

55 Egg folate concentration and indices of folate status in laying hens supplemented with dietary folic acid and 5-methyltetrahydrofolate. G. B. Tactacan¹, M. Jing¹, S. Thiessen¹, D. L. O'Connor², J. C. Rodriguez-Lecompte¹, W. Guenter¹, and J. D. House¹, ¹University of Manitoba, Winnipeg, MB, Canada, ²Hospital for Sick Children, Toronto, ON, Canada.

The deposition of dietary folic acid (FA) into the chicken egg is likely regulated by its conversion to 5-methyltetrahydrofolate (5-MTHF), the predominant form of this vitamin in eggs (>80%). Supplementation of 5-MTHF in the laying hens' diet may therefore enhance total egg folate concentrations. To this end, a study was conducted using equimolar concentration of either FA or 5-MTHF in diets fed to both Shaver White and Shaver Brown laying hens, to investigate their influence on total egg folate concentrations, and indices of performance and folate status. A total of 24 laying hens (24 weeks) from each strain were randomly assigned to receive 1 of 3 (n=8) dietary treatments: 1) basal diet with no supplemental folate and 2) basal diet + 10 mg/kg FA and 3) basal diet + 11.30 mg/kg 5-MTHF, for 21 days. Production performance, plasma homocysteine, liver, serum and egg folate concentrations were measured. Feed efficiency for birds consuming diet 3 improved (P<0.05) by 11.9 and 10.7% respectively, compared to hens consuming diet 1 and diet 2; while egg weight increased (P<0.05) by 6% compared to diet 1. Plasma homocysteine was lower (P<0.05) by 14.2%, while serum and egg folate were higher (P<0.05) by 78.3 and 61.8% in hens consuming either folate species as compared to control. Liver 5-MTHF was not affected by folate supplementation. Together, these data provide evidence that supplementation of FA and 5-MTHF have equivalent effects in enhancing egg folate concentrations and improving folate status in laying hens. Supplementation of 5-MTHF may improve production performance, but this remains to be determined in larger production studies. The data also support a point of regulation of egg folate deposition that is prior to hepatic folate metabolism, possibly at the level of intestinal folate uptake and metabolism.

Key Words: folate, folic acid, 5-methyltetrahydrofolate, egg, laying hen

56 Molecular characterization, tissue distribution and gene expression of reduced folate carrier in laying hens fed the folate supplemented diet. M. Jing*, G. B. Tactacan, J. C. Rodriguez-Lecompte, A. Kroeker, and J. D. House, University of Manitoba, Winnipeg, MB, Canada.

The Reduced Folate Carrier (RFC; SLC19A1) is regarded as an important folate transporter in humans and other mammals. However, its importance in avian systems is unclear. In the present study, the molecular cloning and tissue distribution of RFC and the impact of dietary folate supplementation on the mRNA expression of this transporter were investigated in the chicken. Twenty-four (n = 8/treatment) Shaver White laying hens were randomly divided to receive one of three dietary treatments: a) basal diet with no supplemental folate; b) basal diet + 10 mg/kg crystalline folic acid; or c) basal diet + 11.30 mg/kg 5-methyltetrahydrofolate (5-MTHF) for 21 d. RFC mRNA levels were analyzed by real-time PCR. The results showed that the RFC cDNA containing the full coding region was cloned from duodenum with 99% identity to the reference gene available in GenBank. RFC transcripts were detected in a variety of chicken tissues (e.g., brain, liver, kidney, intestine, etc). Real-time PCR analysis showed no differences (P > 0.05) due to diet in the duodenal and cecal RFC mRNA levels. However, compared with the basal diet, jejunal RFC mRNA was depressed (P < 0.05) in hens fed the 5-MTHF diet, and a reduction (P = 0.077) was also found in hens fed the folic acid diet. Taken together, these data showed that the RFC cDNA containing the entire coding region was successfully cloned from laying hens. A broad tissue distribution of RFC mRNA may indicate the importance of RFC in the folate transport process in chickens. Furthermore, jejunal RFC mRNA was down-regulated by dietary folate supplementation. These findings contribute to our understanding of folate transport in avian systems, including laying hens.

Key Words: reduced folate carrier (RFC), dietary folate supplementation, cloning and tissue distribution, mRNA expression, laying hens

Physiology, Endocrinology, and Reproduction

57 Proteomic assessment of poultry spermatozoa. J. Long*, T. Conn, and W. Garrett, Beltsville Agricultural Research Center, Beltsville, MD.

