

inoculated at 14 days of age and weighed and lesion scored 6 days post inoculation. Across all isolates there was a linear improvement in performance and decrease in coccidiosis lesions. Within all categories there were not significant differences between the NMI and N125 except for *E. acervulina* lesion scores (NMI 0.0 and N125 0.4). All other treatments and categories were significantly different. Feed conversions were NMU 1.447, NMI 2.594, N36 1.956, N72 1.886, N99 1.667, and N125 1.458. Average percent weight reductions compared to NMU were NMI 58.7, N36 40.8, N72 13.3, N99 6.4, and N125 1.7. Average lesion scores (Avg. of EA+ EM+ ET) were NMU 0.0, NMI 2.9, N36 1.8, N72 0.8, N99 0.3, and N125 0.1. Nicarbazin is often combined with other anticoccidial drugs typically at 36 ppm level. This information can be used to determine coccidiosis control at each level in combination or as a standalone anticoccidial drug.

Key Words: nicarbazin, anticoccidial, coccidiosis, eimeria, lesion

405P Comparison study of an impedance biosensor and rRT-PCR for detection of avian influenza H5N2 from infected chickens.

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The ongoing H5N1 highly pathogenic avian influenza (HPAI) outbreaks, originating from Southeast Asian countries, and spreading to Middle

Eastern, European and African countries, have driven global attentions and battles in fighting with deadly H5N1 virus to domestic poultry and also humans. Early detection or rapid diagnosis and effective control measures are needed to control the HPAI outbreaks and prevent its spread to other areas. This study's objective was to compare a newly developed impedance biosensor with real time RT-PCR (rRT-PCR) for detection of avian influenza virus (AIV) from tracheal and cloacal swab samples collected from experimentally H5N2 AIV infected specific-pathogen-free (SPF) chickens. After inoculation of 11-wk-old SPF chickens with H5N2 AIV, tracheal and cloacal swabs were collected daily for 7 days. rRT-PCR was performed on tracheal swab samples per NVSL protocol. The impedance biosensor was based on the use of a magnetic separator, magnetic nanobeads coated with AIV group and subtype specific monoclonal antibodies, and interdigitated microelectrodes in a polydimethylsiloxane (PDMS) microfluidic channel. After mixing the coated nanobeads with the swab sample, the target virus was captured, separated, and concentrated by the magnetic separator. Sample impedance was then measured using the interdigitated microelectrode. The presence of the virus caused a change in the impedance, which is linearly correlated to virus concentration. Of the 15 positive samples tested using the impedance biosensor and rRT-PCR, 14 were tested positive by both assays, for only 1 sample the biosensor was not in agreement with the rRT-PCR results (false negative). Detection time for the impedance biosensor is less than 1 hour compared to roughly 3-4 hours for rRT-PCR. This study indicates that the biosensor was comparable to rRT-PCR in detection of live AIV virus in poultry swab samples. Also, the impedance biosensor is portable and has great potential for in-field use, which would dramatically reduce the turnaround time for AIV detection.

Key Words: avian influenza, impedance biosensor, rapid screening, rRT-PCR, infected chickens

Poster Session: Physiology, Endocrinology, and Reproduction

406P Retinoic acid-induced meiosis of chicken primordial germ cells (PGCs). J. N. Petite*¹, C. Ge², J. Angerman-Stewart¹, and C. Zhang², ¹North Carolina State University, Raleigh, ²Zhejiang University, Hangzhou, China.

In mammals, germ cells enter into meiosis and arrest at prophase I in the embryonic ovary, whereas the drop of retinoic acid (RA) by Cyp26b1 in the embryonic testis protects male germ cells from initiating meiosis until sexual maturity. Organ culture of embryonic gonads and the long-term culture of chicken PGCs was used to investigate if RA plays a conserved role in regulating entry into meiosis in the chicken and if RA acts directly or indirectly on PGCs. Using organ culture, the addition of RA to stage 27 chicken gonads of both sexes significantly increased the mRNA expression of the premeiotic Stra8, as well as the meiotic markers Sycp3 and Dmc1. Using long-term cultured chicken PGCs, RA dramatically upregulated Stra8 expression in both male and female PGCs with similar increases in the expression of Dmc1 and Sycp3. Flow cytometry analysis for DNA content showed that after 4 days of RA treatment, 29.5% male PGCs and 58.37% female PGCs were at sub G1 phase, indicating that the cells had entered meiosis. Analysis of giemsa stained cells suggested a small difference between female and male PGCs in their capability to enter into meiosis where male PGCs appeared to have a lower capability to progress beyond zygotene/pachytene stages. This study shows that RA could induce chicken PGCs

of both sexes to enter meiosis and that RA acts directly on the PGCs of both sexes to enter meiosis.

Key Words: meiosis, germ cells, chicken, retinoic acid

407P Detection of SED1 and GalTase-I in rooster gonadal tissue: an investigation of two key molecules involved in sperm-egg fusion. A. P. Benson*¹, A. J. Davis², and B. D. Shur³, ¹Georgia Gwinnett College, Lawrenceville, ²University of Georgia, Athens, ³Emory University, Atlanta, GA.

Fertilization in eutherian mammals is dependent upon the binding of spermatazoa to the egg coat, or zona pellucida, prior to penetration of the ovum. Two key molecules, Beta 1,4-Galactosyltransferase-I (GalTase-I) and SED1 (MFG-E8 or lactadherin), have been determined to play significant roles in facilitating this binding and are thus critical in mediating sperm-egg interactions. Specifically, in mice, SED1 is involved in the initial binding of spermatazoa to the zona pellucida while the receptor protein GalTase-I promotes the acrosome reaction upon binding of the oocyte's zona pellucida protein, ZP3. Due to the paucity of information that exists regarding the function of SED1 and GalTase-I in avian sperm-egg interactions, we investigated the presence of these molecules in the testes of the domestic rooster, *Gallus gallus*.

