

By the mid-1970s, the industry had evolved into its modern state with mechanization and automation technologies. By the early 1980s, consumers preferred cut-up and further-processed chickens to the traditional whole bird. Chicken surpassed pork consumption in 1985. Chicken consumption surpassed beef consumption in the United States in 1992. In 1998, USDA required the Hazard Analysis and Critical Control Point (HACCP) process control system program in all large poultry slaughter establishments. The HACCP regulation totally changed the

way chicken was inspected. No longer did the inspectors tell the plant how they need to accomplish something, now the plants must decide the best way to produce a safe product and be able to prove scientifically that their process is valid. The role of the Extension specialist has had to change and will need to continue to change to meet the changing needs of the industry.

Key Words: processing, history, extension

Physiology, Endocrinology, and Reproduction: Physiology I

42 Effects of genetics, transport stress, and *Escherichia coli* challenge on hematology and clinical chemistry parameters of turkeys. G. R. Huff*¹, W. E. Huff¹, N. C. Rath¹, N. B. Anthony², and K. E. Nestor³, ¹USDA/ARS/PPPSRU, Fayetteville, AR, ²University of Arkansas, Fayetteville, ³The Ohio State University, Wooster.

Males and females from 3 genetic lines of turkeys were compared for their response to an *Escherichia coli* airsac challenge followed by transport stress (Transport). The turkey lines were a slow growing line selected for increased egg production (Egg line), a fast growing line selected for increased 16 wk body weight (F line), and a commercial line (Comm line). Birds from each line were challenged at 14 wk with an air sac injection of 5,000 to 10,000 cfu of *E. coli* and were subjected to a transport stress procedure 8 d after the challenge that included a total of 12 h holding time in a transport vehicle. At the end of Transport birds were returned to their pens and provided with feed and water. The following morning all birds ($n = 10$ to 19 birds/line) were bled, which was 1 d after the start of Transport and 9 d after challenge with *E. coli*. Whole blood was analyzed using the Cell-Dyn 3500 blood analysis system (Abbott Diagnostics) and serum chemistry was measured using the Express Plus analyzer (Ciba-Corning Diagnostics Corp). Transport decreased hematocrit, hemoglobin, mean cell volume, mean corpuscular hemoglobin, glucose, triglycerides, cholesterol, phosphorus, iron, albumin, and alkaline phosphatase (ALP) levels and increased uric acid, blood urea nitrogen, alanine aminotransferase, aspartate aminotransferase, and creatine kinase (CK). Line differences were variable, but the levels of both iron and ALP were indirectly correlated with growth rate. Iron and ALP were also the only parameters influenced by gender, with males having higher levels of both compared to females. CK levels were over 6-fold higher in transported Comm line birds and iron levels of transported Comm males were 3-fold lower than controls. Previously, the growth rate of these lines was positively correlated with increased heterophil to lymphocyte ratios and susceptibility to colibacillosis. The highly significant differences seen in the Comm line for these commonly measured blood parameters suggest that they may be useful for profiling flocks to determine their response to transport stress and feed withdrawal.

Key Words: turkeys, transport stress, clinical chemistry

43 Circadian expression of clock genes in the pineal gland, visual suprachiasmatic nucleus (vSCN) and premammillary nucleus (PMM) in photostimulated turkey hens. B. Leclerc*, S. Kang, C. Howell, L. Mauro, and M. E. El Halawani, *University of Minnesota, St. Paul.*

Dopamine-melatonin (DA-MEL) neurons in the PMM have previously been described to be light/dark-inducible at 14 hours after light