Fully characterizing the protein composition of spermatozoa is the first step in utilizing proteomics to delineate the function of sperm proteins. To date, sperm proteome maps have been partially developed for the human, mouse, rat, bull and several invertebrates. Here we report the first proteomic analysis of turkey and rooster spermatozoa, using MALDI-TOF and LC-MS/MS. Semen was centrifuged through a discontinuous Accudenz gradient to remove seminal plasma. Protein was extracted from isolated sperm cells and the soluble fraction separated by 2-D SDS-PAGE (pI 5-8). Excised spots were digested with trypsin and prepared for MALDI-TOF analysis. Proteins were identified from Peptide Mass Fingerprints using the MASCOT search engine (Matrix Science). Samples yielding non-significant Mowse probability scores were subjected to LC-MS/MS analysis. When necessary, homology searches were performed for unnamed protein products via BLAST searching. A total of 94 and 36 proteins were identified from turkey and rooster spermatozoa, respectively. All proteins identifications were

limited to *Gallus gallus* and/or *Meleagris gallopavo*. For turkey sperm, 9 hypothetical proteins (6 matching to chicken chromosome open reading frames via BLAST search) were identified, while another 16 were predicted proteins of unknown function. For rooster sperm, 5 hypothetical proteins (2 matching to chicken chromosome open reading frames) were identified, and an additional predicted protein of unknown function was positively identified. Identified proteins were associated with the acrosome (pro-acrosin), mitochondria (enolase I, voltage-dependent anion channel 2, creatine kinase), and flagellum (capping protein, dynein, tektins 1-5). Several chaperone (heat shock protein 70) and calcium-binding (EF-hand protein) proteins also were identified. Three proteins not previously found in sperm were identified: dihydropyrimidinase, mitofilin and mitochondrial tri-functional protein. While the latter 2 mitochondrial proteins most likely exist in sperm from other species, the discovery of dihydropyrimidinase as a predominant soluble protein in poultry sperm poses interesting functional implications.

Key Words: turkey, rooster, sperm, proteome, dihydropyrimidinase

58 Germline replacement by transferring primordial germ cells into sterilized embryos in the chicken. Y. Nakamura*^{1,2}, F. Usui¹, K. Takeda², T. Ono¹, K. Nirasawa², H. Kagami¹, and T. Tagami², ¹*Shinshu University, Minamiminowa, Nagano, Japan*, ²*National Institute of Livestock and Grassland Science, Tsukuba, Ibaraki, Japan*.

It is possible to produce avian germline chimeras by transfer of exogenous primordial germ cells (PGCs) into host embryos. However, a lack of definitive method to incorporate donor PGCs into the host germline has been due to the limitation of applied researches. Here, we report a generally applicable strategy for replacement of the host germline with donor germ cells in the chicken. Barred Plymouth Rock (BPR) and White Leghorn embryos were used as donors and hosts, respectively. Both males and females of PGCs were collected separately from blood of donor embryos incubated for 52 h. Additionally, some PGCs were labeled with the fluorescent dye to monitor the gonadal migration. To deplete endogenous PGCs of host embryos, 100 µg of busulfan was applied at 0 h of incubation. Each of 200 donor PGCs were transferred into bloodstream of busulfan treated- or untreated control host embryos at 55 h of incubation with the same sex combination of donors and hosts. Manipulated embryos were incubated for a total of 6 d, or until they hatched. Germline chimerism in the gonads at 6 d of incubation was evaluated by immunohistochemical analysis using anti-chicken vasa homolog antibody. After sexual maturation of hatched chickens, germline chimerism was evaluated by testcross analysis using artificial insemination with BPR. The proportion of donor PGCs in the embryonic gonads in busulfan treated hosts ($98.8 \pm 1.7\%$) was significantly higher than that in untreated control hosts ($6.9 \pm 1.4\%$) ($P < 0.01$). The hatchability in busulfan treated hosts (40.4%) was significantly lower than that in untreated control hosts (76.5%) ($P < 0.05$). Testcross analysis showed that the germline transmission rate in busulfan treated hosts was 100%. In contrast, the rate that in untreated control host was $5.3 \pm 4.8\%$. These results suggested that donor PGCs were successfully settled to host gonads where endogenous PGCs had been depleted and differentiated normally into functional gametes. In conclusion, the present method enables to produce chimeric chickens in which host germline has been almost totally replaced by donor germ cells.

Key Words: germline replacement, primordial germ cells, sterilization, chicken, busulfan

59 Cryopreservation and transplantation of ovarian tissue in Japanese quail. J. Liu*¹, Y. Song^{1,2}, K. M. Cheng¹, and F. G. Fred², ¹*Avian Research Centre, University of British Columbia, Vancouver, BC, Canada*, ²*Agriculture and Agri-Food Canada, Agassiz, BC, Canada*.

A protocol for the transplantation of frozen-thawed gonadal tissue has been developed as an effective method for germplasm conservation in chickens. We attempted to adapt this protocol for conservation of Japanese quail. Ovaries of week-old quail were removed and frozen in 0.5 ml straws with 10% (v/v) DMSO, using a programmable freezer. The straws were then stored in liquid nitrogen. Vitrification was done by submerging pieces of tissue in 7.5% (v/v) DMSO for 10 min and then 15% DMSO for 2 min at room temperature (RT). The tissue was then quick-frozen and stored in sealed cryovials in liquid nitrogen for at least 48 hr. Straws containing slow-frozen samples were thawed in ice water, and the thawed tissue was rinsed in DMEM containing 10% FBS. Vitrified samples were removed from the vials and transferred in sequence into 1M, 0.5M, and 0.25M sucrose and DPBS, each for 5 min at RT. Viability after freezing was estimated as the viability of disassociated cells using a hemacytometer and by histological examination of frozen ovarian tissue. Frozen-thawed tissue from WB (recessive plum-

