
Bioenergetics (aerobic respiration and glycolysis) can be determined simultaneously in intact cells or tissue explants by measuring O2 consumption rate (OCR) and extracellular acidification rate (ECAR) in a microtiter plate platform (Seahorse Biosciences, North Billerica, MA). The XF24 Flux Analyzer mixes content and creates a transient 7 μL chamber in each well for rapid and sensitive OCR and ECAR detection. After establishing baseline values, chemicals can be introduced into each well through ports in the Seahorse Flux Pak cartridges. We began bioenergetic studies with chicken embryo fibroblasts (CEFs) and DF-1 cells (spontaneously immortalized CEFs) that exhibit differences in growth rate, mitochondrial function, and response to oxidative stress. After optimizing cell numbers and chemical concentrations, studies were conducted with CEF and DF-1 cells (100,000 cells seeded/well) treated sequentially with oligomycin (to inhibit ATP synthase), FCCP (to uncouple oxidative phosphorylation), and antimycin A (to inhibit electron transport) to assess OCR linked to ATP synthesis, proton leak, reserve capacity, and non-mitochondrial (see Hill et al. 2009; Biochem. J. 424:99–107). Regardless of energy source (glucose with or without pyruvate) in the media, DF-1 cells exhibited; a) greater maximal respiratory capacity, b) higher reserve capacity, c) higher ATP-linked OCR, d) lower proton leak, and e) lower non-mitochondrial OCR compared with CEF cells. These bioenergetic differences likely contribute to the faster growth rate in DF-1 compared with CEF cells. We are planning to initiate bioenergetic studies in primary cell culture (e.g., hepatocytes, enterocytes, lymphocytes) in the near future.

Key Words: cell bioenergetics, chicken embryo fibroblasts, DF-1 cells


Studies were conducted to investigate the effect of 4-hydroxynonenal (HNE, a secondary product of lipid oxidation) on bioenergetics of CEF and DF-1 cells. Time course changes in O2 consumption rate (OCR) and extracellular acidification rate (ECAR) in CEF and DF-1 cells were made with an XF24 Flux Analyzer (Seahorse Biosciences, North Billerica, MA) in response to media (Control), 5, 10, 20, or 30 μM HNE. DF-1 cells were more susceptible to oxidative stress than CEF cells as determined by OCR and ECAR response to 20 and 30 μM HNE. Bioenergetic assessment of CEF and DF-1 cells was made 2 h post-HNE treatment by measuring the OCR response to oligomycin (ATP synthase inhibition), FCCP (to uncouple oxidative phosphorylation), and antimycin A (electron transport inhibition). This sequence of chemical treatments enables determination of O2 consumption linked to ATP synthesis, non-ATP linked O2 consumption (proton leak), maximal respiratory capacity, and non-mitochondrial mechanisms. Compared with CEF cells, DF-1 cells given media (0 μM HNE) exhibited a) lower O2 consumption linked to proton leak and non-mitochondrial source, and b) higher ATP-linked O2 consumption as well as greater reserve capacity (determined by the difference between uncoupled and basal OCR). DF-1 cells lost the capability of producing cellular energy above 10 μM HNE whereas CEF cells were still functional at the end of 240 min with 20 μM but not 30 μM HNE. These bioenergetic studies provide additional insight into characteristics of faster growth rates and greater susceptibility to oxidative stress in DF-1 compared with CEF cells.

Key Words: cell bioenergetics, chicken embryo fibroblasts, DF-1 cells, oxidative stress, 4-hydroxynonenal

33 Expression of the EphA4 receptor in the bursa of Fabricius. R. Jacob*SC, E. D. Peebles1, R. L. Taylor, Jr.2, S. L. Branton3, B. Weathers1, and G. T. Pharr1, 1Mississippi State University, Mississippi State; 2University of New Hampshire, Durham, 3USDA ARS, Poultry Research Unit, Mississippi State, MS.