on during the photosensitive phase. It is hypothesized that these PMM DA-MEL neurons may be a component of a biological clock involved in the reception of photoperiodic information critical for the regulation of seasonal reproduction in turkeys. To establish whether clock and melanopsin genes rhythms are related to the activation of these neurons, night interruption studies and circadian expression profile of these genes were conducted. In the first experiment, short day (6L:18D) birds were subjected to a light pulse of 30, 60 and 180 minutes duration at circadian times (CT) 8, 14, and 20. In the second experiment, short (8L:16D) and long day (16L:8D) birds were both sampled at CT1, CT5, CT9, CT13, CT17 and CT21. Brains were collected from each bird, the PMM and the vSCN were isolated using micropuncture technique and total RNA was extracted from nuclei and pineal glands. RT-PCR analysis was performed to determine the fold change in *bmal1*, *clock*, *cry1*, *cry2*, *per2*, *per3*, and melanopsin mRNA transcripts. In the night interruption study, our results suggest that 1 hour light stimulation induced ($P < 0.05$) *per3* gene expression in the pineal and PMM, but repressed it ($P < 0.05$) in the vSCN. A similar trend was observed for *bmal1* and *cry1* genes in the vSCN and PMM. In the circadian profile study, pineal *cry1* gene reached a nadir at CT21 and peaked at CT13 in the PMM, whereas melanopsin gene reached a zenith at CT21 and nadir at CT13 in the respective tissues. In the PMM, *per2* gene peaked at CT5, whereas *per3* gene was repressed at CT9. In the pineal and vSCN, both genes were positively related at CT1 and CT9-13, respectively. This study suggests that light is an effective entrainer of clock genes which are differently expressed in these neuronal targets.

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Key Words: gene expression, circadian rhythm, reproduction

44 Influences of maternal corticosterone and selection for divergent adrenocortical responsiveness to stress in Japanese quail on egg incubation length and hatchling body weight. J. B. Schmidt*, S. T. Treese, K. A. Davis, R. M. Andree, and D. G. Satterlee, *Louisiana State University Agricultural Center, Baton Rouge.*

While changes in corticosterone (B) during embryogenesis are thought to play a role in egg hatching, only a scant and controversial literature exists concerning the effects of in ovo (embryonic and maternally derived) B on the length of egg incubation (LEI) and chick body weight (BWT) at hatching. Because quail hens selected for exaggerated (high stress, HS) rather than reduced (low stress, LS) plasma B response to stress deposit more B into their eggs, these lines provide a good model to study the relationship between embryonic B and the above hatch variables. Therefore, we conducted studies that investigated the interactive effects of line (LS vs. HS) and maternal B treatment on LEI and hatchling

BWT. In Exp. 1, a preliminary investigation that used approximately 150 eggs per line, only the effects of line were studied; while Exp. 2, a large scale trial that used about 400 eggs per treatment combination, served to confirm Exp. 1 line effects on LEI as well as study any line interaction with hen B-treatment (sub-Q implants of B or no-B, CONs, during egg formation). In Exp. 1, on average, eggs from HS hens hatched 3.7 h sooner ($P < 0.0003$) than did eggs from LS hens while mean BWT was unaffected by line. In Exp. 2, the line effects on LEI were confirmed (HS eggs hatching 4.5 h sooner than LS eggs; $P < 0.0001$). In addition, eggs laid by B-treated hens hatched 2.9 h sooner than did CON eggs. Moreover, line*hen B-implant treatment effects on mean LEI partitioned ($P < 0.05$) as follows: LS-CON > LS-B > HS-CON > HS-B. BWT was not examined in Exp. 2 since line did not alter BWT in Exp. 1. Thus, selection for exaggerated adrenocortical responsiveness is associated with a reduction in the LEI without altering hatchling BWT. The findings of further shortenings of the LEIs in eggs of B-treated hens of both stress lines in Exp. 2 are particularly important to the poultry industry because they serve to warn layer, breeder farm and hatchery managers that unless stress in hens during egg formation is avoided, an abbreviated egg incubation period may result.