age colour) chicks was transplanted into week-old ovariectomized QO (wild type plumage) chicks with some chicks receiving fresh tissue as a control. At sexual maturity, QO recipients were mated to WB males and the production of WB offspring demonstrates successful cryopreservation and transplantation. Both freezing methods led to significantly ($P < 0.05$) lower cell viability compared to fresh cells. Vitrified quick frozen cells survived (77%) significantly ($P < 0.05$) better than step-wise frozen cells (70%). Histological examination showed reduced follicle counts in both methods. Two out of 9 birds receiving slow-frozen grafts produced donor-derived offspring. Further optimization will be needed to improve the efficiency of this cryopreservation method.

Key Words: Japanese quail, ovarian tissue, cryopreservation, transplantation

60 The effect of acute corticosterone injections on offspring sex of laying hens. S. E. Pinson*, C. Parr, and K. J. Navara, *University of Georgia, Athens*.

In the layer and broiler industries, approximately 50% of all chicks that hatch are killed immediately because they are the non-preferred sex. Manipulation of the hens such that they preferentially produce more female or male offspring has the potential to increase efficiency and productivity in the industry. Previous studies in both wild and captive birds suggest that treatment with hormones can stimulate females to manipulate the sex of their offspring before eggs are even ovulated. In particular, chronic treatments with corticosterone, the primary stress hormone produced by birds, stimulated significant skews towards female offspring in avian species and it has been suggested that corticosterone acts by influencing which sex chromosome is donated by the heterogametic female bird into the ovulated ovarian follicle. However, the corticosterone treatment was given over a long period of time, making it impossible to pinpoint when its effects on offspring sex occurred. We treated laying hens with acute high-dose corticosterone injections 5h prior to ovulation and quantified the sexes of the subsequently ovulated eggs. We hypothesized that an injection of corticosterone coincident with the segregation of the sex chromosomes would stimulate females to produce more female offspring than male offspring. Contrary to our predictions, hens injected with corticosterone produced nearly 76% males, a significant bias towards male offspring. Based on the results, we propose that an acute increase in corticosterone during genetic sex determination is a possible mechanism for sex ratio manipulation in birds, and that acute corticosterone exposure, compared with chronic exposure, may act through a different mechanism to skew offspring sex.

Key Words: sex ratio, corticosterone, stress hormones, sex determination, meiosis

61 Use of transponder implants for the accurate determination of air cell temperature, eggshell conductance and their functional relationships in embryonated broiler hatching eggs. R. Pulikanti* and E. D. Peebles, *Mississippi State University, Mississippi State*.

Broiler hatching eggs obtained from a young breeder flock (Ross X Ross 308; 34 wk of age) were weighed and set on 8 replicate trays (incubator levels; approximately 20 eggs per tray) of a single incubator. On Day 11 of incubation, all eggs were weighed, and temperature transponders were implanted in the air cells of 4 randomly selected embryonated eggs per tray for determination of internal egg temperature (IT). Two water filled vials per tray containing transponders were also used for determination of external egg temperature (ET). In the morning (AM)

and afternoon (PM) on each of Days 12, 13, 14, 15, 16, 17 and 18 of incubation, IT and ET were determined. Determinations of embryo livability on Days 10 and 18, and egg weight on Days 11 and 18 of incubation were performed for the calculation of percentage incubational weight loss of embryonated eggs (PEWL). Readings of IT and ET were used for the accurate determination of the water vapor pressure gradient across the eggshell, which was subsequently used with PEWL for the calculation of eggshell conductance (G). Beginning on Day 12 of incubation, average IT and the average difference between IT and ET (IT-ET; DT) increased progressively, with significant increases observed for IT on the AM of Days 15 and 17, and for DT on the AM of Day 13. Both PEWL and average daily PEWL were positively correlated with G and relative G. However, IT on Day 17 of incubation was negatively correlated with G. It was concluded that temperature transponders may be successfully implanted in the air cells of broiler hatching eggs in order to more accurately determine IT and G, that DT increases with embryogenesis after Day 11 of incubation, and that a functional relationship may exist between IT and G.

Key Words: broiler, conductance, embryo, temperature, transponder

62 Preparing the broiler embryo for post hatch hot environmental conditions. Y. Piestun¹, O. Halevy², and S. Yahav*¹, ¹*Institute of Animal Science ARO the Volcani Center, Bet Dagan, Israel*, ²*Department of Animal Sciences, Faculty of Agriculture, the Hebrew University of Jerusalem, Rehovot, Israel*.