Testicular tissue was isolated from 6 35-week old broiler breeder roosters. Total RNA was extracted and DNase treated for two step real-time PCR. Expression of SED1 and GalTase-I was examined using custom Taqman minor groove-binding probes and primers created based on chicken cDNA sequences for the two genes (Primer Express version 2.0). Detection of both genes was confirmed in the rooster's testicular tissue in conjunction with previous findings in mammalian species. In addition to examining gene expression of GalTase-I in testicular tissue, immunohistochemistry with a chicken GalTase-I antibody also confirmed the localization of GalTase-I in the seminiferous tubules of the rooster. Lastly, far-Western analysis determined that recombinant mammalian SED1 binds the avian sperm receptor protein ZP1 in zona pellucida from broiler breeder hens' F1 hierarchical follicles. These results demonstrate the testicular expression of SED1 and the presence of GalTase-I at the level of both gene and protein expression in rooster gonadal tissue. As is the case in mammalian species, these molecules may play key roles in promoting fusion of spermatozoa and ova. Future studies will expand upon differences in GalTase-I and SED1 expression in roosters varying in fertility.

Key Words: SED1, GalTase-I, sperm, fertilization

408P Effects of synthetic ice blocker and low-density lipoprotein liposomes on frozen-thawed rooster sperm. D. C. Bongalhardo*, M. Schenkel, S. K. Cunha, J. M. Silva, and J. C. Deschamps, *Universidade Federal de Pelotas, Pelotas, RS, Brazil.*

Synthetic ice blockers have been used to prevent cryoinjuries in oocytes and embryos, and could help to decrease sperm membrane damage during the freezing process. Previous work showed that adding low-density lipoprotein liposomes (LDL) to the cooling diluent helped to maintain rooster sperm membrane integrity during refrigeration for 96h at 5°C. The present work aimed to evaluate motility (MOT) and membrane integrity (MI) of rooster sperm frozen with dimethylacetamide (DMA), with or without synthetic ice blocker (SIB) and LDL in the freezing diluent. After collection, semen was pooled, split in four aliquots, and diluted 1:1 (vol/vol) in one of four solutions: Lake's diluent (control, T1), Lake's diluent + 3% LDL (T2), Lake's diluent + 1% SIB (T3), or Lake's diluent + 3% LDL + 1% SIB (T4). Treatments were equilibrated at 5°C for 10 min, and DMA (6% final concentration) was added. After 1 min exposed to the cryoprotectant, semen was packed into 0.25 mL straws, which were placed in nitrogen vapor for 1 min and then plunged into liquid nitrogen. Straws were thawed with swirling motion into water bath at 40°C for 15 s. After thawing, samples were maintained at room temperature for analyses. Semen was analyzed before and after cryopreservation. MOT was measured subjectively at microscope and MI was observed at fluorescent microscope after staining the samples with SYBR-14 and PI. Before freezing, MOT and MI had overall mean and SE of 93.3 ± 2.7% and 95.5 ± 0.8%, which markedly decreased in all treatments after cryopreservation. Means and SE for MOT after thawing were 7.5 ± 4.3 (T1), 9.2 ± 0.8 (T2), 20.0 ± 5.0 (T3), and 56.7 ± 1.7 % (T4). For MI, means and SE were 6.7 ± 3.3 (T1), 15.0 ± 10.4 (T2), 25.0 ± 5.0 (T3), and 61.7 ± 10.1 % (T4). Analysis of variance showed that T4 was superior to the other treatments in both semen parameters (Tukey test, p < 0.05). These results indicate that adding a combination of LDL liposomes and synthetic ice blocker into the freezing diluent improves rooster sperm motility and membrane integrity after thawing.

Key Words: LDL liposomes, synthetic ice blocker, sperm motility, sperm membrane integrity, rooster semen

409P Turkey line effect on avidin, avidin-related protein 2 and progesterone receptor expression in the hens oviductal sperm storage region following artificial insemination. O. T. Foye-Jackson*, J. A. Long¹, L. A. Blomberg¹, M. R. Bakst¹, M. V. B. Silva^{1,3}, K. G. Becker², W. H. Wood², and J. P. McMurtry¹, ¹*United States Dept. of Agriculture, Agriculture Research Service, Beltsville, MD*, ²*National Institute of Health-National Institute of Aging, Baltimore, MD*, ³*Embrapa Dairy Cattle, Juiz de Fora, MG, Brazil.*

Current in vitro semen storage methods maintain turkey sperm fecundity for 6-8 h. In contrast, sperm can be stored in vivo in the turkey hens sperm-storage tubules (SST) up to 10 wk. Yet, little is known about the cellular and molecular mechanisms supporting sperm survival in the SST. It has been shown that the presence of sperm in the turkeys SST was associated with up-regulation of avidin. To determine if avidin was a SST-specific, sperm-responsive gene across turkey lines, mRNA expression of avidin and two avidin associated factors, avidin-related protein-2 (AVR2) and progesterone receptor (PR), was determined in the SST and adjoining vaginal epithelium (VGE). At 38 wks of age, turkey hens (Hybrid Grade Maker and Converter) were artificially inseminated with Beltsville Poultry Semen Extender with (AI) or without semen [sham-inseminated (SI)]. Forty-eight hr after insemination, total RNA was extracted from the VGE and SST of SI and AI hens. Real-time PCR was utilized to analyze the expression of the avidin, AVR2 and PR transcripts. Grade Maker hens exhibited a tissue-dependent effect on avidin and AVR2 mRNA expression, with a 40 to 70-fold increase in avidin expression in the SST versus VGE of AI and SI turkey hens, respectively. A sperm dependent effect was found only in the VGE tissues of Grade Maker hens. In contrast, only the tissue dependent effect was present in Converter turkey hens; avidin, AVR2 and PR mRNA expression were higher in the SST than in the VGE of AI and SI hens. The up-regulation of avidin and AVR2 within the sperm storage region indicates these factors may be involved in the sustained storage of sperm in the SST. Alternatively, there is no known metabolic role for avidin in the oviduct. Yet, avidin's ability to bind biotin renders it antibacterial properties. Interestingly, *Campylobacter* and *Salmonella enteritidis* have been isolated from semen of commercial turkey toms. The possibility that avidin in and around the SSTs may inhibit infiltration of bacteria should be considered.

