

## Physiology: Physiology I

**50 Migratory ability of the somatic nuclear transferred gonadal germ cells (nt-GGCs) in domestic chicken.** T. Minematsu, A. Tajima\*, and Y. Kanai, *University of Tsukuba, Tsukuba, Ibaraki, Japan.*

The migratory ability of the somatic nuclear transferred gonadal germ cells (nt-GGCs) was investigated in domestic chicken.

### Experiment 1

The gonadal germ cells (GGCs) were collected from the gonads of seven-day-old chick embryos and the nucleus was inactivated by exposing to ultraviolet (UV). The embryonic blood cells (EBCs), collected from four-day-old chick embryos, were then transferred to UV-irradiated GGCs by electrofusion. The efficiency to produce nt-GGC was  $3.58 \pm 0.63\%$ .

### Experiment 2

The cell suspension containing fluorescent-labeled nt-GGCs was injected into dorsal aorta of two-day-old embryos, and recipient embryos were incubated for five days after the injection. Five days later, fluorescent-labeled cells were observed from gonads of recipient embryos. Furthermore, W chromosome specific DNA sequence was detected from the gonad of male recipient, when EBCs were from female embryo and GGCs were from male embryo.

### Conclusion

The results obtained in the present study strongly indicate that UV treated nt-GGCs maintain their migratory ability even after somatic nuclear transfer.

**Key Words:** Somatic nuclear transfer, Gonadal germ cells, Chicken

**51 Gonadotropin inhibiting hormone does not affect circulating luteinizing hormone levels in the chicken.** C. Senthilkumaran\*, S. Peterson, and G. Bedecarrats, *University of Guelph, Guelph, ON, Canada.*

Gonadotropin inhibiting hormone (GnIH) has previously been reported to inhibit the release of luteinizing hormone (LH) in the white crown sparrow. However, its effectiveness in the chicken remains controversial. The objective of this study was to determine the effect of a GnIH bolus injection on circulating levels of LH in the chicken. A catheter was implanted in the brachial vein of immature (18 weeks old; n=16) and mature (36 weeks old, n=18) male, and immature (16 weeks old; n=17) and mature (52 weeks old; n=18) female Barred Rock chickens. A vehicle control (saline solution) or a bolus dose of GnIH (500 ng or 50 ng) were then injected in the opposite wing vein, and blood samples were collected from the catheterized vein before, and 2, 5, 10, and 20 min after injection. Levels of LH in plasma samples were subsequently analysed by radioimmunoassay. The GnIH used in this experiment was synthesised based on the published chicken sequence (Ser-Ile-Lys-Pro-Ser-Ala-Tyr-Leu-Pro-Leu-Arg-Phe-NH<sub>2</sub>). GnIH did not significantly induce any changes in LH concentration, irrespective of the dose, the sex, or the reproductive status of the birds. In all groups tested (including controls), LH concentrations tended to decrease between the pre-injection levels and the last time point measured. This decrease was likely the result of stress experienced during blood collection. In conclusion, unlike in the white crown sparrow, GnIH does not alter circulating LH levels in male and female chickens at different reproductive stages.

**Key Words:** GnIH, LH, Chicken

**52 Role of the eyes in gonadal development in male chicks (*Gallus gallus*) following photostimulation.** T. Rathinam\* and W. Kuenzel, *University of Arkansas, Fayetteville.*

Several species of birds in temperate latitudes show recrudescence of gonads in response to long photoperiods. Compelling evidence suggests the presence of non-retinal photoreceptors located in the ventral brain. The role of eyes, however, in gonadal development of poultry is not clearly known. The objective of this study was to examine the effect of bilateral enucleation or optic nerve transec-

tion on testicular development of male broiler chicks. Birds were kept on a short photoperiod (LD 8:16) and were given chick starter and water ad libitum. Birds were divided into three groups based on the surgery performed on them, namely enucleation (EX), eyelid suture (EL) and eyes intact (EI) (Experiment 1). First experiment was done in three replicates. Surgeries were done during first week of age. At the end of two weeks, a long photoperiod was initiated (LD 16:8) and half of all birds in each group received a chick starter ration mixed with 0.2% sulfamethazine (SMZ), a compound known to augment the effects of photostimulation in male chicks. The other half of birds in each group remained on a control ration. At the end of three weeks, birds were sacrificed, testes collected and weighed. In two out of three repeats of the experiment conducted, there was no difference in testes weights among enucleated, eyelid-sutured and intact birds fed SMZ ( $p>0.05$ ) nor among the same three groups fed a control diet ( $p>0.05$ ). In the second experiment, the EX group was replaced with a group that underwent optic nerve transection (OX). Testes weights of OX, EL and EI birds among the 3 groups fed the SMZ diet and among the same groups fed a standard poultry ration, were not different from each other. In all studies, as expected, SMZ fed birds had larger testes than birds fed the control diet ( $p<0.05$ ). Results suggest little, if any, involvement of the eyes on gonadal development in the chicken. Supported in part by NSF grant #IBN-0315793 to WJK.