During the last decade the incubation period of broilers has gotten more attention. It can be related to the recognition that during this period various manipulations may induce long-lasting-physiological-memory caused by epigenetic adaptation. Thermal (heat) manipulations during the period of the hypothalamus-pituitary-thyroid or adrenal axes development and maturation have demonstrated a significant improvement in thermotolerance acquisition of broiler chickens up to marketing age. However, the effect of these manipulations on the embryos' thermoregulation is still not clear. This study aimed at elucidating the effect of continuously or intermittent (12 h/day) thermal manipulations (TMs) at 39.5°C between days 7 to 16 (included) of embryogenesis on egg shell temperature, oxygen consumption and heart rate of the embryos, as well as on the plasma concentration of the thyroid and corticosterone hormones. During the ectothermic period of incubation, egg shell temperature and oxygen consumption increased along with the elevation of incubation temperature. However, heart rate was relatively constant and plasma concentrations of thyroxine, triiodothyronine and corticosterone were significantly lower in comparison with the control. During the endothermic period of incubation, on days 18 onwards, all parameters were significantly lower than that of control, suggesting a significant decline in the embryo's metabolic rate which lasted along the post-hatch growth period of the chickens. It can be concluded that TM during incubation at periods critical for the hypothalamus-pituitary-thyroid development, induces a long-lasting-thermoregulatory response characterized by significant decline in metabolic rate on pre- (from day 18 of incubation) and post-hatch periods.

Key Words: broilers, embryogenesis, thermal manipulations, long lasting physiological memory

63 Comparison of Cobb and Ross strains in embryo physiology and chick juvenile growth. K. Tona*¹, O. M. Onagbesan², V. Bruggeman³, B. Kamers³, N. Everaert³, and E. Decuyper³, ¹*University of Lome,*

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Broiler performance is known to be related to embryonic developmental parameters. However, strain or genotype differences with regard to embryo physiological parameters and juvenile growth have received little attention. A total of 1,200 hatching eggs produced by Cobb and Ross broiler breeders of the same age were studied. At setting for incubation and between 66 and 130 h of incubation, eggs resonance frequency (RF) was measured as indicator of embryonic development. From d 10 to 18 of incubation, remaining albumen was weighed and at d 18 of incubation, eggs were weighed. During the last days of incubation, hatching events were monitored every two hours. Hatched chicks were recorded and weighed. At internal pipping (IP) stage, gas partial pressures in egg air chamber were measured. Hatched chicks were reared for 7 d and weighed. Results indicate that RF of Ross eggs were lower than that of Cobb eggs ($P < 0.01$) and starting time point of RF decrease occurred earlier in Cobb eggs than in Ross eggs. Relative egg weight loss up to 18 d of incubation was lower in Cobb than in Ross ($P < 0.05$). At IP, pCO₂ was higher in Cobb than in Ross ($P < 0.05$) with shorter incubation duration in Cobb. Between 6 and 60 h post-hatch, heat production (HP) was higher in Cobb than in Ross ($P < 0.05$). At 7 d post-hatch Cobb chicks were heavier than Ross chicks ($P < 0.05$). It is concluded that Cobb and Ross embryos/chicks have different growth trajectories leading in different patterns of growth resulted from differences in physiological parameters.

Key Words: Cobb, Ross, embryo physiology, hatching events, juvenile growth

64 The role of syndecan-4 covalently attached chains in muscle growth and development. Y. Song* and S. G. Velleman, *Ohio Agricultural Research and Development Center, The Ohio State University, Wooster*.

Skeletal muscle formation is a complex process involving the interactions between cells and their extracellular matrix (ECM). Syndecan-4 is a cell membrane heparan sulfate proteoglycan that translocates signals from the ECM into the cell. Syndecan-4 is composed of a transmembrane core protein and covalently attached glycosaminoglycan (GAG) chains and N glycosylation (N) chains. The GAG chains have been shown not to be required for syndecan-4 to regulate muscle cell proliferation and differentiation. The N chains have been reported to be involved in protein proper folding, and cell surface localization of membrane proteins. It is possible that N chains influence syndecan-4 core protein three dimensional structure and the availability of the GAG chains to interact with other molecules. In this study, the role of syndecan-4 N chains and the interaction between GAG and N chains in muscle cell proliferation and differentiation were explored. Turkey syndecan-4 with or without GAG chains was cloned into the pCMS-EGFP vector and used as a template to generate syndecan-4 N chain mutants with or without GAG chains. Syndecan-4 N chains are attached to the core protein at Asn124 and Asn136. The N chain one-chain and no-chain mutants were obtained by changing each Asn to Ala. Wild type syndecan-4, syndecan-4 N chain mutants with or without GAG chains, and the pCMS-EGFP empty vector were transfected into turkey skeletal muscle satellite cells. After transfection, cell proliferation and differentiation were measured. The overexpression of syndecan-4 N chain mutants with GAG chains did not change cell proliferation, while syndecan-4 N chain mutants without GAG chains increased cell proliferation. Cell differentiation was not changed when cells were transfected with syndecan-4 N chain mutants with or without GAG chains compared to wild type syndecan-4. These results suggest

that syndecan-4 GAG chains and N chains function together to regulate muscle cell proliferation, but not differentiation.