Key Words: sperm storage tubules, vaginal epithelium, uterovaginal junction

410P Effects of chicken dexamethasone-induced Ras-related 1 (cDexas1) on growth hormone (GH) gene expression in embryonic pituitary cells. J. Narayana* and T. E. Porter, *University of Maryland, College Park.*

Corticosterone (CORT) induction of GH in the anterior pituitary of the embryonic chicken requires ongoing protein synthesis, suggesting involvement of intermediary proteins in CORT regulation of GH expression. Microarray screening conducted on 14,053 unique chicken cDNAs, identified 14 candidate genes that may be involved in CORT induction of GH gene expression, including cDexas1. The aim of this project was to characterize the effects of cDexas1 on GH promoter activity and GH mRNA levels. The effect of over-expression of cDexas1 on GH promoter activity was tested using a luciferase reporter assay. A CORT-responsive 1727-bp 5'-flanking region of the GH gene was used as the reporter construct. Embryonic day 11 chicken pituitary cells were co-transfected with the GH-luciferase reporter, the cDexas1 expression vector and renilla luciferase control plasmid. The cells were then cultured with or without CORT. Dexas1 did not affect GH promoter activity.

Effects of over-expression on endogenous GH mRNA levels was tested by co-transfecting cells with cDexas1 expression vector and a green-fluorescent protein (GFP) expression vector. Positively transfected cells were sorted and collected by fluorescence-activated cell sorting. Total RNA was extracted from the GFP-positive cells, and GH mRNA levels were measured by qRT-PCR. Over-expression of Dexas1 (1 µg of vector) significantly inhibited CORT induced GH expression ($p < 0.05$). This effect was specific to GH, since Dexas1 did not affect mRNA levels of other genes tested. To determine if Dexas1 has a dose-dependent effect on GH regulation, varying doses of Dexas1 expression vector (1-100ng) were tested, with or without CORT. Preliminary data indicate that Dexas1 at lower doses may stimulate basal GH expression. These results indicate that Dexas1 may play a key role in GH regulation that has not been identified before in any species.

Key Words: dexas1, pituitary, somatotroph, corticosterone, development

411P Effects of *in ovo* injection of L-carnitine on subsequent broiler slaughter yield. M. M. Keralapurath, E. D. Peebles*, A. Corzo, S. K. Womack, and R. Pulikanti, *Mississippi State University, Mississippi State.*

The effects of *in ovo* injection of L-carnitine on the meat yield of Ross x Ross 308 broilers hatched from eggs laid by a young breeder flock were determined. Fertilized eggs were injected in the amnion with L-carnitine (0.5, 2.0 or 8.0 mg dissolved in 100 µl of a commercial diluent) on Day 18 of incubation using an AviTech Intellilab™ egg injector. Three control groups (non-injected and injected with or without diluent) were also included. Incubation length (hatch time) was recorded every 4 h beginning at 19.5 and ending at 21.5 days of incubation. Hatched chicks from each replicate treatment group were transferred to pens in Petersime batteries for brooding and further growout. Organ and muscle tissue samples were collected at frequent intervals during growout for determination of tissue nutrient profiles provided in a companion report. On Day 47 posthatch, approximately 4 birds from each pen were slaughtered and processed for determination of total carcass and abdominal fat pad weight. Furthermore, the slaughter yields of all major commercial cuts including the thigh, drumstick, wing, and breast muscle were determined. There were no treatment effects on relative carcass or abdominal fat pad weights, or for any of the commercial cut yields. Although the doses of L-carnitine used affected tissue nutrient profiles, as reported in a companion report, they had no effect on subsequent slaughter yield.

Key Words: amnion, broiler, *in ovo* injection, L-carnitine, slaughter yield

412P Effects of *in ovo* injection of L-carnitine on subsequent broiler chick tissue nutrient profiles. M. M. Keralapurath, E. D. Peebles*, R. W. Keirs, A. Corzo, L. W. Bennett, S. K. Womack, and R. Pulikanti, *Mississippi State University, Mississippi State.*

The effects of *in ovo* injection of L-carnitine on subsequent tissue nutrient profiles of Ross x Ross 308 broiler chicks from a young breeder flock were determined. L-carnitine at 3 dose levels (0.5, 2.0 or 8.0 mg dissolved in 100 µl of a commercial diluent) were injected into the amnion of fertilized eggs on Day 18 of incubation using an AviTech Intellilab™ egg injector. Three control groups (non-injected and injected with or without diluent) were also included. Hatch time was recorded

every 4 h beginning at 19.5 and ending at 21.5 days of incubation. On Day 0 posthatch (day of hatch), samples of chick liver, and breast, thigh and pipping muscles were collected and stored in perchloric acid. On Days 10 and 48 of posthatch growout, samples of chick liver, and breast and thigh muscles were also collected and stored in perchloric acid. Biochemical analyses were conducted on the samples collected to determine their glycogen, glucose, protein, and fat concentrations. On Day 0 posthatch, the 0.5, 2.0 and 8.0 mg doses of L-carnitine resulted in a significantly lower liver glucose concentration when compared to control eggs that were injected without diluent. On Day 3 posthatch, the 0.5 and 2.0 mg doses of L-carnitine resulted in a significantly higher moisture content of the pipping muscle when compared to diluent-injected controls. Furthermore, on Day 48 of posthatch growout, L-carnitine at the 2.0 mg dose resulted in a significantly higher thigh muscle glycogen concentration when compared to all 3 control groups. However, the variations in the tissue nutrient profiles produced by the L-carnitine doses reported in this study did not have any subsequent effects on hatchability, posthatch performance, or slaughter yield that were reported in other companion reports.

Key Words: amnion, broiler chick, *in ovo* injection, L-carnitine, nutrient profiles

413P A comparative study of chickens' auditory brainstem responses (ABR): The effect of age, sex and production type on the morphology of the waveforms. E. Otu-Nyarko*¹, P. Scheifele², D. Miller¹, and M. Darre¹, ¹*University of Connecticut, Storrs,* ²*University of Cincinnati, Cincinnati, OH.*

The Auditory brainstem response (ABR) comprises several amplitude deflections occurring within the the first 15ms after onset of an acoustic stimulus. These deflections are peaks and troughs which represent far-field synchronous activity produced by onset responses of neural elements in the neural fiber tracts of the eighth cranial nerve and the auditory brainstem pathway. The ABR provides a tool to reveal the collective activity of auditory neurons and an opportunity to evaluate the overall development of the auditory system. The hearing receptors along the auditory pathway are time locked with the passage of acoustic stimuli and indicate how well the chicken can hear. The objective of this study was to determine the latency-intensity function of the chicken ABR relative to age, sex and production type. Five White Leghorn chicks (4 weeks), hens (22 weeks), roosters (74 weeks) and broiler-breeder type chickens (10 weeks) were used for the study. To obtain electric potential along the auditory pathway, three subdermal needle-receiving electrodes were placed under the skin of the comb, auricular area and base of the skull. Using the GSI audera auditory evoked potential screening system an ABR test was run to obtain evoked potentials. Stimulus intensities presented were 102, 82 and 62 dB peSPL. The study was designed as a factorial RCBD. PROC mixed procedure of SAS was used for the statistical analysis at a significance level of $P < 0.05$. The morphology of the waveforms for all the categories of chickens tested showed up to seven peaks occurring within the first 10ms. Significant difference in the latencies of the peaks (I, III, V) between all age groups tested was observed. This is consistent with the fact that maturation of receptors for hearing along the auditory pathway occurs as the bird ages. Sex, however, did not significantly change the peak latencies in broilers. The waveforms of all the chicken categories tested showed specific morphology typical to the group.