Key Words: Japanese quail, corticosterone, egg incubation length

45 Influences of maternal corticosterone in Japanese quail selected for contrasting adrenocortical responsiveness on the fertility, hatchability, and embryonic mortality of their eggs. D. G. Satterlee* and J. B. Schmidt, *Louisiana State University Agricultural Center, Baton Rouge.*

In avians, either no effects or severe negative consequences on reproductive performance have been associated with chronic corticosterone (B) treatment of hens. Furthermore, hens selected for exaggerated (HS, high stress) rather than reduced (LS, low stress) plasma B stress responsiveness deposit more B into their eggs than do LS hens, and female progeny of HS B-implanted hens show reduced egg production when compared to their LS and HS control and LS-B counterparts. Herein, we examined the interactive influences of maternal B-treatment, either sub-Q implants filled with no-B (controls, CON) or B, with quail stress line on egg fertility (FRT), and total and fertile egg hatchability (TOTTHATCH and FRTHATCH, respectively), as well as on the percentages of embryonic mortality (early deads, ED; late deads, LD) and pipped (PIP) eggs. FRT was dramatically reduced in eggs of HS compared to LS hens ($P < 0.0001$) and B-implant compared to CON-treated hens ($P < 0.0001$), with line*implant treatment FRT outcomes ($P < 0.05$) as follows: LS-CON = LS-B > HS-CON > HS-B. TOTTHATCH was also affected by line (LS > HS; $P < 0.0001$), implant treatment (CON > B-implant; $P < 0.0002$) and their interaction (LS-CON = LS-B = HS-CON > HS-B; $P < 0.05$), while line and maternal B-treatment did not affect FRTHATCH and only a trend for FRTHATCH to be reduced was detected in eggs from HS-B-treated mothers. While the percentages of LD and PIP eggs were unaffected by any of the treatments, embryonic ED was higher ($P < 0.05$) in eggs laid by HS-B-implanted hens than in eggs from any of the other 3 treatment groups. These findings are important to avian geneticists as they further emphasize the overall benefit that selection for reduced adrenocortical responsiveness would likely have on the reproductive performance of hens. In addition, the maternal B findings serve to warn layer, breeder farm and hatchery managers that unless stress in hens during egg formation is avoided, negative consequences in FRT, TOTALHATCH, and ED can also result.

Key Words: Japanese quail, corticosterone, egg hatchability

46 Alleviation of heat stress in market age broilers by strategic elevation of incubation temperature. S. Yahav*^{1,2}, E. Oviedo-Rondon², and J. Brake², ¹*Animal Science, Bet Dagan, Israel*, ²*Department of Poultry Science, University of North Carolina, Raleigh.*

The potential of inducing thermotolerance in broilers was elucidated by exposing embryos to elevated temperature during specific critical periods of incubation. Fertile eggs were divided into 2 treatments: the control treatment was incubated under standard single-stage conditions; while the thermal manipulated (TM) treatment was incubated as the control except from 7 to 16 d when eggs were incubated for 12 h/d at 39.5°C and 53% RH. At 17 d eggs were returned to standard conditions, transferred, hatched, and reared under standard conditions. At 35 d of age, broilers were heat challenged (35°C, low RH). The TM eggs exhibited slightly reduced hatchability (85.8% vs. 88.7%) as compared to the controls, but with no effect on chick BW. Body temperature (T_b) of the TM males and females was lower than that of the control treatment at hatching. Up to 35 d of age there were no significant differences between treatments for BW or FCR observed. However, a numerically heavier breast muscle was observed in the TM males and females. Prior to heat challenge at 35 d of age, T_b of the TM males was significantly lower than that of the controls suggesting that the TM males had a lower metabolic rate. Heat challenge for 5 h produced a severe hyperthermia in all males but hyperthermia was significantly less evident in the TM group than in the control group. No significant differences were observed in mortality. The females demonstrated less sensitivity to heat challenge with almost no mortality. Following heat challenge, significantly greater breast muscle was observed in both genders of the TM group at 39 d of age, which suggested reduced utilization of breast muscle during heat stress. It was concluded that exposure to elevated temperature during a specific period of embryogenesis improved acquisition of thermotolerance.