Key Words: syndecan-4, muscle, turkey, glycosaminoglycan, N glycosylation

65 Coenzyme Q10 content varies based upon muscle phenotype and age in turkeys. L. S. Nierobisz*, N. G. Hentz, J. V. Felts, and P. E. Mozdziaik, *North Carolina State University, Raleigh.*

The cellular antioxidant Coenzyme Q10 (CoQ10) is one of the major components of mitochondrial electron transport and plays an important role in the production of cellular energy in the form of ATP. Histological alterations in muscle fibers have been correlated with a type of cellular energy. The objective of this study was to determine the relationship between mitochondrial protein, CoQ10 content, and muscle type in turkeys. Anterior Latissimus Dorsi (ALD; slow/oxidative muscle), Posterior Latissimus Dorsi (PLD; fast/glycolytic muscle), Pectoralis Major (PM), and Biceps Femoris (BF) muscles were analyzed in 9 and 20-week old turkey toms. The amount of muscle mitochondria was determined using Bradford assay and CoQ10 content was measured using HPLC-UV. The amount of mitochondrial protein relative to total protein significantly decreased ($P < 0.05$) between 9 and 20 wk of age. Significant differences ($P < 0.05$) in total muscle and mitochondrial CoQ10 content were also observed with age. Additionally, total muscle and mitochondrial CoQ10 content in ALD and BF muscles was significantly higher ($P < 0.05$) than that in PLD and PM muscles. The mitochondrial CoQ10 content significantly increased ($P < 0.05$) between 9 and 20 wks in ALD and BF muscles, but it significantly decreased ($P < 0.05$) in PLD and PM muscles. On week 20, the mitochondrial to total muscle CoQ10 ratio was significantly higher ($P < 0.05$) in ALD and BF muscles than that in PLD and PM muscles. It appears that there is an age-related decrease in mitochondrial and CoQ10 content, and that muscles with a slow phenotypic profile contain a higher proportion of CoQ10 than muscles with a fast phenotypic profile.

Key Words: Coenzyme Q10, HPLC, mitochondria, muscle phenotype, turkeys

66 Mitochondrial proton leak kinetics and relationship to feed efficiency within a single genetic line of male broilers. W. G. Bottje*¹, M. D. Brand², C. Ojano-Dirain³, K. Lassiter¹, M. Toyomizu⁴, and T. Wing⁵, ¹*University of Arkansas, Department of Poultry Science, Center of Excellence for Poultry Science, Fayetteville,* ²*Buck Institute for Age Research, Novato, CA,* ³*Mitochondrial Disease Research Lab, College of Medicine, University of Florida, Gainesville,* ⁴*Graduate School of Agricultural Science, Tohoku University, Sendai, Japan,* ⁵*Cobb-Vantress, Inc., Siloam Springs, AR.*

Studies were conducted to assess proton leak kinetics (proton conductance) in breast muscle mitochondria isolated from broiler breeder males within a single genetic line exhibiting either high (HFE) or low (LFE) feed efficiency. Proton conductance was determined by simultaneously measuring mitochondrial membrane potential (MMP) and State II (resting) respiration rate in breast muscle mitochondria as succinate oxidation was progressively decreased by malonate. Control proton conductance was similar in HFE and LFE mitochondria and decreased in both groups in response to bovine serum albumin (BSA). Whereas treatment of mitochondria with glutamate (Glut) or guanosine diphosphate (GDP) had no effect, retinal (RET) increased and carboxyatracylate (CAT) alone or in

combination with glutamate (Glut) decreased proton conductance relative to control proton conductance in both HFE and LFE mitochondria. Following treatment with either GDP or CAT alone, proton conductance was lower in HFE compared to LFE mitochondria. With the exception of BSA, proton conductance in HFE mitochondria following the various chemical treatments was either less than or equal to, and never greater than proton conductance in the LFE mitochondria. The results suggest that there are subtle differences in membrane characteristics (e.g. lipid composition or integral membrane proteins) that affect proton conductance in broiler muscle mitochondria that may in turn play a role in the phenotypic expression of feed efficiency in broilers.

Key Words: broiler, feed efficiency, mitochondria, proton leak kinetics

67 Functional characterization of the chicken glucocorticoid and mineralocorticoid receptors. M. Proszkowiec-Weglarz* and T. E. Porter, *University of Maryland, College Park.*