Key Words: auditory brainstem response, waveform, latency, evoked potential

414P Removed**415P Photoperiodic modulation of clock gene expression in the avian premammillary nucleus dopamine-melatonin neurons.**

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The premammillary nucleus (PMM) has been shown to direct an endogenous dual-oscillation in dopamine (DA)/melatonin (MEL) rhythm and c-fos mRNA expression associated with the photo-inducible phase of gonad growth in turkeys. In this study, avian clock genes (Bmal1, Clock, Cry1, Cry2, Per2 & Per3) mRNA were colocalized in the PMM tyrosine hydroxylase (TH) immunoreactive (ir) neurons using in situ hybridization (ISH), double-label ISH/immunocytochemistry (ICC). The expression of circadian-driven clock genes in the PMM was determined under short (8L:16D) and long (16L:8D) photoperiods relative to changes associated with the diurnal rhythm of DA and MEL Constant darkness (0L:24D cycle) were also used to assess clock genes endogenous response. In addition, light pulses were given at zeitgeber time (ZT) 8, 14 & 20 to ascertain that clock genes are modulated by light pulses stimulation. In the PMM, the temporal clock gene expression profiles were similar under short and long photoperiods, except Per3 gene was phase shift induced in long photoperiod. In addition, Cry1 & Per3 genes were light-induced at ZT14 for reproductive stimulation while Clock gene was repressed. On a comparative basis, clock genes in the pineal gland were rhythmic under both photoperiods, but were not altered following light pulses at ZT14 which suggests that pineal clock genes may not be associated with the photosensitive phase and reproductive activities. In the vSCN, clock gene expression was phase shift dependent photoperiods by showing apexes at night in short daylength and during the day in long daylength. Also, light pulses induced Per2 gene while it repressed Bmal1 gene at ZT14. Taken together, Cry1 & Per3 could be the primary genes involved in the photic response associated with the PMM neuronal activation and stimulation of the reproductive neuroendocrine system. *Supported by National Research Initiative Grant (2007-35203-18072) from the USDA Cooperative State Research, Education, and Extension Service*

Key Words: clock genes, photoperiod, DA-MEL neurons, reproduction

416P Changes in vasoactive intestinal peptide and gonadotropin releasing hormone-I immunoreactivity in the brain of nest-deprived native Thai hen. N. Prakobsaeng¹, N. Sartsoongnoen¹, S. Kosonsiriluk², I. Rozenboim³, M. E. El Halawani², T. E. Porter⁴, and Y. Chaiseha*¹, ¹*Suranaree University of Technology, Muang, Nakhon Ratchasima, Thailand,* ²*University of Minnesota, St. Paul,* ³*The Hebrew University of Jerusalem, Rehovot, Israel,* ⁴*University of Maryland, College Park.*

Hyperprolactinemia has been associated with incubation behavior in native Thai chicken. This study compared the changes in the numbers of vasoactive intestinal peptide-immunoreactive (VIP-ir) neurons in the nucleus infundibuli hypothalami (IN) and nucleus inferioris hypothalami (IH) and gonadotropin releasing hormone-I-immunoreactive (GnRH-I-ir) neurons in the nucleus commissurae pallii (nCPa) of incubating native Thai hens (B) with those of nest-deprived hens (NB; n=4). Plasma prolactin (PRL) levels were determined by enzyme-linked immunosorbent assay. The numbers of VIP-ir neurons in the IN-IH on days 6 (B6), 10 (B10), and 18 (B18) of incubation were 128.5±13.0, 138.4±13.0, and 100.8±7.3 cells, respectively. When hens were nest deprived, VIP-ir neurons decreased (P<0.05, NB6=82.4±11.2; NB10=53.9±6.6; NB18=40.3±5.7 cells). This disruption of broodiness was accompanied by a precipitous decline in PRL levels (P<0.05, B6 vs NB6; 329.8±18.6 vs 34.1±6.6, B10 vs NB10; 481.5±40.1 vs 15.4±1.4, B18 vs NB18; 101.1±41.8 vs 24.8±3.8 ng/ml). The numbers of GnRH-I-ir neurons in the nCPa increased after nest deprivation (B6 vs NB6; 0.7±0.5 vs 2.1±0.7, B10 vs NB10; 0.9±0.3 vs 3.3±1.6, B18 vs NB18; 0.6±0.3 vs 2.0±0.6 cells). This study indicates an association between VIP neurons in the IN-IH and GnRH-I neurons in the nCPa with the degree of hyperprolactinemia, suggesting that the differential expression of VIP neurons in the IN-IH might play a regulatory role in year-round reproductive activity and subsequent PRL release. The increase in GnRH-I neurons may effect the changes in gonadotropins secretion that consequently affects egg production. *Supported by The Royal Golden Jubilee Ph.D. Program; #PHD/0176/2547(YC/NP).*

Key Words: birds, GnRH-I, nest-deprived, PRL, VIP

417P Cloning and characterization of chicken galanin type I receptors. J. C-W. Ho, D. Zhao, A. H-Y. Kwok, Y. Wang, and F. C. Leung*, *University of Hong Kong, Hong Kong, HK-SAR, China.*