The bursa of Fabricius is the major organ responsible for the development of B-cells in the chicken. A major B-cell differentiation event which initiates immunoglobulin gene diversification occurs in the bursa during embryonic days (ED) 15–18. In previous studies to identify factors in the bursa which mediate B-cell differentiation we obtained evidence for the expression of ephrin (Eph) receptor A4. Eph receptors and Eph ligands control cellular organization within tissues and mediate cell growth and differentiation. We hypothesize that Eph receptors and their ligands regulate the critical cell contacts between developing B-cells and stromal cells in the embryonic bursal follicle. To determine the role of EphA4 in B-cell differentiation, further basic studies are required to confirm and extend previous results suggesting EphA4 expression in the embryonic bursa. Therefore, the objective of this project was to examine EphA4 expression with reverse transcriptase-PCR and Western blotting in the bursa at ED15 and ED18. RT-PCR experiments were conducted with bursal cDNA amplified transcripts from the EphA4 gene, which were confirmed by nucleotide sequencing. The anti-EphA4 antibodies recognized proteins of the expected molecular mass (120 kDa) on Western blots of whole tissue proteins from the ED15 and ED18 bursa, suggesting a role of the EphA4 receptor in guiding B-cell differentiation during ED15 and ED18. Future studies will evaluate the cell type(s) expressing the EphA4 receptor in the embryonic bursa.

Key Words: B-cell, bursa of Fabricius, chicken embryo, ephrin receptor, immunology


This study is the first proteomics analysis of the muscularis complexus (pipping muscle) in broiler embryos. The pipping muscle is responsible for the embryo’s pipping action during hatching. The pipping muscle undergoes rapid developmental changes during embryonic life. Typical early muscle development occurs in the pipping muscle on d 13 of incubation before the rapid increase in lymph infiltration beginning between d 14 and 15 of incubation. The objective of this research was to characterize the pipping muscle proteome in the d 13 broiler embryo using Differential Detergent Fractionation (DDF) and nano-LC MS/MS analysis. A pipping muscle sample was extracted from each of 3 d 13 Ross 708 broiler embryos. These triplicate samples were washed in physiological saline, snap-frozen in liquid nitrogen, and stored at −80°C. Four DDF extractions were performed per replicate sample resulting in 12 technical samples that were analyzed using nano-LC MS/MS. Proteins with a single peptide were considered, if the peptide occurred multiple times. A total of 676 proteins were identified. Gene Ontology (GO) annotations for the identified proteins were collected from the AgBase database. For molecular function classification, 331 pipping muscle proteins (97.6% of annotated protein) had assigned 1298 GO
molecular function terms belonging to 325 diverse functions, summarized into 21 GO slim categories. A total of 302 proteins had assigned 1617 GO cellular component terms belonging to 231 cellular compartments, grouped into 17 GO slim categories. A total of 275 proteins had designated 2256 GO biological process terms from 690 biological processes, fused into 18 GO slim categories. This description of the pipping muscle proteome provides an important insight into various proteins involved in its cellular functions and other signal pathways.

Key Words: animal proteomics, broiler, embryo, nano-LC MS/MS, pipping muscle

35 Effects of turning frequency during different periods of incubation on broiler embryonic development. Y. M. Lin*, S. Yahav1, O. Eliboi3, and J. Brackle1, 3Dept. of Poultry Science, North Carolina State University, Raleigh, 2Institute of Animal Science, ARO, The Volcani Center, Bet-Dagan, Israel, 3Department of Animal Science, Faculty of Agriculture, University of Ankara, Ankara, Turkey.