Glucocorticoid receptors (GR) and mineralocorticoid receptors (MR) are ligand-activated transcription factors that belong to the nuclear hormone receptor superfamily. Once activated by their steroid hormone ligands, they bind to specific glucocorticoid response elements (GRE) in gene promoters to regulate transcription. Chicken GR (cGR) was recently cloned, while chicken MR (cMR) was only partially sequenced. Little is known about the functional properties of cGR and cMR. Therefore, the aims of this project were to 1) clone the full length cMR, 2) determine whether cGR and cMR proteins have the ability to bind glucocorticoids and mineralocorticoids and induce transcription through a classical GRE, and 3) define the dose-response relationships for cGR and cMR to steroids. Cos-7 cells, lacking endogenous GR and MR, were transiently transfected with pCMVSPORT6.1-cGR or -cMR expression vectors, MMTV-Luc GRE-reporter construct, and pSV40 renilla construct (to correct for transfection efficiency). Cells were then treated with increasing doses of corticosterone (CORT) or aldosterone (ALDO), and transactivation of cGR or cMR was evaluated by luciferase assay. CORT and ALDO induced cGR- and cMR-driven MMTV transcriptional activity in a dose-dependent manner. Each receptor responded to both steroids, but cMR responded to lower levels of CORT and ALDO than cGR. Dose-responses for the two steroids differed significantly ($P < 0.05$) at the 1 and 100 nM doses for cGR and at the 0.1 nM dose for cMR. Furthermore, CORT-dependent transactivation of cGR was significantly ($P < 0.05$) blocked by its antagonist (ZK98299). Our results indicate that cGR and cMR genes express functional, ligand-activated receptor proteins that have the ability to bind to the classic GRE sequence and induce transcription. Better understanding of cGR and cMR function will be helpful in defining their physiological roles in avian species. *Supported by USDA-CSREES Grant #2009-35206-05189.*

Key Words: GR, MR, steroids, nuclear receptor

68 A novel antibody for the detection of gonadotropin releasing hormone receptor-2 protein in chickens. H. O. McFarlane* and G. Y. Bédécarrats, *University of Guelph, Guelph, ON, Canada.*

Two gonadotropin releasing hormone receptors have been characterized in chickens (cGnRHR-1 and cGnRHR-2), with cGnRHR-2 being pituitary specific. The purpose of this study was to validate a novel antibody specific to cGnRHR-2 and, using this antibody, determine if pituitary protein levels change during sexual maturation.

Two antibodies were developed using the BioPerformancePlus protocol (Affinity BioReagents) by immunizing rabbits with synthetic peptides corresponding to portions of the cGnRHR-2 N-terminal extracellular domain. Specific immuno-globulins (Igs) were affinity purified and evaluated through dot-blot analysis. Western blots were performed on pituitaries harvested from immature and sexually mature male and female Barred Plymouth Rock chickens to further quantify any changes in receptor levels during sexual maturation. Furthermore, Igs were also tested on COS-7 cells transfected with either cGnRHR-2, cGnRHR-1, chicken gonadotropin inhibiting hormone receptor (cGnIHR) or empty pcDNA3 expression vectors.

Initial dot-blot analyses revealed that Igs against peptide 1 had the highest affinity and titre. Thus, these Igs were selected for further validation. Western blots showed a strong specific band in pituitary samples at around 50kDa that was competed out by pre-incubation with peptide 1. In pituitaries, the lowest receptor levels were observed in immature males, significantly increasing post photostimulation. In females, cGnRHR-2 levels also increased post photostimulation with levels consistently higher than in males. In addition, a shorter receptor variant, possibly corresponding to an mRNA splice variant previously identified, was also detected. However the amount was 6-10 fold lower than that of the full length receptor. Immunocytology experiments revealed that in live cells, Igs are specific to cGnRHR-2.

In summary, our antibody is specific to cGnRHR-2 and can be used for western blotting as well as immunohistology. In addition, levels of receptor in chicken pituitaries appear to be higher in females than males and increase in both sexes post-photostimulation.

Key Words: gonadotropin releasing hormone, receptor, chicken, sexual maturation, pituitaries

69 Melanopsin in the premammillary nucleus of the avian hypothalamus may trigger seasonal reproduction. S. W. Kang*, B. Leclerc, and M. E. El Halawani, *University of Minnesota, St. Paul.*

Melanopsin (cOPN4) has been proposed as an important photoreceptive molecule regulating the avian circadian system. Previous studies in our laboratory have shown that co-localized dopamine-melatonin (DA-MEL) neurons in the hypothalamic premammillary nucleus (PMM) are photosensitive and exhibit circadian rhythms. This study investigates chicken OPN4 (cOPN4) mRNA distribution in the turkey hypothalamus and brainstem, and characterizes cOPN4 mRNA expression in PMM DA-MEL neurons, using in situ hybridization (ISH), double-label immunocytochemistry (ICC), double ISH/ICC, and real time-PCR. cOPN4 mRNA was found in anatomically discrete areas in or near the hypothalamus and the brainstem, including POM (nucleus preopticus medialis), SL (nucleus septalis lateralis), PMM and the pineal gland. Double ICC, using tyrosine hydroxylase (TH)/cOPN4 antibodies, confirmed that the cOPN4 protein coexisted in the DA-MEL neurons and cOPN4 mRNA expression was verified with double ISH/ICC using cOPN4 mRNA and TH immunoreactivity. PMM and pineal gland cOPN4 mRNA expression levels were high during the night and low during the day, indicating circadian rhythmicity. Stimulation with light during the dark period in short day hens downregulated cOPN4 expression level significantly at the avian photosensitive phase (circadian time 14 h; CT14), more so than it did at CT8 and CT20. There was a significantly lower level of cOPN4 mRNA in PMM neurons in photorefractory hens as compared with short and long day hens. The present study is the first to show that cOPN4 is expressed in the DA-MEL neurons controlling seasonal reproduction and also the first to show cOPN4 expression peaking at night as part of the

circadian rhythm. The results suggest that cOPN4 in the PMM DA-MEL neurons in the hypothalamus might constitute an important photoreceptive system for regulating reproductive function in the female turkey. *Supported by National Research Initiative Grant (2007-35203-18072) from the USDA Cooperative State Research, Education, and Extension Service*