Key Words: embryogenesis, thermotolerance, heat stress

47 Serotonergic and catecholaminergic interactions with co-localized dopamine-melatonin neurons in the hypothalamic preamillary nucleus of the female turkey during different reproductive states. S. W. Kang*, B. Leclerc, L. J. Mauro, and M. E. El Halawani, *University of Minnesota, St. Paul.*

Serotonin (5-HT) and catecholamines (CAs; epinephrine and norepinephrine) appear to have important roles as neurotransmitters in avian reproduction, but their neuroanatomical relationship to the neuroendocrine circuitry that regulates reproduction is poorly understood. Our previous studies lead to the hypothesis that 5-HTergic/CAergic neurons in the brainstem and their interaction with PMM DA-MEL neurons might be an important circuit for reproductive function in the female turkey. The retrograde fluorescent tract tracer, DiI was injected into the PMM, and combined with 5-HT, tyrosine hydroxylase (TH), dopamine β-hydroxylase (DBH), and phenyl N-methyltransferase (PNMT) immunocytochemistry (ICC) to reveal neuroanatomical connections. Changes in the activities of 5-HTergic, and CAergic neuronal systems projecting to the PMM were measured at different reproductive states with in situ hybridization technique, using tryptophan hydroxylase 2 (TPH2) and TH mRNA expression, respectively. Cells labeled with DiI were found in anatomically discrete areas in or near the hypothalamus and the brainstem. Double ICC, using TH/5-HT, TH/DBH and TH/PNMT antibodies, confirmed that there were 5-HT, DBH, and PNMT immunoreactive fibers in close approximation to DA-MEL neurons. TPH2 mRNA expression in 5-HT neurons was found in the AVT, nDBC, LC, LoC, SCv and R.

TPH2 mRNA expression level was greatest in laying and incubating hens and least in the sexually inactive short day and photorefractory hens in all areas, except in the R. The most abundant expression of TPH2 mRNA was seen in the LoC of laying and incubating hens. Among the CAergic areas labeled with Dil, the nTS showed the highest level of TH mRNA expression in photorefractory hens and the lowest level in incubating hens. The patterns of 5-HT/CA neuronal distribution and their variable interactions with PMM DA-MEL neurons during different reproductive states may offer a significant neuroanatomical basis for understanding the control of avian reproduction. USDA Grant No. 2007-35203-18072

Key Words: dopamine neuron, serotonin, catecholamines

48 Caprylic acid maintains rooster sperm viability during in vitro storage. G. Liu^{*1,2}, A. M. Donoghue², K. Venkitanarayanan³, and D. J. Donoghue⁴, ¹Yangzhou University, Yangzhou, Jiangsu, P.R. China, ²PPPSRU, ARS, USDA, Fayetteville, AR, ³University of Connecticut, Storrs, ⁴University of Arkansas, Fayetteville.

Campylobacter and *Salmonella* are leading bacterial causes of human food-borne infections. Studies suggest that these microorganisms are highly prevalent in poultry semen and may contribute to vertical transmission between the breeder hen and offspring. Unfortunately, strategies to reduce or eliminate these pathogens in poultry semen negatively impact sperm viability. Caprylic acid, an 8-carbon fatty acid, has shown bactericidal effects against many pathogens in vitro, including *Campylobacter* and *Salmonella*. Before testing the efficacy of this compound against pathogens in semen, determining the effect of caprylic acid on poultry sperm viability is necessary. Therefore, the objective of this study was to test various concentrations of caprylic acid on chicken sperm viability during in vitro storage. Semen collected from multiple roosters was pooled and diluted in poultry semen extender alone (control) or treatments containing serial dilutions of a 1.4% caprylic acid stock solution (1:1, 1:2, 1:4, 1:8, 1:16, 1:32, 1:64, 1:128, 1:256, 1:512). Semen was stored at 4°C and assessed for viability utilizing SYBR 14/Propidium iodide live/dead stain and fluorescent microscopy after 6 h, d 2, d 5, and d 7 in vitro storage. At 6 h viability was maintained in controls and caprylic acid groups. By d 2 caprylic acid dilutions of 1:32 to 1:512 maintained viability equal or greater than controls ranging from 80–90% viable sperm. By d 7 the caprylic acid treatments of 1:32 to 1:512 maintained 70% viable sperm whereas control viability was 40%. Caprylic acid at optimal doses maintained sperm viability during in vitro storage better than controls and may provide an effective treatment for reducing pathogens in poultry semen.