**Key Words:** Sexual maturation, Optic nerve transection, Sulfamethazine

**53 Association of duodenum mitochondrial complex activity, protein expression and protein oxidation with feed efficiency in broilers.** C. Ojano-Dirain\*, N. R. Pumford<sup>1</sup>, K. Lassiter<sup>1</sup>, T. Wing<sup>2</sup>, M. Cooper<sup>2</sup>, and W. Bottje<sup>1</sup>, <sup>1</sup>*University of Arkansas, Fayetteville,* <sup>2</sup>*Cobb-Vantress Inc., Siloam Springs, Arkansas.*

Differences in duodenum mitochondrial function and biochemistry were observed in broilers with low and high feed efficiency (FE, gain:feed). The objective of this study was to evaluate correlations of FE with mitochondrial complex activity, protein expression, and protein oxidation (carbonyl). Duodenal mitochondria were isolated from male broiler breeders with low ( $0.52 \pm 0.01$ ) and high ( $0.68 \pm 0.01$ ) FE (n = 8/group). Mitochondrial complex activity (Complex I to V) was measured spectrophotometrically. Western blots were used to assess carbonyls and expression of specific respiratory chain subunits. Protein bands were detected by chemiluminescence with a charge-coupled device camera and band intensities quantified with Scion® software. Complex I, II, III and V (ATPase) activities were positively correlated with FE. A positive correlation was also observed between FE and steady-state levels of ND4 and ND6-C subunits (mitochondrial-encoded) while 70S(FP), Core I Core II, cyt c1, and ISP subunits (nuclear-encoded) showed a negative correlation with FE. ATPase-a subunit tended to be negatively correlated ( $P = 0.08$ ) with FE while 5 other subunits were not correlated with FE. There was no correlation between duodenal protein carbonyls and FE, but a negative correlation with Complex V activity and a positive correlation with 70S(FP), Core II and ISP subunits were observed with carbonyls. These results indicate that some mitochondrial proteins are well correlated with the phenotypic expression of FE in broilers and may provide clues in finding FE biomarker(s).

**Key Words:** Feed efficiency, Duodenal mitochondria, Complex activity and protein expression

**54 Expression of the activin type IA and type IB receptors during follicular development in broiler breeder hens.** J. Hoffman\*, A. Davis, and M. Freeman, *University of Georgia, Athens.*

Activin is a glycoprotein hormone that was originally identified as a gonadally produced, positive regulator of pituitary FSH secretion. Subsequently, activin has been found to have significant paracrine and autocrine functions within the ovary. Activin induces its biological effects by binding to a serine/threonine kinase receptor complex. There are two type I activin receptors (ActRIA and ActRIIB) and two type II activin receptors (ActRIIA and ActRIIB). An activin

receptor complex capable of intracellular signaling forms when activin binds to a type II receptor which stimulates the recruitment of a type I receptor to complete the receptor complex. Activation of the type I receptor results in the phosphorylation of one or more intracellular receptor Smad proteins. The mRNA expression of ActRIA and ActRIB was investigated in the developing follicles of broiler breeder hens. Individual granulosa and theca layers were collected from the F<sub>1</sub> through F<sub>4</sub> follicles, from a pool of small yellow follicles and from a pool of large white follicles from 6 hens. The isolated theca and granulosa layers were combined from 2 birds to create 3 replicate granulosa and theca samples for each follicle size. Total RNA was extracted from each sample for subsequent real-time RT-PCR analyses of activin type I mRNA expression. Primer pairs and Taqman minor groove-binding (MGB) probes were designed for ActRIA, ActRIB and GAPDH (endogenous control) with Primer Express (Version 2.0, Applied Biosystems) based on chicken mRNA sequences for these three genes. Expression of both ActRIA and ActRIB were detected in all of the theca and granulosa samples. Previously, our laboratory reported that ActRIIA mRNA expression could be detected by Northern blot analysis in both the theca and granulosa layers of a follicle of any size. Therefore, when the current data is combined with our previous findings, it suggests that functional activin receptor complexes could form in both the theca and granulosa cells of hierarchical and non-hierarchical follicles of broiler breeder hens.