The effects of turning broiler hatching eggs 24 (24X) or 96 (96X) times daily to E3, E9, or E15 of incubation followed by 24X to E18 was studied. Eggs were obtained from Ross 344 male x Ross 708 female broiler breeders at 51 wk of age. Freshly laid eggs were weighed and distributed so as to provide equal egg weights in each turning treatment before storage for 2–5 d at 16 C and 60% RH followed by preheating at 27 C and 53% RH for 12 h before setting. Each individually weighed egg in each turning treatment constituted a replicate. Incubators were operated at 53% RH at all times and at an air temperature of 37.5 C until E12, and 37.3 C from E13 to E 18 in Natureform NMC-1000 incubators. Eggs were transferred to individual pedigree bags at E18 and hatched in a Natureform NMC-2000 hatchers at 36.9 C. At E15 of incubation, 40 embryos from each treatment were necropsied to determine embryo length, and weights of the egg, embryo, yolk sac, and embryonic fluids. Chick BW and length were measured on all chicks at hatching. Smoking time was monitored. A t-test was used to compare means and variances. Embryo length at E15 was greater \((P < 0.05)\) in the 96X treatments but with a greater variance \((P < 0.05)\) as well as a greater variance \((P < 0.05)\) in relative weight of the yolk sac and embryo. This effect emerged as length of 96X turning increased. The 96X treatments hatched earlier than the 24X control. Further, chick BW relative to initial egg weight and chick length were greater \((P < 0.05)\) in the 24X treatment at hatching. These data indicated that more frequent turning before E15 resulted in a faster growing but less uniform embryo at E15. These data suggested that early embryo growth and development was accelerated by 96X turning during early incubation.

Key Words: broilers, incubation, turning frequency, chick weight, chick length

36 Physiological responses to right pulmonary artery occlusion of male broiler and Leghorn chickens. J. Bautista-Ortega1, G. Casco-Montenegro, and C. A. Ruiz-Feria, Texas A&M University, College Station.

Physiological responses to unilateral pulmonary artery occlusion (PAO) were evaluated in male broiler (B) and Leghorn (L) chickens. Chicks were purchased from a local hatchery and were fed a diet containing 3200 Kcal ME and 23% CP with free access to water. Broilers (18–20 d-old; 718 g BW) and L (61–63 d-old, 933 g BW) had surgical PAO (B-PAO and L-PAO; n = 12 each) or were sham-operated (B-SHAM and L-SHAM; n = 12 each). Hematocrit (% Hct), specific lung weight (wet right + left lung weight / BW) and right ventricle weight to total ventricle weight ratio (RV/TV) were measured in 6 chicks per group one d pre–surgery, and at 7 and 14 d post–surgery. Data was analyzed using One–Way ANOVA. Specific lung weight, a parameter of ventilation capacity, was higher in L–chickens than in B–chickens \((1.4 \pm 0.09 vs 1.0 \pm 0.04; 1.2 \pm 0.06 vs 1.0 \pm 0.05; 1.2 \pm 0.06 vs 1.0 \pm 0.06, \) for pre–surgery, d 7 and d 14, respectively). The Hct increases with hypoxia; there were no differences in Hct between B and L pre–surgery (31 ± 0.6 vs 32 ± 0.6), however the Hct was highest in the L–PAO chickens at d 7 (35 ± 1.4) and d 14 (40 ± 1.9); the B–SHAM had a lower Hct (d 7, 28 ± 1.2; d 14, 29 ± 1.0) than the L–SHAM (d 7, 32 ± 1.2; d 14, 34 ± 1.1) and B–PAO (d 7, 32 ± 1.2; d 14, 34 ± 2.0) chickens, with no significant differences between L–SHAM and B–PAO. The RV/TV ratio is related to the degree of pulmonary hypertension and was highest in the B–PAO at d 7 (0.31 ± 0.06) and d 14 (0.32 ± 0.03); the average RV/TV ratio of the other groups was: 0.21 ± 0.02 and 0.23 ± 0.01, at d 7 and d 14, respectively. Broilers had a lower ventilation capacity than L, and after PAO they developed right ventricular hypertrophy, whereas the L–PAO showed a higher degree of hypoxia (high Hct) but had lower RV/TV ratios suggesting that L–chickens had a better pulmonary arterial vasodilatory capacity.

Key Words: male Leghorn, broiler chicken, right pulmonary artery occlusion, pulmonary hypertension syndrome

37 Incidence of plexiform lesion formation in lines divergently selected for ascites. J. G. Mason*, N. B. Anthony, and R. Wideman Jr., University of Arkansas, Fayetteville.