Key Words: melanopsin, dopamine, turkey, avian reproduction, real-time PCR

70 Evaluation of the minimum dose of dietary thyroxin sufficient to induce molt in turkey breeder hens. V. A. L. Gulde*¹, R. Renema², and G. Y. Bedecarrats¹, ¹*University of Guelph, Guelph, ON, Canada,* ²*University of Alberta, Edmonton, AB, Canada.*

In the turkey industry, the standard method of molting consists of removing feed and water and reducing the photoperiod. As this results in severe stress, an alternative needs to be developed. Our previous study showed that 10 days of 40 ppm dietary thyroxin (T4) supplementation induces a complete moult in turkeys when combined with a reduction in photoperiod. The present study aimed at determining the minimal dose of T4 sufficient to induce molt while allowing hens to return into production. Spent White turkey hens (75 weeks old; n=220) were randomly split into 8 groups (5 floor pens (replicates)/group, 5 hens each). During the first 10d, all groups were kept under 15h light and, while 2 control groups were fed a breeder's diet, the 6 remaining groups were given either 1, 10 or 20 ppm T4 (2 groups/dose). On d10, all groups were switched to 6h light and fed a holding diet. After a 6 or 12 wks holding period, 1 group per treatment (control, 1, 10 and 20 ppm T4) was photostimulated with an abrupt change to 15h light and was fed the breeder's diet. Egg laying, feed intake, body weight, molt and behavior were monitored. Hens from the 20 ppm group ceased laying by d23 while all other treated hens ceased laying by d28 and control hens by d33. All hens resumed lay 30d after stimulation, reaching peak production 20d later. During the T4 supplementation period, feed intake for the 20 ppm groups was lower (P<0.05) than for the control groups. By d5, all hens fed T4 showed a rapid body weight loss (P<0.001). However, control hens also lost weight (P<0.001) by d13. By d18, the rate of molt was significantly higher for hens fed 10 and 20 ppm (P<0.01). Although all hens completed molt by d68, completion was faster for hens fed 20 ppm T4 (P<0.05). Throughout the experiment, no hens showed increased aggression, hunger, or stereotypies and there was no difference in heat stress between groups. In summary, although a drop in photoperiod was sufficient, a more rapid complete molt was successfully induced by 10 ppm T4 supplementation.

Key Words: molting, turkey hen, breeder, diet, thyroxin

71 Influence of two different molting methods on productive performance and the immune response of two different strains of laying hens. M. A. Elmenawey, H. M. Safaa*, A. S. Hassan, A. O. Abbas, H. B. Gharieb, and A. M. Abdou, *Animal Production Department, Faculty of Agriculture, Cairo University, Giza, Egypt.*

An experiment of 3 x 2 factorial arrangement was conducted to determine the influence of 2 different molting methods compared with non-molting group (20'000 ppm zinc oxide per kg diet "Zinc method" vs. California method vs. non-molting) on productive performance and the immune response of 2 different strains of laying hens (Bovans Brown "commercial strain-BB" vs. Red Baladi "Egyptian native strain

developed from a cross between Red Rhode Island males and Fayoumi females-RB"). At 65 wk of age 285 hens (153 from BB and 132 from RB) were housed in individual cages and fed a commercial layer ration (17% crude protein, 2800 kcal AMEn/kg, 3.7% calcium and 0.66% total phosphorus) except during the induced molting periods. Productive performance traits were recorded daily from 71 to 88 wk of age. At 0, 5, 10 and 30 days from the beginning of treatments, 10 birds were selected randomly per treatment per period and injected intramuscularly with 1 ml of 10% sheep red blood cell's (SRBC's) suspension prepared in 0.9% physiological saline to measure the antibody production titer against SRBC's and Newcastle Disease Virus. Results indicated that induce molting improve productive performance whereas, no differences were detected between molting methods. Non-molting group recorded lower

values for egg rate (48.0 vs. 59.7 vs. 60.8%; $P < 0.0001$) and feed conversion ratio (4.74 vs. 2.88 vs. 2.95; $P < 0.0001$) compared to Zinc and California methods, respectively. Hens molted with California method recorded higher antibody titer against SRBC's (8.04 vs. 5.96 vs. 5.21; $P < 0.0001$) than hens treated with Zinc method or non-molting hens, respectively. However, the antibody titer against Newcastle Disease Virus was not affected. Hens of RB have lower productive performance but higher antibody titer against SRBC's (7.56 vs. 5.25; $P < 0.0001$) than BB hens, respectively. We conclude that both molting methods have the same impact on hen's productivity and California is better than Zinc for affecting humoral immune response. In addition, RB hens have lower productive performance but higher immunity than BB hens.