In the present study, we report the identification of 2 chicken genes with considerable homology to galanin receptor type 1 (GalR1). Galanin, a 29- to 30- amino acid neuropeptide with diverse physiological effects, has been reported to be widely distributed in mammalian nervous systems and peripheral tissues. Through the interactions with the 3 known distinct G protein-coupled receptors (GPCRs), i.e. GalR1, 2 and 3, galanin was found to be involved in a broad spectrum of biological functions including modulation of hormone release, nociception, cognitive and feeding behaviour in mammalian species. To our knowledge, galanin receptors have yet to be cloned and characterized in any avian species. Using reverse-transcription polymerase chain reaction (RT-PCR), 2 full-length cDNAs of GalR1 homologues, which we termed GalR1a and GalR1b, were cloned from chicken brain and intestine tissue respectively. GalR1a encodes a 357- amino acid precursor protein that shares 84%, 82% and 84% sequence identities to the human, mouse and rat homologues respectively. On the other hand, GalR1b is 363 amino acids in length with comparatively lower homologies to the mammalian homologues (human, 53%; mouse, 53%; rat, 52%). Using RT-PCR, we also examined the expression of the two receptors in adult chicken tissues. Both GalR1a and GalR1b were found to be

expressed in most of the tissues examined with similar patterns. Using a pGL3-CRE-luciferase reporter system, forskolin-stimulated luciferase activity in Chinese hamster ovary (CHO) cells expressing GalR1a was inhibited by galanin in a dose-dependent manner, confirming the functional coupling of G_i protein to GalR1a. The same functional assay is under way for GalR1b. Cloning and characterization of chicken galanin receptors would provide a better insight into the physiological functions of galanin in the avian species.

Key Words: chicken, galanin, galanin receptor, GPCR

418P Fine mapping of the glucocorticoid-responsive region of the chicken growth hormone (GH) gene in embryonic pituitary cells.

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Normal differentiation of somatotrophs (GH-producing cells) in the chicken pituitary occurs on embryonic day (e) 14 and can be induced earlier by treatment with the glucocorticoid, corticosterone (CORT). Inspection of 10 kilobase (kb) upstream and 5 kb downstream of the GH transcription start site (TSS) indicates no consensus full length glucocorticoid response element (GRE), only several imperfect half sites. Furthermore, induction of GH mRNA by CORT can be blocked by a protein synthesis inhibitor, indicating an indirect effect requiring synthesis of an intermediary protein(s). Identification of the unknown protein(s) would be aided by fine mapping of the CORT-responsive region of the GH gene using a luciferase reporter construct. Previous research showed that a luciferase reporter containing 1727 base pairs (bp) upstream of the chicken GH gene was minimally responsive to glucocorticoids (2-fold) in a rat pituitary cell line. In the present study, e11 chicken pituitary cells were transfected with the same 1727 bp GH reporter construct and treated with 100 nM CORT for 20 h. Reporter activity with the 1727 bp construct increased 12-fold over basal when treated with CORT ($p < 0.05$; $n = 5$). Deletion of the 1727 bp region down to 1467 bp resulted in ablation of reporter activity ($p < 0.05$; $n = 5$). 12 additional constructs (1663, 1620, 1544, 1496, 1477, 1430, 1398, 1201, 1042, 954, 807, and 382 bp) were made to aid in fine mapping of the GH gene. All constructs were CORT-responsive, except the 954, 807, and 382 bp constructs ($p < 0.05$; $n = 5$), indicating the presence of a glucocorticoid-responsive region. The proximal glucocorticoid responsive region was found between 1042 and 954 bp upstream of the TSS ($p < 0.05$; $n = 3$). Addition of this 88-base-pair region in the antisense direction to the 382 bp construct resulted in partial recovery of the CORT-response ($n = 3$). This region is being tested for protein binding in gel shift assays. Positive identification of a glucocorticoid responsive region in the chicken GH gene will aid in understanding the regulation of GH cell differentiation during embryonic development.

Key Words: corticosterone, luciferase, reporter, GRE, embryonic

419P Involvement of Pit-1 isoforms in growth hormone gene expression in chickens: Characterization of a novel Pit-1 β isoform.

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The transcription factor Pit-1 is expressed in three types of cells in the anterior pituitary gland, where it binds to specific consensus sequences present in the promoter region and activates transcription of Prolactin (PrL), Growth hormone (GH) and thyroid-stimulating hormone beta

subunit (TSH β) genes. Alternative forms of Pit-1 have been reported in mammals and in chickens, which differ from each other in their N-terminal transactivation domain. Pit-1 is known to regulate chicken GH promoter activity, but it is not known which of the three isoforms, α , β or γ are involved. The aim of this study was to functionally characterize the isoforms for their ability to transactivate GH gene expression. We identified a novel Pit-1 β isoform containing a 58-bp β -specific insert instead of 84-bp as previously reported. This shorter cDNA makes the β -isoform the shortest and is predicted to code for a 315 amino acid protein which shows homology to rat (72% identity), human (72% identity), sheep (71% identity) and mouse (68% identity) Pit-1. We used a dual luciferase reporter assay system to evaluate the ability of the three isoforms to individually regulate chicken GH promoter activation in HeLa cells. Expression vectors for each isoform (1000ng) were transfected into HeLa cells along with 1000ng of reporter vector containing 1727bp of the 5'-flanking region of the chicken GH gene, which contains the Pit-1 binding site. Both Pit-1 α and the novel Pit-1 β isoforms increased GH promoter activity (4.8 ± 1.6 fold and 7.6 ± 1.6 fold, respectively; $n = 3$; $p < 0.05$), while Pit-1 γ had no effect (1.1 ± 1.6 fold; $n = 3$; $p > 0.05$). Thus, Pit-1 α and the novel Pit-1 β isoform identified could stimulate chicken GH gene expression in a heterologous system. Future research in our laboratory will focus on potential synergistic or inhibitory interactions between these isoforms in the transcriptional regulation of genes controlled by Pit-1.

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Key Words: POU-homeodomain, Pit-1, growth hormone, anterior pituitary, promoter activation

420P Ghrelin and obestatin influence on food and water intake in lines of chickens selected for high or low body weight.

