3 strains under various heat stress regimens. Heat stress was created by a temperature controlled chamber. In one study, birds of each strain were exposed for one hour at 35C, while others remained at thermoneutral conditions (22C). Blood samples were collected before and after each temperature exposure and analyzed for circulating estradiol, LH, and progesterone. After that, the hens were kept at 22C for five days, and then all birds were exposed to 35C for 18 hours. Hens were euthanized by cervical dislocation and immediately a 3-cm segment from the mid-duodenal loop was cut into six thin slices (1.5 cm x 2mm wide) and incubated in calcium transport buffer for 10 min at 37C and then for 4 and 9 min in 45Ca (25,000cpm/100mL). The reaction was terminated by transferring the slices to mannitol solutions. 45Calcium was extracted from the tissue with trichloroacetic acid (TCA), the activity of the 45Ca was counted, and calcium uptake rates were expressed as nanomoles per gram of duodenal tissue per min. In a second series of studies, hens were exposed to various heat stress regimens, with blood samples collected before and after exposure. Immediately after exposure, livers were removed and processed for heat shock protein analysis using Western blots with β -actin as the housekeeping protein. The results show a strain effect ($p \leq 0.09$), with the higher rate of calcium transport in the W36 followed by the W98 and the Browns. In a third study, hormone concentrations, which are depressed within the first day of heat stress in all 3 strains, recovered during exposure to chronic (2wk) stress. Initial results indicated that transient pre-exposure to heat stress did not induce changes in heat shock protein expression in the liver. However, the results show that the response to heat stress differs by strain with regard to endocrine status and calcium homeostasis representing an important economical factor to be considered.

Key Words: Laying hen, Calcium uptake, Heat stress

356 Testicular responses of male quail to heat stress: effects on steroidogenesis. H. Taira*, L. G. Robeson, and M. M. Beck, *University of Nebraska-Lincoln, Lincoln, Nebraska.*

Heat stress is known to have significant effects on spermatogenesis in male birds. In this series of studies, the effects of heat stress on Leydig cell function were investigated. 3-hydroxysteroid dehydrogenase (3b-HSD)

is one of the critical enzymes in Leydig cells steroidogenesis. Comparisons were made between young, not yet sexually mature (1 month old) quail and adults (> 1 year of age) and between adult males subjected to heat stress or thermoneutral conditions. Blood samples were taken at the beginning of each study and before and after heat stress, when appropriate. Testes were removed, weighed, and frozen. Frozen testes were sectioned at 8 μ m on a cryostat. Sections were incubated in the presence of the steroid precursor, dehydroepiandrosterone (DHA), and a dye, nitro blue tetrazolium; the cells in which 3 β -HSD is active accumulate blue formazan deposits. Sections were lightly counterstained with fast green for identification of Leydig cells and the activity of 3 β -HSD was determined using oil emersion. Enzyme activity for control birds (adult, not heat stressed) was set at 100%, and the activity of the experimental birds (young or heat stressed) was expressed as a relative percentage. In these studies, the enzyme activity in young birds was 80%. Although two of the young birds already had a small foam gland, the amount of formazan present in Leydig cells was not different from those without foam gland development. Heat stress reduced the activity of 3 β -HSD; in the heat-stressed birds, no positive cells or only a few positive cells were found. In comparison, positive enzyme staining was extensive in sections of testes from non-heat stressed birds. The blood samples that were collected before and after heat-stress were frozen for testosterone analysis and an aliquot was used to determine heterophil/lymphocyte (H/L) ratios as indicators of general stress. H/L ratios were significantly elevated by heat stress (P=0.0003; 0.1950 & 0.9706, respectively). Reductions in testosterone by heat stress, thus appear to be mediated at least in part by direct effects on enzyme activity in Leydig cells.

Key Words: Leydig cell function, 3 β -HSD, Male quail

357 *Eimeria acervulina* infection elicits an elevation in plasma ghrelin and changes in other metabolic hormones. J. McMurtry*, P. Allen, M. Richards, S. Poch, and D. Brocht, ARS, ANRI, USDA, Beltsville, MD.