Key Words: sperm viability, caprylic acid

49 Differential gene expression in the hypothalamus of neonatal chicks during feeding and fasting. S. E. Higgins^{*1}, L. E. Ellestad¹, N. Trakooljul², J. Saliba¹, F. McCarthy³, L. A. Cogburn², and T. E. Porter¹, ¹University of Maryland, College Park, ²University of Delaware, Newark, ³Mississippi State University, Starkville.

Delayed access to feed following hatching has been associated with reduction in growth performance in chicks. However, the gene networks within the hypothalamus that regulate feed intake and metabolism, and

the effects of fasting on those pathways are not well understood. The present experiment evaluated global hypothalamic gene expression in neonatal chicks using the Arizona *Gallus gallus* 20.7K Oligo Array v1.0 to elucidate genes and pathways regulated by feeding, fasting, and refeeding. Ten groups of chicks were sampled over 4 days posthatch, including: control (at hatch), 24-h fed, 24-h fasted, 48-h fed, 48-h fasted, 48-h fasted then 4-h refed, 72-h fed, 48-h fasted then 24-h refed, 96-h fed, and 48-h fasted then 48-h refed. Nonesterified fatty acids were elevated, and triglycerides were decreased in fasted chicks at 24 and 48 h, indicating that fasting induced physiological changes. Hypothalamic samples were collected from 8 chicks per group, and total RNA was extracted and pooled for hybridization (n = 4). Expression patterns of selected genes were confirmed by quantitative real-time PCR. Twofold differences in gene expression were detected in 1,272 genes between treatment groups, and of those, 119 genes were significantly ($P < 0.05$) different. Assignment of gene ontology terms to the significant genes resulted in 34 different categories of biological processes, with 24% of genes participating in signal transduction, transport, or metabolic processes. Genes that were up-regulated during fasting (and confirmed by qPCR) include FK506BP51, which is involved in the formation of steroid receptor complexes, and deiodinase type II, which is responsible for converting the thyroid hormone T4 into T3. Confirmed genes, down-regulated due to fasting, included proopiomelanocortin and fatty acid binding protein 7. Other regulated genes were identified that play a role in feeding and obesity in other species, such as relaxin 3 and adrenergic receptor- $\beta 2$. Further analysis of differentially regulated genes could provide new information regarding the role of the hypothalamus in feed intake and metabolism.

Key Words: hypothalamus, microarray, fasting

50 Physiological status of the broiler embryo during various phases of incubation with a special emphasis on the pipping muscle. R. Pulikanti^{*}, E. D. Peebles, R. W. Keirs, L. W. Bennett, S. K. Whitmarsh, and M. M. Keralapurath, *Mississippi State University, Mississippi State.*

The developmental relationships between liver and pipping muscle compositions of broiler embryos during incubation were determined. Ninety eggs obtained from a commercial source were incubated on 3 replicate tray levels (30 eggs per tray) for 19 d. During incubation, internal incubator conditions were monitored daily and egg weight loss between Days 0 and 19 was determined. On Days 15 and 19 of incubation, eggs were candled, and at least 10 eggs were randomly selected from each tray level for live embryo analysis. Whole livers and pipping muscles were obtained from the live embryos and were then analyzed to determine their relative weights, and their protein, fat, glucose, and glycogen contents. Also, the Day 19 pipping muscles were analyzed to determine their calcium concentrations. Between Days 15 and 19 of incubation, relative liver and pipping muscle weights, and liver fat and pipping muscle glucose, glycogen, and protein concentrations increased; whereas, liver protein and pipping muscle fat concentrations decreased. At Day 19, relative pipping muscle weight was positively correlated with its calcium concentration and negatively correlated with its fat concentration. Also, at Day 19, liver fat concentration was positively correlated with relative pipping muscle weight, but was negatively correlated with pipping muscle glycogen concentration. These results suggest that glycogen and glucose accumulate in the pipping muscle in preparation for pipping. Furthermore, by Day 19, increased relative pipping muscle weight was associated with increased liver fat depots

and respective increases and decreases in pipping muscle calcium and fat concentrations.