Complex vascular lesions (CVLs) are a common vascular change associated with humans exhibiting idiopathic pulmonary arterial hypertension (IPAH). CVLs have been characterized in animal model studies involving, but not limited to, canines, swine and poultry. IPAH has been documented in poultry although no connection has been made with CVLs. Birds suffering from ascites exhibit pulmonary arterial pressures (mmHg) consistent with pressures found in humans with IPAH. The current study characterizes CVLs in the lungs from broilers derived from lines divergently selected 15 generations for ascites susceptibility under simulated high altitude conditions. The ascites resistant and susceptible lines were sampled for CVL incidence over time. Both lines were reared in a common environment and provided feed and water ad libitum. At ages 2, 4, 6, 8, 10, and 12 weeks post hatch, 5 males and 5 females from each line were sampled and lung tissue fixed, sectioned and stained for microscopy. Lung sections were then scored for CVL incidence by 2 independent researchers. CVL data were analyzed based on age post hatch, gender, lung location, and line. Findings indicate the susceptible line exhibited a generally higher CVL incidence than the resistant line at all ages. Data suggests lesion incidence is associated with selection for ascites susceptibility. These broiler lines may prove valuable in future animal model research.

Key Words: domestic fowl, broilers, pulmonary arterial hypertension, plexiform lesion, ascites

38 Evaluation of the effects of L-carnitine in ovo injection followed by L-carnitine feed supplementation on broiler hatching and growing characteristics. M. Dooley*, E. D. Peebles, W. Zhai, L. Mejia, and A. Corzo, Mississippi State University, Mississippi State.

On Day 18 of incubation, Ross × Ross 708 eggs were injected immediately before transfer with a solution containing L-carnitine (8, 16, or 32 mg dissolved in 100 μL of a commercial diluent) using an automated multi-egg injector. Three control groups (non-injected and injected with or without diluent) were also included. After hatch, 1,080 male and female broiler chicks were placed in an experimental broiler house
and distributed into 90 pens with 10 replicate pens per treatment. In the grow-out phase of the experiment, L-carnitine feed supplementation (50 ppm) overlaid on each of the 3 L-carnitine egg-injected treatments. Body weight and feed consumption were determined on Days 21 and 45. At 44 d of age, 3 male and 3 female birds per replicate pen were randomly selected for processing. L-carnitine injection had no effect on incubation time or hatchability of fertilized eggs. Birds that were fed supplemental L-carnitine that had been injected with in ovo L-carnitine had a lower BW on Days 21 ($P < 0.01$), and 42 ($P < 0.001$). Feed consumption was reduced at 21 ($P < 0.001$) and 42 ($P < 0.0001$) d of age. However, the feed conversion ratio of the L-carnitine feed-supplemented birds was improved ($P < 0.05$) when compared with most control treatments. Livability was not affected at hatch, 21 or 45 d of age by the experimental treatments imposed. After processing, absolute and relative weights of carcass, back-half and abdominal fat were found to be unaffected by treatment. Breast meat yield was also unaffected, but its absolute weight was reduced ($P < 0.05$) in response to the administration of L-carnitine through in ovo injection and feed supplementation. Based on the growth, feed consumption and breast meat yield results of this study, supplementing broilers with 50 ppm of L-carnitine in the feed appeared to be toxic to the birds if provided subsequent to its in ovo injection at the specified dosages.

Key Words: L-carnitine, in ovo injection, hatchability


Unfertilized chicken, turkey, and quail eggs are capable of developing embryos by parthenogenesis. It is unknown if the physiological mechanisms regulating parthenogenesis in virgin hens may actually work against fertilization, embryonic development and hatchability of eggs from these same hens following mating. Additionally, because most parthenogenic development closely resembles early embryonic mortality in fertilized eggs during the first 2–3 d of incubation, it is possible that many unhatched eggs classified as containing early dead embryos may actually be unfertilized eggs that contain parthenogenetic development. Therefore the objective of this study was to examine the relationship of parthenogenesis before mating with embryonic development and hatchability characteristics after mating. Based upon their ability to produce unfertilized eggs that exhibit parthenogenesis, 340 virgin Chinese painted quail hens were divided into 7 groups as follows: 0, 10, 20, 30, 40, 50, and 60% or greater parthenogenesis. Males were then paired with these hens so that fertility, embryonic mortality, and hatchability could be evaluated for each hen. Hatchability of eggs set, hatchability of fertile eggs, and late embryonic mortality decreased dramatically as the incidence of parthenogenesis increased. On the other hand, early embryonic mortality increased as parthenogenesis increased. Fertility was not different across the 7 parthenogenesis hen groups perhaps because unfertilized eggs that exhibited parthenogenesis resembled and were therefore classified as early embryonic mortality. In conclusion, virgin quail hens that exhibit parthenogenesis appear to have impaired embryonic development and hatchability following mating. Additional sperm egg interaction and embryonic research is needed to determine if a large portion of the early embryo death experienced by virgin hens exhibiting parthenogenesis is in fact infertility.