Key Words: molting, laying hen performance, immunity

Environment and Management II

72 Differences in growth parameters and response to yeast components in chicks seeded with gut microflora from high and low weight broilers. R. Van Wyhe*¹, M. Bedford², R. Dalloul¹, and A. P. McElroy¹, ¹Virginia Polytechnic Institute and State University, Blacksburg, ²Ab Vista, Marlborough, Wiltshire, United Kingdom.

Research has shown that gut microflora in obese and normal weight animals differs in composition. The objectives of this trial were to 1) evaluate the effect of feeding cecal droppings collected from heavy (HW) or low weight (LW) broilers on performance and 2) to determine if dietary supplementation with yeast derivatives would effect growth and gut morphology in broilers fed the cecal droppings from HW or LW populations. Cobb 500 broiler chicks were given a standard commercial diet and raised to 28 d of age. At d28, birds of top 10% and bottom 10% body weight (BW) were moved into batteries and cecal contents were collected for a period of 24 hours. Cecal droppings collected from the HW and LW populations were collected for a period of 24 hours and then fed to Cobb 500 chicks (n=1400/group; HW or LW microflora) for a period of 48 hours. After 48 hours, chicks from each microflora treatment were weighed and placed in floor pens (n=42/pen) according to 4 dietary yeast treatments. Diets were 1) control (C); 2) HCT, (C+ 0.1% HCT); 3) PO24 (C + 0.1% PO24); 4) RNA (C + 0.1% RNA) for a resulting 8 total treatments (n=8 reps/diet). BW and feed intake were measured for the feeding periods of starter (d0-10), and grower (d10-28), and cumulative (d0-28). On d10 and d28, 1 bird per pen was selected for the measurement of villus height (VH), crypt depth (CD) and villus height: crypt depth ratio (VCR) in the duodenum, jejunum and ileum. Cumulatively and during the grower period feed conversion was reduced ($P < 0.05$) and BW was increased ($P < 0.05$) in the control compared to HCT or PO24. There were no differences in microflora treatments or dietary and microflora interactions for performance. On d28, in the ileum, VCR of control was less ($P < 0.05$) than that of the HCT diet. In the jejunum and ileum, VCR was higher ($P < 0.05$) and CD was lower ($P < 0.05$) in the LW group on d 28. These results suggested that in a non-challenge setting early feeding of microflora from HW or LW broilers or dietary yeast products effected intestinal morphology, and yeast derivative feeding was not beneficial for growth performance.

Key Words: bacteria, yeast, poultry, gut, performance

73 Evaluation of length of finisher Maxiban® withdrawal period on broiler performance. R. Lehman*, C. Walk, J. Sottosanti, R. Van

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Coccidiosis costs the poultry industry billions of dollars annually due to anticoccidial medications, disease-invoked losses in bird performance and mortality, and treatment. To reduce anticoccidial costs, increasing the length of the non-medicated, withdrawal period may be considered; however this raises concern regarding late-breaking coccidial infections. This experiment evaluated the effect of Maxiban® withdrawal period length on bird performance during a mild environmental coccidia exposure. Day-old Cobb 500 male broilers were placed in floor pens (43 birds/pen) on pine shavings previously seeded with 3 species of *Eimeria* and raised to day 40. Each pen received one of 5 dietary treatments (n=12 reps) including a non-medicated control diet or one of 4 diets consisting of the control supplemented with the anticoccidial Maxiban® from day 1 to day 25, 28, 31, or 34. Birds were fed non-medicated finisher diets on the day of Maxiban® removal. Performance parameters included body weight (BW), body weight gain (BWG), feed intake (FI), feed conversion (FC), and mortality, which were measured on days 18, 25, 28, 31, 34, and 40. Until day 34, birds on the control diet had lower ($P \leq 0.05$) BW and BWG than the groups receiving Maxiban®, but no differences existed by day 40. Feed intake only differed between day 0 and 25 between the control and the group receiving Maxiban® to day 28 ($P \leq 0.05$). At day 40, no differences in FC existed among any of the groups receiving Maxiban®, whereas birds on Maxiban® diets generally had better FC than birds on the control diet. There was no difference in mortality throughout the trial. Birds in this trial performed at or above Cobb performance standards, which indicated that the litter exposure to coccidia resulted in a mild infection. Results suggest that early withdrawal of Maxiban® caused no significant decrease in bird performance during a mild coccidia infection; however, results may vary with a more severe challenge.

Key Words: anticoccidial, withdrawal period, coccidiosis, performance, Maxiban®

74 Identification and evaluation of candidate *Bacillus* probiotics (DFM) for use in commercial turkey feed. R. E. Wolfenden*, N. R. Pumford, M. J. Morgan, A. D. Wolfenden, G. Tellez, and B. M. Hargis, University of Arkansas, Fayetteville.

As effective probiotic *Bacillus* spores are identified, these may offer advantages of in terms of stability, cost, and feed application over current