**Key Words:** Activin receptors, Broiler breeder hens, Theca, granulosa

**55 Effects of dopamine and oxytocin receptor antagonists on the transition to brooding of young in turkeys.** A. Thayananuphat<sup>\*1</sup>, O. Youngren<sup>1</sup>, S. Kang<sup>1</sup>, T. Bakken<sup>1</sup>, J. Proudman<sup>2</sup>, and M. El Halawani<sup>1</sup>, <sup>1</sup>University of Minnesota, St Paul, <sup>2</sup>United States Department of Agriculture, Beltsville, Maryland.

Both dopamine (DA) and oxytocin (OT) have been shown to be involved in maternal behavior in rats. Both D<sub>1</sub> and D<sub>2</sub> DA receptor antagonists attenuated this behavior. Intracerebroventricular (ICV) injection of an OT antagonist delayed the onset of maternal behavior. Mesotocin (MT) is an oxytocin-like peptide in birds. Our recent work found the induction of a neuronal activation marker, c-fos mRNA, in DA and MT neurons during the transition from incubating eggs to brooding of poults in turkeys. This finding suggested the involvement of DA and MT with this transition. The roles of DA and MT were further investigated in the present study, using ICV administration of D<sub>1</sub> DA or D<sub>2</sub> DA receptor antagonist, OT receptor antagonist, or vehicle in incubating hens prior to the transition to poult brooding behavior. Brooding behavior and c-fos mRNA induction were observed and compared among treatment groups. All the hens that received vehicle and the majority of the hens that received D<sub>1</sub> DA receptor antagonist showed poult brooding behavior within the 30-minute experimental period. Hens receiving D<sub>2</sub> DA or OT receptor antagonist showed partial or no brooding behavior within the 30-minute experimental period. C-fos mRNA induction was markedly less in the preoptic nucleus, the critical nucleus involved in the neural control of maternal behavior in rats, in hens receiving D<sub>2</sub> DA or OT receptor antagonist as compared to that observed in vehicle treated control hens. These data support the hypothesis that DA and MT are two of the key neurotransmitters/neurohormones involved in the transition from incubating to brooding behavior in the turkey. USDA grant # 04-35203-14771

**Key Words:** Dopamine, Mesotocin, Avian reproduction

**56 The effect of in ovo injection of L-carnitine on hatch rate of White Leghorn eggs.** W. Zhai<sup>\*1</sup>, S. L. Neuman<sup>2</sup>, M. A. Latour<sup>1</sup>, and P. Y. Hester<sup>1</sup>, <sup>1</sup>Purdue University, West Lafayette, Indiana, <sup>2</sup>Astra Zeneca, Raleigh, North Carolina.

Five experiments (EXP) were conducted to determine if carnitine injected in ovo affected hatch of fertile eggs collected from White Leghorns. The first 4 EXP evaluated injection protocol, diluent, and sealing the injection site. The injection site of all eggs of EXP 2 and 3 were sealed with water-based glue. Volume of injectable was 100 µL with injections at either 17 (EXP 1 and 2) or 18 d of incubation. Data were analyzed using ANOVA. EXP 1 used 20 eggs injected with a purple dye to verify that the injectable was placed in the amnion. When opened, all 20 embryos had dye within their amnions. For EXP 2,