Key Words: parthenogenesis, fertility, hatchability, embryonic mortality, quail

40 Novel use of an in vivo reagent to transfect germ line stem cells in chicken. B. Jordan*SC, R. Beckstead1, and M. Stark2, 1University of Georgia, Athens, 2Brigham Young University, Provo, UT.

The chicken is a well-established model system for studying vertebrate embryogenesis, but creating transgenics to study mutants has proven difficult. Viral infections have been predominantly used to create transgenic chicks and disrupt the natural genetic code. Use of virus has been moderately successful but the rate of infection is low even with high titers of viral particles. Additionally, there are many biohazard concerns when working with virus. To circumvent viral problems, we are using a transposable element (TE) system paired with an in vivo transfection reagent to generate transgenic chicks. The TE system incorporates a transposase enzyme, which recognizes a specific DNA sequence called a transposon. The enzyme excises the transposon from its original location and inserts it into another genomic location. The transposon contains a GFP gene for tracking of insertion into active genes by fluorescent microscopy. Cells expressing GFP can be followed throughout development. The TE system is delivered to cells using an in vivo transfection reagent, JetPEI. This reagent interacts with DNA to form a bundle, which is then endocytosed by cells. Once inside, the bundles rupture and the TE DNA is released into the cell where integration can occur. The current question with this system is the ability to transfert germ line stem cells for the production of transgenic chicks. To test the in vivo delivery system, we prepared a mixture of in vivo reagent with a constitutively expressed GFP gene. This mixture was injected into freshly laid Stage X white leghorn embryos and incubated for 5 d. The embryos were removed from the shell, imaged for GFP expression and sectioned and stained with germ cell markers. Imaging showed localization of GFP to the germinal ridge, along with other tissues. Staining with antibodies to germ cell markers confirmed that the reagent transfected DNA into developing germ cells. With transfection possible, we can now pair the JetPEI reagent with the TE system to utilize a novel and powerful tool for creating transgenic chicks.

Key Words: transgenic, transfection reagent, transposable elements, embryogenesis, germ cells

41 Ingenuity Pathway Analysis of feed efficiency microarray data. W. G. Bottje*1, B-W. Kong1, J. Y. Lee1, J. J. Song1, K. Lassiter1, and T. Wing2, 1Dept. of Poultry Science, University of Arkansas, Fayetteville, 2Cobb-Vantress, Inc., Siloam Springs, AR.

Feed efficiency (FE) remains an important trait in poultry production. Therefore, we investigated global gene expression in breast muscle obtained from male broilers phenotyped for FE in a previous study (Poult. Sci. 88: 1683–1693). Amplified cRNA from pooled RNA (from high and low FE tissues) were labeled with Cy3 or Cy5 fluorescent dyes and hybridized to a 4 × 44 K Aligent chicken oligo microarray. After background-corrected red and green array intensities were normalized, polynomial regression (loess) was applied so only biological variations remained. Setting a 1.3 fold differential expression level cutoff, a total of 782 genes or 20% of the differentially expressed genes (4011 total) was obtained. Network, functional and canonical pathway analyses were generated using Ingenuity Pathways Analysis (IPA, Ingenuity Systems, www.ingenuity.com). The top 10 upregulated genes in the high FE group could be broadly grouped into anabolic-related processes whereas 7 of the top 10 upregulated genes in the low FE group (or downregulated in the high FE group) could be broadly classified into muscle fiber or cytoskeletal development/function. With the pathway designer function in IPA, a ‘cellular’ view of genes of the top 5 gene networks both
individuals and collectively (merged) was obtained. The merging of the top 5 networks incorporated a total of 38 upregulated and 84 downregulated genes present in the high FE broiler phenotype (there were also 39 other genes in these networks that were not differentially expressed). The relevance of this differential expression and top canonical pathways will be presented.