Key Words: broiler embryo, nutrient profile, pipping muscle

51 Changes in cellular localization of Nampt/PBEF/Visfatin in the chicken testis: Influence of sexual maturation. S. M. Krzysik-Walker*, O. M. Ocón-Grove, S. R. Maddineni, G. L. Hendricks III, and R. Ramachandran, *Pennsylvania State University, University Park.*

Sperm cells require vast amounts of energy upon maturation for successful fertilization. Visfatin, also known as Nampt and PBEF, is a cytokine-like enzyme involved in nicotinamide adenine dinucleotide (NAD) production, a coenzyme necessary for energy metabolism. We hypothesized that testicular visfatin expression would increase with sexual maturation and spermatogenesis. The objective of the present study was to investigate testicular and sperm cell visfatin expression, as well as plasma visfatin levels, in prepubertal and sexually mature broiler breeders. By RT-PCR, visfatin cDNA expression was detected in prepubertal and sexually mature breeder testes. Using immunohistochemistry, visfatin immunoreactive granules were predominantly observed in the nucleus of seminiferous peritubular and interstitial cells in prepubertal breeder testis. In sexually mature breeders, visfatin immunoreactivity was observed in interstitial cells as well as in the adluminal and luminal compartments of seminiferous tubules. Visfatin immunoreactivity was also observed in Sertoli cellular syncytia, as well as in spermatocytes and sperm cells, but not in the cytoplasm or nucleus of spermatogonial cells. Using real-time quantitative PCR, sexually mature breeder testis was found to contain 10-fold higher visfatin mRNA quantity compared to 14-week-old prepubertal breeders ($n = 4-6$; $P < 0.05$). Testicular visfatin protein quantities determined by Western blotting were not different between sexually mature and prepubertal breeder testes ($n = 5$; $P > 0.05$). Using immunoblotting, visfatin was detected in protein extracts of sperm cells obtained from breeder semen. Plasma visfatin levels, determined by enzyme immunoassay, were at least 20-fold higher in sexually mature breeders compared to 4- or 14-week-old prepubertal male breeders ($n = 6$; $P < 0.05$). Collectively, our results provide novel evidence that testicular visfatin expression undergoes dramatic changes as the broiler breeder undergoes sexual maturation possibly indicating that visfatin is involved in spermatogenesis, Sertoli cell function, NAD production, steroidogenesis, and sperm cell viability.

Key Words: visfatin, spermatogenesis, breeder

52 Impaired intestinal villi growth in broiler chicks with navel buttons. L. T. Kawalilak*, A. M. Franco, and G. M. Fasenko, *University of Alberta, Edmonton, AB, Canada.*

By the time of hatching the residual yolk sac (RYS) has been internalized into the chick's body cavity and the navel should be closed (healed). After hatching the RYS is absorbed and used for maintenance and growth. This research examined the relationship between unhealed navels and intestinal villi growth in chicks. Hatching eggs containing live embryos were collected from a commercial hatchery at 18, 19 and 20 d of incubation ($n = 5$ /day). At 21 d of incubation chicks with healed navels (H) and chicks with navel buttons no larger than 3 mm in diameter (B) ($n = 30$ /group) were collected. The chicks were grown out and sampled over a 5 d period. Embryos (5/day) and chicks (5/group/

day) were weighed, euthanized, and the RYS removed and weighed. Samples of the small intestine (duodenum and ileum) were processed for histological analysis and villi height were measured using MetaMorph software. The trial was replicated in time using eggs and chicks from the same flock. Data were analyzed using SAS proc Mixed ($P \leq 0.05$). No significant differences in average weight were observed between H and B chicks; however, the average RYS (as a percentage of chick weight) was greater in B ($5.0 \pm 0.3\%$) than in H ($3.6 \pm 0.3\%$) chicks. Significant increases in duodenal and ileal villi height were observed daily from 18 d of incubation until hatching. Duodenal villi height was shorter in H than in B chicks ($386.0 \pm 17.5 \mu\text{m}$ vs. $453.4 \pm 17.5 \mu\text{m}$, respectively) at hatching but longer on each day posthatch until 4 d. Ileal villi were longer in the H vs. the B chicks from hatching through to 5 d. The average posthatch villi heights were longer in the H chicks vs. the B chicks in both the duodenum ($571.4 \pm 7.2 \mu\text{m}$ vs. $545.0 \pm 7.2 \mu\text{m}$, respectively) and in the ileum ($320.5 \pm 4.0 \mu\text{m}$ vs. $303.0 \pm 4.0 \mu\text{m}$, respectively). The presence of even small navel buttons at hatching may lead to a subclinical yolk sac infection (omphalitis), and this may ultimately impair the absorption of the RYS causing a decrease in intestinal villi growth. Further research on the relationship between navel buttons and intestinal physiology is advised.