107 eggs injected with ethanol or 2 µmoles of carnitine (Lonza, Inc.) dissolved in ethanol had similar hatch rates (77% for carnitine and 78% for ethanol eggs), but hatch rates for both groups were low. Use of saline instead of ethanol as the diluent improved hatch rate of EXP 3 as compared to EXP 2 with no differences among treatments (92, 90, 90, and 91% for injection of 2 µmoles carnitine, 0.85% saline, shams, and non-injected controls, respectively). EXP 4 (450 eggs), in which hatch rates were low for all groups due to a power failure, showed that sealing the hole of the injection site of eggs did not affect hatch rate (61% for 0.50 µmoles of carnitine sealed eggs; 68% for unsealed carnitine injected eggs, 56% for saline sealed, 67% for saline unsealed, and 63% for non-injected controls). Eggs (1,680) of EXP 5 were injected with sterilized saline (0.85%) or carnitine (0.25, 0.50, 1.00 or 2.00 µmoles dissolved in saline) without sealing or served as non-injected controls. Hatch was unaffected by treatment (94% for non-injected controls, 94% for saline injected eggs, and 87, 87, 88, and 88% for eggs injected with 0.25, 0.50, 1.00 or 2.00 µmoles of carnitine, respectively). It is concluded that the in ovo injection of carnitine into fertile chicken eggs at 18 d of incubation did not affect hatch rate. Supported by the U. S. Poultry & Egg Association & Lonza, Inc.

**Key Words:** Carnitine, Hatch rate, In ovo injection

**57 Expression of the mRNA for zona pellucida proteins D and B2 in two genetic lines of turkey hens that differ in fertility.** A. Benson<sup>\*1</sup>, A. Davis<sup>1</sup>, M. Compton<sup>1</sup>, B. Fairchild<sup>1</sup>, and V. Christensen<sup>2</sup>, <sup>1</sup>University of Georgia, Athens, <sup>2</sup>North Carolina State University, Raleigh.

The freshly ovulated ovum in avian species is surrounded by a protein layer called the inner perivitelline layer (IPVL). For successful fertilization, sperm must attach to and penetrate the IPVL. The IPVL contains the zona pellucida family of proteins and members of this family of proteins have been shown to possess sperm binding activity and to assist with sperm penetration of the follicle. In the domestic chicken six distinct zona pellucida genes have been characterized (ZPA, ZPB1, ZPB2, ZPC, ZPD and ZPX). Previously our laboratory characterized the mRNA expression of ZPB1 and ZPC by Northern blot analysis in two lines of turkey hens selected for over 40 generations for either increased egg production (E) or increased body weight (F). In the present research, the expression of the mRNA for ZPB2 and ZPD was investigated in these two lines of turkey hens. Total RNA was extracted from the individual granulosa and theca layers of the four largest follicles of four, 46 week old hens from each genetic line. Northern blot analysis for ZPD was performed using a chicken cDNA probe. Equality of RNA loading and transfer was verified with a cDNA probe of chicken GAPDH. Expression of ZPD was equal in the four largest hierarchical follicles, and equal between the genetic lines of turkey hens. ZPB2 mRNA expression could not be detected by Northern blot analysis; however, using RT-PCR methodology, a partial cDNA clone for turkey ZPB2 was obtained and sequenced. PCR primers were designed based on this sequence and subsequently used to detect ZPB2 expression by RT-PCR in both granulosa and theca samples from hierarchical follicles. The abundant mRNA expression of ZPD in the largest preovulatory follicles suggests that it may be an important constituent of the IPVL and thus play a role in successful fertilization.

**Key Words:** Zona pellucida proteins, Turkeys, mRNA expression

**58 The effects of semen diluent and rate of dilution on the Sperm Quality Index and on ATP utilization, gas exchange, and ionic balance of broiler breeder sperm immediately following dilution.** H. Parker<sup>\*</sup> and C. McDaniel, Mississippi State University, Mississippi State.

The Sperm Quality Index (SQI) is a method used to predict overall rooster semen quality, fertility, and hatchability at lower semen dilutions. Therefore the present study was undertaken to determine why the SQI is not as predictive of fertility at higher semen dilutions and whether semen diluent type alters the SQI, ATP utilization, gas exchange, and ionic balance of broiler breeder sperm. The diluent types used were saline, seminal plasma (SP), and minimum essential medium (MEM). Pooled semen was diluted 0- (neat), 2-, 4-, 8-, 10-, 12-, 25-, 50-, 75-, 100-, 150-, and 200-fold. The following parameters were measured for each diluent type at each dilution: SQI, ATP, Na<sup>+</sup>, Ca<sup>2+</sup>, K<sup>+</sup>, Cl<sup>-</sup>, CO<sub>2</sub>, and O<sub>2</sub>. To examine motility of individual sperm cells, the SQI was expressed as SQI/million sperm/mL (SQI/sperm). There was an interaction between diluent