Key Words: feed efficiency, broilers, global gene expression, microarray, Ingenuity Pathway Analysis

42 Sperm residing in turkey sperm storage tubules are motile. M. R. Bakst*1 and J. P. Brillard2, 1Mississippi State, 2Clemson University, Clemson, SC.

Sperm residing in the hen's sperm storage tubules (SSTs) located at the uterovaginal junction (UVJ) are released over the course the daily ovulatory cycle, ascend to the site of fertilization, and fertilize the daily succession of ovulated ova. A proposed mechanism of sperm release from the SSTs infers that resident sperm swim against a fluid current originating from the SST epithelium and flowing toward the UVJ lumen. Sperm exit the SSTs when their velocity fails to exceed the velocity of the fluid generated by the SST. In the course of our studies with isolated UVJ mucosa containing SSTs, we have observed that sperm at the base of SSTs slowly oscillate in synchrony. This suggests that sperm are in a fluid environment and in fact are not quiescent but maintain some degree of motility. In this study we attempted to record by videomicroscopy movement of sperm within the SST lumen and record the egress of sperm out of the SSTs. Turkey hens were killed within 3 d of artificial insemination and the UVJ UVJ mucosa containing SSTs were excised and small pieces placed on a slide. A coverslip was placed over the tissue and gently squashed spreading the mucosa thereby rendering the SST and its lumen discernible by light microscopy. While we observed sperm at all levels of the SST slowly oscillating, sperm at the base of the SST were generally aligned head-to-head slowly oscillating in synchrony. When viewed from the UVJ surface, the cilia surrounding the SST orifice beat in a circular manner around the orifice. We failed to note the egress of sperm from the SSTs examined. This may be due to the squash preparation destroying tissue integrity, and the possible creation of a SST luminal environment not conducive to normal sperm and SST epithelia behavior.

Key Words: sperm, motility, oviduct

43 Effects of commercial in ovo injection of carbohydrates on liver metabolic profile. W. Zhai*1, L. W. Bennett1, P. D. Gerard2, R. Pulikanti1, and E. D. Peebles1, 1Mississippi State University, Mississippi State, 2Clemson University, Clemson, SC.

Effects of the in ovo injection of commercial diluent supplemented with dextrin or with dextrin in combination with various other carbohydrates on liver metabolic profile of Ross × 708 broiler chicks were investigated. Eggs containing live embryos were injected in the amnion on d 18 of incubation using an automated multiple-egg injector for the delivery of the following carbohydrates dissolved in 0.4 mL of diluent: 1) 6.25% glucose, 18.75% dextrin; 2) 6.25% sucrose, 18.75% dextrin; 3) 6.25% maltose, 18.75% dextrin; and 4) 25% dextrin. Also, a non-injected control, a commercial control (0.1 mL diluent-injected), and an experimental control (0.4 mL diluent-injected) were included. The results showed that hatchability of fertilized eggs was not affected by any injection treatment, but that differences in glycogen, glucose, and fat concentrations, and total glycogen and fat contents in the liver were observed on d 19 of incubation (E19). As compared with the 0.4 mL diluent-injected group, use of the supplementary carbohydrates increased liver glycogen and glucose concentrations (except for the glucose and dextrin combination group), and decreased fat concentration (except for the dextrin group) on E19. From E19 to day of hatch, glycogen concentrations dropped dramatically from an average of 3.2% to 0.6%. Despite the differences in glycogen, glucose, and fat concentrations on E19, these differences were lost by day of hatch. Liver glycogen and protein concentrations were negatively correlated on E19, and glycogen and glucose concentrations were positively correlated on day of hatch. In conclusion, the in ovo injection of various supplemental carbohydrates in 0.4 mL of commercial diluent examined in this study altered liver composition, embryonic metabolism, and the pattern of energy utilization by the embryo during the hatching process.