One of the most costly enteric parasitic diseases of broilers is coccidiosis. Impaired feed consumption is a major consequence of coccidiosis, which negatively impacts growth and feed efficiency. The cause of the suppression of appetite in coccidial infected birds is unknown. Ghrelin is a recently discovered peptide hormone that has unique properties separate from mammals. In birds, ghrelin is mainly produced in the proventriculus, and has been shown to inhibit feed intake. This study was conducted to monitor plasma ghrelin and other metabolic hormones during a coccidial infection. At 24 days of age, male chicks were inoculated with 500,000 sporulated oocysts of *E. acervulina* (strain #12) per chick (I). A control (C) group received vehicle. Blood and tissue samples were taken prior to inoculation (day 0), and on days 4, 8, and 14 post infection. Feed consumption was determined daily. Body weights were determined at sampling. The extent of infection was confirmed by histology. Plasma levels of ghrelin, IGF-I and -II, GLP-1, glucagon and thyroid hormones were determined by RIA. At 4 days post infection, ghrelin levels in the I chicks were 3-fold higher than in the C group. This is also the period when feed intake was suppressed to the greatest extent. By day 8, ghrelin levels were similar in both groups. On day 4 glucagon levels were greater in the I group compared to the C birds. Conversely, IGF-I and triiodothyronine concentrations were lower in the I birds on day 4. By day 8 post infection, any changes in circulating hormone levels were not different between the two groups, and remained similar through day 14. Infection did not alter IGF-II, GLP-1 or thyroxine levels. It is evident from this study that coccidial infection elicits a dramatic increase in circulating ghrelin, and with the concomitant decrease in feed intake, strongly suggests that ghrelin may be the causative factor. However, confirmation of this association awaits further study.

Key Words: Coccidiosis, Feed intake

358 The development of a homologous radioimmunoassay for chicken leptin. J. P. McMurtry*¹, D. M. Brocht¹, C. Ashwell¹, P. Allen¹, R. Leach², and C. Coon³, ¹ARS, USDA, ²The Pennsylvania State University, ³University of Arkansas.

Leptin is involved in appetite and energy balance regulation, reproduction, and immune function in mammals. Whether leptin has similar roles in birds is largely unknown. To facilitate studies to delineate leptin's function in domestic fowl, an homologous double antibody RIA for chicken leptin has been developed. Recombinantly-derived chicken leptin

(rcL) was used as antigen, radiolabeled tracer, and standard. The resulting RIA has a minimum detection limit of 0.15 ng and effective dose of 1.25 ng. Dose response curves of chicken and turkey plasma were parallel with rcL. The antiserum exhibits a cross-reactivity of 0.25 and 2.0 percent with human and mouse leptin, respectively. A series of experiments was conducted in chickens to evaluate changes in circulating leptin in which alterations in blood levels would be anticipated. A significant decrease in blood leptin was noted following 8 hrs of fasting, and levels returned to pre-fasting levels within 2 hrs of refeeding. Coccidial infections elicit changes in food intake. At 4 days post *E. acervulina* plasma leptin was significantly depressed compared to control chicks, and returned to pre-infection levels at approximately 11 days post infection. Broiler breeder hens reared on a feed restriction regime exhibited significantly lower blood leptin concentrations compared to breeders fed ad libitum. Chicks reared on a zinc deficient diet displayed elevated plasma leptin compared to birds fed a control diet and a pair-fed group. Circulating leptin concentrations were found to be greater in chicks exposed to an elevated ambient temperature (37 C) compared to chicks maintained at 22 C. Chicks reared on a phosphorus-deficient diet had elevated leptin levels in conjunction with elevated corticosterone concentrations. It is evident from these studies that leptin secretion is affected by many factors, including nutritional and health status, as well as environmental temperature.

Key Words: Hormone, Appetite, Energy balance

359 Effects of F-strain *Mycoplasma gallisepticum* inoculation at twelve weeks of age on serum vitellogenin concentrations in commercial egg laying hens. E. D. Peebles*¹, M. R. Burnham², S. L. Branton³, K. O. Willeford¹, J. R. Richardson⁴, and P. D. Gerard¹, ¹Mississippi State University, ²USDA-ARS, SPARC, Food and Feed Safety Research Unit, ³USDA-ARS, SCPR, ⁴Emory University.

At 20, 28, 32, 36, 40, 44, 48, and 52 wk of age, blood was collected and serum was extracted for analysis of vitellogenin (VTG) concentration from layers that had either been sham inoculated (control) or inoculated with F-strain *Mycoplasma gallisepticum* (FMG) at 12 wk of age. Ten birds were housed in each of 8 biological isolation units with 4 units per treatment. Each wk, sera from 4 independently tagged birds in each unit were pooled prior to analysis. The molecular weight of VTG in the pooled serum samples from each unit was estimated by comparison with the electrophoretic mobilities of proteins of known molecular weight in polyacrylamide gels. Additional comparisons were made using serum from estrogenized roosters known to have elevated VTG concentrations. Electrophoretic image analysis and VTG quantitation were determined via a multi-image analyzer and associated software. The effects of bird age, FMG treatment, and their interactions on VTG concentration were determined. Serum VTG concentrations significantly increased in birds between 20 and 28 wk of age. This was followed by a significant decrease between 28 and 36 wk, and another significant increase between 36 and 52 wk of age. However, FMG treatment had no significant effect on serum VTG concentration. Therefore, the effects exerted by FMG on layer performance and egg yolk composition, as noted in previous research, may not be attributed to the influences of FMG on serum VTG concentrations.

Key Words: F-strain *Mycoplasma gallisepticum*, Layer, Vitellogenin

360 WITHDRAWN. . .