type and dilution for the SQI, SQI/sperm, CO<sub>2</sub> generated, O<sub>2</sub> utilized, as well as Na<sup>+</sup>, Ca<sup>2+</sup>, and K<sup>+</sup> internalization. For sperm diluted with saline, the SQI declined rapidly with increasing dilution. However, the SQI/sperm increased rapidly when semen was diluted with MEM or SP. Sperm diluted in SP utilized ATP with increasing dilution whereas sperm diluted with saline and MEM generated ATP. Neat semen contained no free O<sub>2</sub>, however each diluent type contained abundant O<sub>2</sub> resulting in more O<sub>2</sub> available as semen was diluted. Sperm diluted in SP produced more CO<sub>2</sub> and utilized more O<sub>2</sub> as compared to saline and MEM. For SQI/sperm, ATP and CO<sub>2</sub> generated, as well as Na<sup>+</sup> and Ca<sup>2+</sup> internalization, differences between diluent types occurred when semen was diluted 50-fold and greater. In conclusion, it appears that sperm motility, ATP utilization, gas exchange, and ionic balance are altered by diluent type and rate of dilution. These alterations are exacerbated at semen dilutions of 50-fold and greater yielding an SQI that is not indicative of sperm motility or fertility.

**Key Words:** Sperm Quality Index, Sperm Motility, Sperm Metabolism

**59 Molecular cloning and tissue expression of chicken adiponectin cDNA: Food deprivation influences adiponectin mRNA expression in adipose tissue.** S. Maddineni\*, S. Metzger, O. Ocon, G. Hendricks, III, and R. Ramachandran, *The Pennsylvania State University, University Park.*

Adiponectin is a cytokine hormone secreted exclusively by the adipose tissue in mammalian species. We have characterized the sequence of chicken adiponectin cDNA and analyzed the expression of adiponectin mRNA in various tissues. We also investigated the effect of food deprivation on adiponectin gene expression in chicken adipose tissue. The open reading frame of chicken adiponectin cDNA consists of 735 nucleotides that was 65-68% homologous to mammalian adiponectin cDNA. The deduced amino acid sequence of chicken adiponectin contains 22 Glycine-X-Y repeats (where X and Y represent any amino acid) at the N terminal end as found in the mammalian adiponectin. By RT-PCR and northern analysis, we detected chicken adiponectin mRNA transcript in the adipose tissue, liver, anterior pituitary gland, diencephalon, skeletal muscle, liver, kidney, ovary, and spleen but not in the blood. Adiponectin mRNA expression in various tissues was quantitated using real-time quantitative PCR and found to be the highest in adipose tissue followed by liver, anterior pituitary, diencephalon, kidney and skeletal muscles. We also found that adiponectin mRNA quantity in chicken adipose tissue was significantly decreased following a 48 hr food deprivation (P<0.05). Our results provide novel evidence that, unlike mammals, adiponectin gene is expressed in multiple tissues in the chicken and that its expression in the adipose tissue is influenced by food deprivation.

**Key Words:** Adiponectin, Adipose Tissue, Liver

**60 Effect of feed withdrawal on mucus and morphology of the small intestine of broilers.** K. L. Thompson\* and T. J. Applegate, *Purdue University, West Lafayette, Indiana.*

In an effort to reduce carcass contamination and consequent reprocessing, market-age broilers are often subjected to feed withdrawal (FW) prior to processing in order to reduce intestinal content and intestinal ruptures during processing. However, little is known regarding the effects of FW on the mucus layer and the intestinal morphology. Therefore, an experiment was conducted to determine the effects of FW on intestinal characteristics. Twenty male broilers were raised in floor pens on standard industry diets. At 42d of age, feed was removed from the individual pens at 24, 12, 8, and 0 hr prior to sampling, respectively (n=5 birds per FW period). Birds remained on litter with access to water for the first 4 hr of the FW period and were then placed in crates. Distal ileal and jejunal segments were collected, fixed in neutral buffered formalin, and processed, sectioned, and stained with hematoxylin and eosin for determination of morphological characteristics. One centimeter distal ileal segments were collected for mucus quantification at 0, 12, and 24 hr. Jejunal villi heights were greater prior to FW (1080.1µm) versus 1371.3, 1415.6, and 1483.3 µm for 0, 12, and 24 hr, respectively (P<0.05), though villi widths were unaffected. Jejunal crypt depths were greater at 12 hr of FW (190.9 µm) than at 0 (137.1µm), 8 (160.7µm), or 24 hr (141.67µm; P<0.05). Ileal villi heights were unaffected by FW but villi width decreased with increasing FW time (175.8, 141.0, 139.8, and 121.8µm at 0, 8, 12, and 24 hr, respectively). Ileal crypt depth was greater prior to FW (162.7µm) than at 8 (137.5µm), 12 (139.0µm), and 24 hr (105.7µm; P<0.05).