Key Words: carbohydrate, embryo, in ovo injection, liver, metabolism

44 Effects of in ovo injection of carbohydrates on broiler metabolism and embryogenesis. W. Zhai*1, P. D. Gerard2, R. Pulikanti1, and E. D. Peebles1, 1Mississippi State University, Mississippi State, 2Clemson University, Clemson, SC.

The effects of in ovo injection of different carbohydrate solutions on internal egg temperature, hatchability of fertilized eggs, time of hatch, liver weight, BW, yolk sac weight (YSW), and yolk-free BW (YFBW) of Ross × 708 broiler chicks were investigated. Eggs containing live embryos were injected in the amnion on d 18.5 of incubation using an automated multiple-egg injector with 1.2 mL of physiological saline (0.85%) or a carbohydrate dissolved in saline. Saline containing 0.3 g/mL of the following carbohydrates were injected into eggs: glucose, fructose, sucrose, maltose, or dextrin. Unfertilized and fertilized eggs were implanted with transponders for detection of internal egg temperature after injection. The egg temperature was monitored at 6, 14, and 22 h after injection. The results showed that the internal temperature of embryonated eggs at 22 h after injection were approximately 1.4°C higher than that of unfertilized eggs. As compared with the dry punch control group, injection of saline or carbohydrates in saline reduced internal egg temperature. The decrease of egg internal temperature was associated with a delay in hatch time, and a decrease in yolk sac absorption and weights of the liver and yolk-free body of hatchlings. Liver weight was negatively related to YSW and positively related to YFBW. The YSW was negatively related to YFBW. Even though the saline and carbohydrate solution injections increased BW as compared with non-injected controls, YFBW responded differently to the various carbohydrates as well as to saline injection. In conclusion, the injection of 1.2 mL of saline or carbohydrate solutions lowered embryonic metabolism as reflected by a lower internal egg temperature and delay in time of hatch. However, the effects of the different carbohydrate solutions on yolk absorption and tissue deposition in yolk-free-embryos varied.

Key Words: carbohydrate, embryo, in ovo injection, metabolism, temperature

45 Comparative transcriptomic analysis of *Salmonella* Insights into the adaptation of *Salmonella enterica* Kentuckiana in chicken cecum. Y. Cheng*1, S Porwollik2, M McClelland2, M Lee3, A Pedros3, and J Maurer4, 1University of Georgia, Athens, 2Vaccine Research Institute of San Diego, San Diego, CA.

Numerous studies have shown that *Salmonella enterica* serovar Kentucky is the most prevalent serovar in poultry in the US However, it is rarely associated with human illnesses although it has become such a successful colonizer of chickens. Epidemiologic evidences have suggested that S. Kentucky may represent a host-specific serovar adapted to chickens. But the molecular mechanisms underlying its success as colo-
izer in chicken remain unknown. Our previous data have revealed that S. Kentucky presents a growth advantage in chicken cecum compared with serovar Typhimurium. Therefore, it is hypothesized that the adaptability of S. Kentucky is due to its metabolic advantage in utilization of carbon or energy source present in chicken cecum. To determine whether differentiated expression of genes associated with the uptake and utilization of carbon source accounts for the adaptability of serovar Kentucky in chicken cecal environment, a comparative transcriptomic analysis of S. Kentucky and S. Typhimurium grown in medium supplemented with chicken cecal content was performed. We found that expression of 96 genes in S. Kentucky was upregulated in response to the chicken cecal content while in S. Typhimurium there are only 36 upregulated genes. Among the upregulated genes common to both serovar Kentucky and serovar Typhimurium, 26 genes were upregulated more significantly in S. Kentucky than in S. Typhimurium, including a prpBCDE operon which is involved in the propionate catabolism. In conclusion, our results suggest that high expression of genes associated with uptake and utilization of carbon sources available in chicken cecum may allow S. Kentucky to better colonize in chicken.

Key Words: Salmonella Kentucky, Salmonella Typhimurium, gene expression, propionate, cecum