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Ghrelin, a 28-amino acid peptide produced mainly by the stomach, is involved in the regulation of body weight and food intake in both mammals and chickens. Obestatin, a 23 amino acid peptide encoded by the same gene as ghrelin, was reported to reduce food intake and body weight gain in rats and is considered to be a physiological opponent of ghrelin. The aim of the present study was to investigate the role of ghrelin and obestatin on food intake in lines of chickens that had undergone long-term divergent selection from a common founder population for high (HW) or low (LW) eight week body weight. Ghrelin (0, 0.1, 0.2, and 0.4 nmole) and obestatin (0, 0.016, 0.032, and 0.064 nmole) were intracerebroventricularly (ICV) administered to males of each line. Ghrelin decreased food intake in both lines, with low doses of ghrelin being more efficacious in the LW chickens. Ghrelin had no effect on water intake in either line. Obestatin increased feed intake in HW chickens while obestatin had no effect on food intake in LW chickens. Water intake was not affected by obestatin in HW chickens while 0.016 nmole of obestatin significantly decreased water intake at 180 min post-injection in LW chickens. These data support the hypothesis that selection for body weight has resulted in alterations in the central ghrelin and obestatin system, and these differences may contribute to differences in body weight between these lines. *This project was supported by National Research Initiative Competitive Grant no. 2007-35206-17899 from the USDA Cooperative State Research, Education, and Extension Service.*

Key Words: ghrelin, obestatin, food intake

421P Differential response in feed intake regulation to centrally-administered insulin in layer-type versus broiler-type chicks. J.-I. Shiraishi*¹, K. Yanagita¹, M. Yanagawa¹, R. Fukumori¹, T. Sugino¹, M. Fujita¹, J. P. McMurtry², and T. Bungo¹, ¹*Hiroshima University, Higashi-Hiroshima, Japan*, ²*USDA-ARS, Beltsville, MD*.

The aim of this study is to elucidate whether insulin (INS) acts differentially within the central nervous system (CNS) of two types of commercial chicks to control ingestive behavior. In trial 1, male layer-type and broiler-type chicks (4-day-old) were ICV injected with saline, INS (20 or 100 ng) under fed conditions. Feed intake was measured at 30, 60 and 120min after treatment. In trial 2, blood and hypothalamus were collected from both chick types after fasting for 24h. Plasma INS concentration was measured by time-resolved fluoro-immunoassay. Hypothalamic INS receptor (IR), pro-opiomelanocortin (POMC), agouti-related peptide (AGRP) and neuropeptide Y (NPY) mRNA expression levels were measured by quantitative RT-PCR. The ICV injection of INS significantly inhibited feed consumption in layer-type chicks when compared with saline ($P < 0.05$), but not broiler-type chicks ($P > 0.1$). Plasma INS concentration of both chick types significantly decreased following 24 h of fasting, while INS levels in the broiler-type were significantly higher compared to the layer-type under ad libitum conditions. Although hypothalamic IR mRNA expression levels in layer-type were unaffected by 24h of feed deprivation ($P > 0.1$), significantly higher expression levels were evident in broiler-type chicks ($P < 0.05$). Conversely, POMC, AGRP and NPY mRNA expression levels were changed in both layer- and broiler-type chicks following 24h of fasting ($P < 0.05$). These results suggest that INS resistance exists in the CNS of broiler-type chicks, possibly due to persistent hyperinsulinemia, which results in a down-regulation of CNS INS receptor expression compared to that in layer-type chicks. In addition, possible dissimilarities in brain INS signaling mechanisms may be involved in the differences of feeding regulation and energy metabolism between layer- and broiler-type chicks.

Key Words: broiler-type chick, layer-type chick, feed intake, insulin, central nervous system

422P Removed

423P In vitro myogenicity of chicken muscle cell isolates. P. E. Mozdziak*, R. Malheiros, M. Gustillo, and J. Petite, *North Carolina State University, Department of Poultry Science, Raleigh*.

Isolation of postnatal chick muscle precursor cells is confounded by the inclusion of fibroblasts in the isolates. However, recent work has suggested that myogenic cells may also be located within the fibroblastic compartment. The objective of these studies was to assess the in vitro myogenicity of muscle cell isolates from chick Pectoralis thoracicus. The second objective was to assess the proliferative compartment of the total uni-nucleate cell population with age using 5-Bromo-2 prime-deoxyuridine (BrdU) labeling. Briefly, cells were isolated from the Pectoralis thoracicus of young (41 gram) chicks and mature chickens (15 weeks) and inoculated into culture. After a 24 hour attachment period, cells were fixed, and stained for Pax7, MyoD, myogenin, myosin, BrdU, and desmin. Furthermore, cells were also grown to confluency, induced to fuse into myotubes and a fusion index was calculated based upon the number of nuclei lying within myotubes. It was revealed for young chicks that $14 \pm 4\%$ were Pax7 positive, $17 \pm 6\%$ were myogenin positive, $74 \pm 2\%$ were MyoD positive, 100% ($n=4$ animals 6473 cells) were myogenin positive, and there was a fusion index of $99 \pm .4\%$. In young chick muscle $1.3 \pm .4$ were BrdU positive, whereas in mature chicken muscle $4.9 \pm 1.7\%$ cells were BrdU positive. The results suggest that there is an age-related increase in the proportion of proliferating cells in the chicken, and that the majority of the cells chicken muscle cell isolates are myogenic in vitro.

Key Words: satellite cell, avian, muscle, myoblast, BrdU

424P Bone density of laying chickens fed increasing levels of omega-3 PUFA DHA (22:6) using algae as vehicle of diet enrichment. N. P. Johnston*, R. T. Davidson, C. Buckley, and C. B. Evans, *Brigham Young University, Provo, UT*.

In recent years a host of health benefits have been associated with the intake of omega-3 fatty acids in general and DHA (22:6 n-3) in particular for both humans and animals. During a 12-wk feeding trial 60 SCWL pullets were fed omega-3 PUFA-rich diets with increasing levels of docosahexaenoic acid (DHA) 22:6 (n-3) to determine the dietary effects on bone mineral density (BMD). Two of the diets were DHA-free including a corn oil-enriched control and a flaxseed-enriched diet and in the remaining four diets a portion of the diet was replaced with increasing levels of algae ranging from 10% to 50%. As a result-dietary DHA ranged from 0 to 0.94% of the diet. The birds were evenly

divided by treatment and housed in individual cages in environmentally controlled rooms where they received feed/water *ad libitum* and a light-dark cycle of 14L:10D. It was hypothesized that with increasing levels of DHA there would be a corresponding increase in bone mineral density (BMD). At the conclusion of the feeding trial both leg quarters and wings were removed and scanned on a GE Lunar DEXA scanner for the BMD of the femur, tibia and humerus bones. Diet had no effect on the BMD of the humerus bones but appeared to positively impact femur and tibia BMD. The BMD of the femur (0.249 g/cm²) and tibia (0.227 g/cm²) of the 40% algae treatment was significantly ($P < 0.05$) greater than the control femurs (0.215 g/cm²) and tibias (0.206 g/cm²) as well as that of all other omega-3 treatments. The 10, 20, and 50% algae-fed birds had more dense ($P < 0.05$) femurs and the 10% and 20% had more dense ($P < 0.05$) tibias than the controls. In conclusion, the replacement of flaxseed with DHA-rich algae had a beneficial effect on the weight bearing bone (femur and tibia) mineral density of laying chickens.