Mucus content of the ileum decreased at 0 and 12 hr from 88.55 and 93.63µg/g alcian blue/g ileum, respectively, versus 24 hr (63.06µg/g; P<0.05). The intestinal morphology alterations and the depletion of intestinal mucus that occur during FW may reduce the integrity of the intestine at processing, which may contribute to pathogen proliferation and shedding, though additional research is necessary to elucidate these roles.

**Key Words:** Broiler, Feed withdrawal, Intestine

**61 Spontaneously occurring fibroid tumors of the laying hen oviduct.** A. Doernte\*, L. Stevenson, S. Oates, and W. Berry, *Auburn University, Auburn, Alabama.*

Spontaneously occurring benign fibroid tumors of the uterus are the most common tumors of women of reproductive age. More than 30% of all women have uterine fibroids and the presence of these tumors is a primary cause of hysterectomies. Research into the causes and treatment of uterine fibroids is hampered by a lack of reliable animal models of the disease. Tumors (leiomyomas) that appear to be essentially identical to human uterine fibroid tumors are known to occur in the oviducts of laying hens. In the course of studies using the aged laying hen as a model for development of ovarian cancer, the authors noticed that fibroid tumors of the oviduct were common in hens over 2 years of age. It was decided to characterize and compare these tumors to human uterine fibroids for the purpose of determining their suitability as a model system for the study of the disease. Fifty five-year-old hens were examined for the presence of oviduct associated fibroid tumors. Fibroids were identified in 100% of these hens. These tumors were attached to the internal surface of the oviduct, embedded in the oviduct wall, or attached to the exterior of the oviduct. Tumor samples were taken and frozen or fixed in formalin for histological analysis and immunohistochemistry for estrogen and progesterone receptor. (Supported by the Alabama Agricultural Experiment Station).

**Key Words:** Oviduct, Liomyoma, Tumor

**62 Nitric oxide measurement in avian blood: Evaluation of two commonly used assays and the impact of aminoguanidine on plasma nitric oxide after injection of lipopolysaccharide.** M. E. Chapman\* and R. F. Wideman, *University of Arkansas, Fayetteville.*

Previous studies demonstrated that bacterial LPS triggers pulmonary vasoconstriction leading to pulmonary hypertension (PHS) in broilers. Among broilers from a single genetic line, some individuals respond to LPS with large increases in pulmonary arterial pressure (PAP), whereas others fail to exhibit a response to the same supra-maximal dose of lipopolysaccharide (LPS). It is our hypothesis that the pulmonary hypertensive response to LPS in broilers is minimal when more vasodilators, such as nitric oxide (NO), than vasoconstrictors, such as thromboxane A<sub>2</sub> (TXA<sub>2</sub>) and serotonin (5HT), are generated during an LPS challenge. The activation of macrophages by (LPS) and the subsequent expression of inducible nitric oxide synthase (iNOS) is a well-established model of inflammation. In the present study we evaluated two methods for the ability to measure plasma levels of i. v. infused NO<sup>2-</sup> (a stable metabolite of NO) in male broilers. The first Griess reaction assay employed copper coated cadmium as a catalyst for the reduction of NO<sup>3-</sup> to NO<sup>2-</sup>, while the second employed nitrate reductase. Secondly we evaluated the two methods for their ability to measure endogenously produced NO<sup>2-</sup> in response to an LPS (*S. typhimurium*) challenge. Thirdly, we measured plasma NO<sup>2-</sup>, using the nitrate reductase assay, in response to the nitric oxide donor sodium nitroprusside (SNP) infused i. v. at levels demonstrated to cause pulmonary vasodilation. Finally, we evaluated the impact of the selective iNOS inhibitor aminoguanidine (AG) on plasma NO<sup>2-</sup> levels in broilers challenged with LPS. Both assays were successful in detecting an increase in NO<sup>2-</sup> as NO<sup>2-</sup> was infused at increasing concentrations, and at 5 hrs post LPS injection. An increase in NO<sup>2-</sup> could not be detected in plasma from male broilers infused with SNP, indicating that endothelial NOS (eNOS) may be present in amounts too low to be detected in vivo. Finally AG reduces the 5 hr response to LPS indicating inhibition of iNOS.