Key Words: chick navel, yolk sac, intestinal villi

53 Effects of electrolytic lesions of the medial bed nucleus of the stria terminalis (BSTM) on sexual behavior in male broiler breeders. D. L. Allen*, W. J. Kuenzel, J. Xie, and A. Jurkevich, *University of Arkansas, Fayetteville.*

An experiment was conducted to test the effect of lesions to subnuclei of the BSTM on appetitive and consummatory sexual behavior in male broiler breeders. The BSTM is known to contain sexually dimorphic cells expressing arginine vasotocin (AVT), an important modulator of male sexual behavior. Male broilers, ranging in age from 28 to 45 weeks were involved in the study. They were feed-restricted according to industry standards and housed in individual battery cages. All males had previous sexual experience. On 3 different days each male was placed for 20 minutes in an observation pen with 8 laying hens, behavior was video recorded, and frequencies of courtship and copulatory behaviors were noted. Males who copulated in all 3 observation replicates received bilateral electrolytic lesions to different portions of the BSTM. Lesions were made for each target site applying 1 mA DC current for 15 seconds. Birds were allowed to recover for 7 days before initiating behavioral observations postoperatively. Again, three 20-minute behavioral observations with 8 hens were recorded to determine if male sexual behavior had been altered. Birds were then perfused with Zamboni's fixative, brains removed, sectioned, and prepared for Nissl staining and immunocytochemistry using antibodies against AVT and aromatase. Estimates of the percent destruction of AVT containing nuclei were determined. In cases affecting sexual behavior, the BSTM2 (ventral medial BSTM) and the most caudal portion of the medial pre-optic nucleus were lesioned. Males with lesions to the BSTM2 showed marked reduction of copulation and mounting behavior; while waltzing and tidbitting were reduced by 84 and 70%, respectively. These data reveal that lesions to the ventral medial BSTM of broiler breeder males virtually eliminate consummatory behavior while some components of appetitive sexual behavior are still displayed. Supported by NRI grant no. 2005-35203-15850 from USDA, CSREES.

Key Words: vasotocin, copulation, septal-hypothalamic region

54 Differential neural activation in the septo-hypothalamic region following sexual and agonistic behavior in male broiler breeders. J. Xie*, W. J. Kuenzel, D. L. Allen, and A. Jurkevich, *University of Arkansas, Fayetteville.*

Sexual and agonistic behaviors are important social variables affecting fertility in male broiler breeders. Studies in birds and mammals have shown that the same brain nuclei, such as the medial bed nucleus of stria terminalis (BSTM) and paraventricular nucleus (PVN), are activated by both copulatory and agonistic behaviors. Our goal was to determine anatomical specificity of neural circuits within the septo-hypothalamic region following activation by copulatory or agonistic behavior. Mature male broiler breeders ($n = 19$) with sexual experience were randomly allocated to 4 groups. Each male was transferred to a testing pen for 20 min and placed either with a sexually mature female (FEM), a taxidermy adult female in a crouching position (DUMMY), a male (MALE), or alone (EMPT). One hour after observation, birds were perfused with a fixative and an immediate early gene product, Fos protein, a marker of cell activation was visualized in brain sections using immunocytochemistry. Number of Fos-immunoreactive cells, stained cell areas and optical density were determined in the BSTM1, BSTM2, PVN, and NCPa (bed nucleus of the pallial commissure). In the BSTM1, the FEM group had significantly more Fos-ir cells than the MALE group. No differences were found in the area and optical density of Fos immunoreactivity of this subnucleus. In contrast, no overall significant effect of treatment was found for Fos cell counts, their area and optical density in the BSTM2. In the PVN, the MALE group had significantly higher Fos-ir cell counts than the other 3 groups. In the NCPa, more Fos-ir cells were found in MALE and DUMMY groups than the FEM birds. Area of Fos-ir cells in the NCPa were significantly larger in the MALE group compared to the remaining ones. In summary, the BSTM1 appears to be activated following copulation, while stimulation of the PVN and NCPa is associated with agonistic behavior or stress. Supported by NRI grant 2005-35203-15850 from USDA, CSREES.