Key Words: bone, density, omega-3, algae

425P Effect of dietary gelatin supplementation on intestinal goblet cells and villi morphometry in broiler chicks. Y. O. Fasina*, F. J. Hoerr, W. Zhai, S. R. Mckee, and E. T. Moran, *Auburn University, Auburn, AL.*

Gelatin is an ingredient that is higher in crude protein (95%) compared to soybean meal (45%), and is rich in mucin-forming amino acids such as glycine, glutamine, proline and serine. Intestinal mucins are secreted by goblet cells located in the villi epithelium. Incorporation of gelatin into chick starter diets may enhance goblet cell size and numbers, and improve villi morphometric indices such as villi surface area and villi:crypt ratio (V:C ratio). Thus, two experiments were conducted, each with 112 day-old broiler chicks. In each experiment, chicks were randomly allocated to 2 dietary treatments. Treatment 1 (SB) consisted of chicks fed a conventional corn-soybean meal (SBM) basal as control, while treatment 2 (SBG) consisted of chicks fed the basal corn-SBM into which 2% gelatin was added. Each treatment consisted of 4 replicate pens with 14 chicks per pen. On day 7 in both experiments and day 14 in experiment 2, jejunal tissue samples were collected, fixed in buffered formalin, routinely processed, sectioned, and mounted on glass slides. The slides were stained with alcian blue, periodic acid-Schiff, or haematoxylin and eosin for the evaluation of acid mucin-producing goblet cells (AGC), neutral mucin-producing goblet cells (NGC), and villi morphometric indices, respectively. Results obtained showed that gelatin has no effect ($P > 0.05$) on AGC size or numbers. However, for NGC, a bigger size was recorded in SBG chicks ($P < 0.05$) compared to SB chicks at 14 days of age. Results for villi morphometric indices at 7 days of age showed that in comparison to SB treatment, chicks in SBG had higher intestinal surface area in Experiment 1 ($P < 0.05$), and higher V:C ratio in Experiment 2 ($P < 0.05$). However, by day 14 in experiment 2, no differences were observed in villi indices between treatments. It was concluded that dietary gelatin supplementation has the potential to enhance early posthatch intestinal development. Further determination of optimal inclusion level is necessary.

Key Words: gelatin, goblet cell, villi indices, broiler chicks

426P Calcitonin directly increases adrenocorticotrophic hormone-stimulated corticosterone production in the chicken adrenal gland. K. Nakagawa-Mizuyachi*¹, T. Takahashi², and M. Kawashima³, ¹*The United Graduate School of Agricultural Science, Gifu, Japan,* ²*Depart-*

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In the chicken, calcitonin (CT) secreted from the ultimobranchial gland is known to regulate calcium homeostasis in an opposite manner to parathyroid hormone (PTH). In addition, adrenocortical cells which secrete glucocorticoids such as corticosterone and cortisol under the influence of adrenocorticotrophic hormone (ACTH) have also been implicated in calcium homeostasis via the parathyroid gland. However, the possible role of CT on this axis has not yet been determined. Thus, the present study was performed to show that CT can have an effect on the adrenal gland function, and to determine its physiological role.

The binding site of CT in the membrane fraction of adrenal glands obtained from hens was determined using a [¹²⁵I] CT binding assay system. The binding properties in the adrenal gland satisfied the criteria of a receptor-ligand interaction in terms of specificity, reversibility and saturation. These findings indicate that the CT receptor is present in the adrenal gland and that CT could directly impact adrenal function. When adrenocortical cells were incubated *in vitro* with chicken ACTH in the presence of CT, greater corticosterone production was observed. In summary, our results suggest that in the hen, by binding to its receptor, CT acts directly on adrenocortical cells to increase the responsiveness to ACTH, thus amplifying the production of corticosterone.

Key Words: adrenocortical cell, calcitonin, adrenocorticotrophic hormone, corticosterone, calcitonin receptor

427P The effect of various concentrations of salt on health of Japanese quail. B. K. Biswas*, N. Phillips, S. Mattice, and K. L. Arora, *Fort Valley State University, Fort Valley, GA.*

The effects of salt on high blood pressure is well known and higher salt consumption can also have a negative influence on kidney, liver, and other metabolic activities including diabetes and obesity. The present investigation was carried out on Japanese quail (*Coturnix japonica*), a lab animal model that has been used in toxicology for a long time. Physiological responses of Japanese quail by feeding two different salt concentrations were studied. The following parameters were examined: blood glucose, total plasma protein, PCV, body weight, and cloacal gland for sexual behavior. Eighteen healthy Japanese quail males of similar body weights were randomly divided into three groups; Group I (Control): received regular water and no salt; Group II: received 0.5% salt (Sodium chloride solution; table salt); and Group III: received 2.5% salt solution. Birds were kept in 36x36x12 inch wire cages, 14:8 L:D photoperiod, and received regular feed *ad libitum*. All birds were looking good for the first two days of the experiment but the birds in Group III appeared disoriented by the morning of the third day. The daily feed consumption of Group III was drastically reduced but consumption of 2.5% salt solution was not reduced. The average body weight, PCV and plasma protein were reduced; 17% of them died by the evening of the third day, 33% of them died within 4 days; and a total 50% birds died within 5 days. The salt solution was stopped and only water was provided to the rest of the birds. No birds died during the remaining part of the experiment. Group II birds tolerated 0.5% salt solution very well throughout the experiment of six weeks. The birds in this group consumed more feed and water as compared to control (Group I). In addition, there was a gain in their body weights, their PCV and plasma protein had also increased. This investigation reflects that the table salt (sodium chloride) is fatal to birds in higher (2.5%) concentrations. However, drinking the lower (0.5%) concentration does increase the

body weight, feed and water consumption of Japanese quail. Further investigations are underway.