**Key Words:** Nitric oxide, Hypertension, Lipopolysaccharide

**63 Effects of egg size and eggshell conductance on poult livability and body weight gain.** S. Funderbunk<sup>\*1</sup>, V. Christensen<sup>1</sup>, J. Grimes<sup>1</sup>, M. Wineland<sup>1</sup>, M. Mann<sup>1</sup>, R. Neely<sup>1</sup>, D. Ort<sup>1</sup>, D. Rives<sup>2</sup>, and G. Campbell<sup>2</sup>, <sup>1</sup>North Carolina State University, Raleigh, <sup>2</sup>Prestage Farms Inc., Clinton, North Carolina.

A high rate of poult mortality can occur within 10 days of placement. A better understanding of how egg size and eggshell conductance (G) affect the livability of a poult is needed. The objective of this study was to determine the effects of egg size and G on the livability of commercial turkey poults. An equal number of eggs from an induced molted flock (large eggs) and from a first cycle flock (small eggs) were weighed, numbered, set and incubated under standard operating procedures in a commercial turkey hatchery. All eggs were reweighed at d25 (transfer) and G (or moisture loss) was calculated. The eggs were then divided and sorted into 3 groups: high, average, and low G. At hatch poults were marked for identification according to G group and flock. The poults were then

processed and sexed. Thirty poults from each group and sex were then randomly selected and individually weighed on the day of hatch (d1) and again at d4 of the brooding period. Poult mortality for each group and sex were also monitored through d8 of brooding. Data were analyzed using the GLM procedure of SAS. Percent mortality was calculated. There was a flock by G interaction for d1 body weights and flock by sex interaction for d4 body weights. The best quality poult came from a large egg with a low G and conversely poor quality poults came from small eggs with low G. The percent mortality was lower for the better quality poults. At d4 tom poults weighed more than hen poults. Within the two flocks each of the low G groups weighed more at hatch than the other G groups. This could be due to the amount of residual yolk that is present in the poult and therefore could have affected the livability of that poult. It is concluded that poult quality may be a function of both egg weight and G.

**Key Words:** Incubation, Poult, Egg size

## Environment and Management: Broilers I

**64 The effect of holding time without water on broiler performance, yolk sac retention and gut integrity.** B. D. Fairchild<sup>\*1</sup>, J. M. Mauldin<sup>1</sup>, J. K. Northcutt<sup>2</sup>, M. B. Cole<sup>3</sup>, M. D. Darby<sup>3</sup>, and R. J. Buhr<sup>2</sup>, <sup>1</sup>University of Georgia, Athens, <sup>2</sup>USDA Russell Research Center, Athens, Georgia, <sup>3</sup>Cole Services, Stockbridge, Georgia.

Providing nutrients to chicks as soon as possible after hatching can influence chick growth and development. Using a device designed to deliver water to chicks in baskets at the hatchery, after removal from the hatcher, the effect of duration of holding time without water was evaluated on broiler performance, unabsorbed yolk sacs and intestine tension strength. Two trials were conducted utilizing 1,200 Cobb 500 male broilers. In Trial 1, chicks were divided into two treatment groups: water or no water, and both held for 24 h before being placed into floor pens. Each treatment group was distributed into eight pens. Body weights and feed consumption, and daily mortality were recorded weekly. At 6-wk-of-age broilers were processed through defeathering but prior to evisceration, the New York dressed carcasses were opened and the presence or absence of an unabsorbed yolk sac was determined. Intestinal strength (maximum load) in the region of the yolk stalk was evaluated on three intestines with and without unabsorbed yolk sac per pen (6 per treatment). In Trial 2, chicks were held for either 24 or 48 h with or without water prior to placement. At placement, each treatment group was distributed in 6 pens and measurements taken were the same as in Trial 1. In both trials, body weights at placement were greater for chicks provided water during the holding period. However, no differences were observed in final body weight, feed consumption, or mortality due to treatment. In Trial 1, the incidence of unabsorbed yolk sacs was 15% for chicks that were provided water and 19% in control chicks. In Trial 2, the incidence of unabsorbed yolk sacs was 28% for chicks that were provided water and 23% in control chicks. No differences were detected in intestine strength. The provision of water during the holding period prior to chick placement did not influence broiler performance, intestinal strength, or the incidence of unabsorbed yolk sacs. These data suggest that the presence of unabsorbed yolk sacs at processing age does not affect intestine strength and therefore would not have a greater tendency to tear during evisceration.

**Key Words:** Broilers, Water, Unabsorbed yolk

**65 Evaluation of lactose on clinical intestinal lesions in poultry with Necrotic Enteritis.** J. McReynolds<sup>\*</sup>, J. Byrd, L. Kubena, and D. Nisbet, USDA-ARS-SPARC, College Station, Texas.

In the commercial poultry industry there are many management tools used to control enteric pathogens including antibiotics, vaccines, and competitive exclusion cultures. Growth promoting antibiotics (GPA) have been used in the poultry industry, to target Gram-positive organisms which are associated with lower levels of performance and health. One target organism controlled with GPA is *Clostridium perfringens* (CP), the etiologic agent of Necrotic Enteritis (NE). Due to increasing consumer demands to remove GPA from the market,

our laboratory is currently evaluating the effects of dietary lactose on the disease condition of NE. Birds were administered CP ( $10^7$  cfu / mL) daily via oral gavage for three consecutive days starting on day seventeen. In Exp. 1, broilers were fed a control, 2.5% lactose, or 5% lactose diet from day-of-hatch until termination of the experiment. When evaluating the intestinal lesions associated with NE, birds fed 2.5% lactose had significantly lower ( $P < 0.05$ ) lesion scores of .70 compared to the control with a mean lesion score of 1.55 on a scale of 0-4. In Exp. 2, day-of-hatch broilers were fed a control, or 2.5% lactose diet. Broilers in the 2.5% lactose treatment group had a significant reduction ( $P < 0.05$ ) in intestinal lesion scores with a mean of 1.26 compared to the control with a mean lesion score of 1.80. These experiments suggest that lactose could be used as a potential alternative to GPA to help control this costly disease.

**Key Words:** Necrotic enteritis, *Clostridium perfringens*, Lactose

**66 Evaluation of lactose on the gastrointestinal microbial ecology in poultry with necrotic enteritis.** J. A. Byrd<sup>\*</sup>, J. L. McReynolds, L. F. Kubena, and D. J. Nisbet, USDA/ARS/SPARC, College Station, Texas.

*Clostridium perfringens* is the etiologic agent of Necrotic enteritis and is normally found in the soil and the intestines of healthy poultry. The incidence of Necrotic enteritis has increased in countries that have stopped using antibiotic growth promoters. The mechanisms of colonization of *Clostridium spp.* and the factors involved in onset of Necrotic enteritis are not fully understood. Previously, our laboratory has demonstrated that lactose could reduce *Salmonella* and *Clostridium spp.* in ceca of poultry. In the present investigation our working hypothesis is that dietary lactose will reduce the clinical signs of Necrotic enteritis and could be used as an alternative to antibiotics. In the present experiment, we evaluated the microbial ecology associated with Necrotic enteritis in the gastrointestinal system of birds being fed dietary lactose. Day-of-hatch broilers were assigned to one of the following groups: negative control, 2.5%, 4.0%, dietary lactose; dietary lactose was fed from day one until termination of the experiment. Experimental animals were euthanized and the gastrointestinal contents were evaluated at 7, 14, and 21 d of age for *Clostridium*, *E. coli*, *Enterococcus* and *Lactobacilli*. By d 14, broilers demonstrating clinical signs for Necrotic enteritis had significant decreases ( $P < 0.05$ ) in *Enterococcus* populations 3.77 and 4.15  $\text{Log}_{10}$  at both concentrations of lactose 2.5%, and 4.0% respectively as compared to the controls 5.93  $\text{Log}_{10}$ . At day 21, broilers fed 2.5% lactose had significant increases in *Lactobacillus* populations (8.31  $\text{Log}_{10}$ ) as compared to the control (6.82  $\text{Log}_{10}$ ). Although there were no significant bacterial predictors in this model, lactose in the diet did significantly reduce the clinical infection of birds. The results of the present study suggest that the incorporation of lactose into the broiler diet may reduce the signs associated with Necrotic enteritis.

**Key Words:** *Clostridium perfringens*, Necrotic enteritis, *Lactobacillus*