Key Words: Japanese quail, *Coturnix japonica*, table salt (NaCl), health effects, lab animal

428P Blood electrolytes and acid-base balance are affected by F–Strain *Mycoplasma gallisepticum* inoculation in commercial egg-laying hens. H. A. Olanrewaju*, J. L. Purswell, S. D. Collier, and S. L. Branton, *USDA-ARS, Starkville, MS.*

Two trials were conducted concurrently to determine and compare, blood pH, blood gases, hematocrit, and hemoglobin in F–strain *Mycoplasma gallisepticum* (FMG) inoculation layers, and FMG contact–infected broilers. FMG–inoculated layers had the highest partial pressure of O₂ and the lowest partial pressure of CO₂ as compared with the other treatment groups. Blood pH values were unaffected by FMG inoculation. Hematocrit and blood concentrations of hemoglobin were slightly higher and HCO₃[–] levels were lowest in FMG contact–infected broilers in comparison to the other treatments groups. *Mycoplasma gallisepticum* inoculation layers also resulted in a significant increase in blood concentrations of K⁺, a decrease in Na⁺, but no significant effects on blood concentrations of Ca²⁺ and Cl[–]. There were no differences in plasma glucose, cholesterol, triglyceride, and anion gap, but osmolality was significantly reduced in FMG contact–infected broilers. Results indicate that inoculation of layers with FMG vaccine results in changes in plasma acid–base status along with changes in other blood metabolic variables. However, the FMG inoculation did not prevent homeostatic regulation of acid–base balance, as indicated by constant blood pH. There was a significant increase in pO₂, which is generally associated with an oxygen–dependent improvement in tissue oxygenation. Elevated arterial partial pressure of oxygen is beneficial to maximize oxygen transport capacity along with high concentrations of hemoglobin and hematocrit to carry oxygen throughout the body. It was concluded that in addition to protecting birds from MG infection, an FMG vaccine may improve the layer chicken's ability to withstand the harmful effects of stressors on their performance and well–being.

Key Words: *Mycoplasma gallisepticum*, acid–base balance, broiler chickens

429P Real-time PCR measuring gut microflora and its association with body weight selection. C. J. Denbow, P. B. Siegel, and D. M. Denbow*, *Virginia Polytechnic Institute and State University, Blacksburg.*

Obesity is a problem in broiler breeders. Developing strategies to control obesity requires a better understanding of its etiology, and recent evidence suggests that the gut microflora has an effect on body fat content. The aim of this study was to develop a real-time PCR quantification method as a tool to investigate whether gut microflora may contribute to obesity in a chicken model. Real-time PCR was used to monitor and compare *E. coli* and enterococcus populations of bacteria in fecal material from high- (HWS) and low-weight (LWS) selected lines of chickens. The HWS and LWS lines were selected for divergent body weight at 8 weeks-of-age for 50 generations. Samples of fecal material were collected from ten chicks from both high- and low-weight lines at weekly intervals from one to five weeks-of-age. The fecal samples were analyzed with real-time PCR using primers based on conserved genus-specific enterococcus 16S rRNA or *E. coli* malB promoter gene sequences. Fecal material (250 µg) was mixed with water, and the suspension mixed using a stomacher. Then, 100 µl was applied to a CloneSaver Card with FTA technology. The filter was allowed to air dry, and a 2.0 mm punch was removed from the sample using a Harris micro-punch. The CloneSaver punch was transferred to a microcentrifuge tube and DNA eluted as per the manufacturer's specifications. Real-time PCR was performed by fluorescence detection and melting-point analysis. For melting point analysis, the intercalating dye SYBR-Green (BioRad) was used for nonspecific labeling of target DNA. Pure strains of *E. coli* and enterococcus bacteria were used to prepare DNA for standard curves. A melt curve was plotted at the end of each run to verify the specificity of the amplification product. There appears to be no difference in enterococcal DNA between HWS males or females fed either ad libitum or restricted diets. However, enterococcal DNA concentration was increased in LWS birds at 4 weeks-of-age compared to high weight birds.

Key Words: obesity, *E. coli*, enterococcus

Poster Session: Processing, Products, and Food Safety Posters

430P *Campylobacter jejuni*, *C. coli*, and *C. lari* naturally present in Leghorn laying hens and the antibiotic resistance profiles of these organisms. N. A. Cox*¹, L. J. Richardson¹, R. J. Buhr¹, and P. J. Fedorka–Cray², ¹*USDA, ARS, PMSRU, Russell Research Center, Athens, GA*, ²*USDA, ARS, BEAR, Russell Research Center, Athens, GA.*

Campylobacter spp. are present in the intestinal tract and internal tissues of broiler breeder and broiler chickens. The objectives were to determine 1) *Campylobacter* spp. presence within internal tissues and organs of commercial Leghorn laying hens, 2) species of *Campylobacter* present, and 3) antimicrobial resistance pattern of *Campylobacter* isolates. In study 1, three flocks ranging from 94–105 wk-of-age were sampled from a commercial laying complex. In study 2, two flocks, 82 and 84 wk-of-age were sampled from a separate complex. Hens (n=30/flock) were euthanized, de-feathered, aseptically necropsied, and the ovarian follicles, spleen, liver/gallbladder, upper (infundibulum, magnum, and isthmus) and lower (shell gland and vagina) reproductive tracts were

aseptically removed prior to the ceca. Samples were packed on ice and transported to the laboratory for evaluation. For speciation, a standard BAX® PCR method was used while susceptibility testing was performed using NCCLS standards and recommended quality control organisms. Isolates were examined for susceptibility using a semi-automated testing system (Sensititre™) to the following nine antimicrobials: azithromycin, clindamycin, ciprofloxacin, erythromycin, florfenicol, gentamicin, nalidixic acid, telithromycin, and tetracycline. In study 1, the isolation rate was 13, 67, 53, 3, 13, and 57% from the ovarian follicles, lower reproductive tract, upper reproductive tract, spleen, liver/gallbladder, and ceca, respectively. In study 2, the isolation rate was 17, 43, 33, 20, 17, and 73% from the ovarian follicles, lower reproductive tract, upper reproductive tract, spleen, liver/gallbladder, and ceca, respectively. Overall, 53% of isolates were *C. coli*, 46% *C. jejuni*, and 1% *C. lari*. In study 1, all of the isolates were pan susceptible. In study 2, 37% of the isolates were resistant to tetracycline. Commercial table egg laying