Key Words: Fos, stress, image analysis

55 Investigating the effects of estrogen on Zebra Finch eggshell thickness and eggshell morphology. S.L. Westmoreland*¹, H. Pourarsalan¹, K. A. Schug³, and J. R. Millam², ¹*Department of Biology, University of Texas, Arlington,* ²*Department of Animal Science, University of California, Davis,* ³*Department of Chemistry, University of Texas, Arlington.*

Female Zebra finch chicks are exposed to estrogen in their daily consumption of seeds. We hypothesized that estrogen causes eggshell thinning in Zebra Finch and eggshells treated with estrogen show a higher mammillary cone density. We then hypothesized that the eggshells treated with estradiol benzoate show a higher concentration of magnesium. Jim Millam at UC Davis administered the experimental female chicks with estradiol benzoate and canola oil for 7 days when they were 5–11 days old. Control females were administered canola oil only. They were allowed to form mating pairs with males at 110 days and 25 eggs were collected: 15 control and 10 experimental. Three samples were removed from each eggshell and digital electron micrographs of the eggs were taken at 500 \times . Thickness of the eggshells was determined using Image Pro Plus. It was found that the treated eggshells were significantly thinner than the control ($P = 0.02$). We then investigated the relationship between eggshell thickness and mammillary cone density. We marked the cone tips on the photos and counted the tips. The mean for treated eggshells was 65.97 (SD = 11.2) while the mean for control was 63 (SD = 14.6). This difference was not significant ($P = 0.228$). We then asked the eggshells to make 8 control and 8 experimental eggshells and measured their magnesium content using the atomic mass spectrometer. There was no significant difference between the treated and control groups ($P = 0.48$). We concluded that estradiol causes eggshell thinning in Zebra Finch, but the mammillary cone and magnesium content are not directly related to thickness.

Key Words: eggshell, estrogen, morphology

Immunology

56 Natural source vitamin E (RRR- α -Tocopheryl Acetate) level in broiler diets modulates expression of some cytokines. S. S. Block*¹, M. G. Kaiser², E. Beach², M. Sifri³, and S. J. Lamont², ¹*ADM Research, Decatur, IN,* ²*Iowa State University, Ames,* ³*ADM Alliance Nutrition, Quincy, IL.*

Level of vitamin E in the broiler diet has been reported to impact both growth and immune function. The objective of the current study was to determine the effects of varied levels of natural-source vitamin E (Nova-ETM) on growth and on mRNA expression level of 13 cytokines in the broiler chicken, with or without immune activation. Broilers, in a randomized block design, were fed 1 of 3 diets. Diets were industry standard, enhanced (standard + 10 \times Natural Source Vitamin E) and reduced (reduced Zn, Mn, Se, and vitamins A and D) for 23 days and a common diet days 24–36. Birds were given either a saline or lipopolysaccharide (LPS) injection on day 23, 3 hours before collecting blood for isolation of white blood cells for cytokine gene expression assays. Cytokines are natural immunomodulators produced in response to immune activation. The LPS source for immune activation was *E. coli*. Diet had a significant

effect on body weight, with birds on the reduced diet being smaller and having a higher death rate than the other 2 diets from 2 weeks of age through trial completion. Birds fed the enhanced vitamin E diet had less reduction in average daily gain after immune activation by LPS than did the industry standard diet group. LPS treatment decreased mRNA expression of interferon-gamma and the major histocompatibility complex IIB; and increased transforming growth factor β -4 and inducible nitric oxide synthase and interleukin-6. Interferon-gamma and transforming growth factor B4 gene expression was greater for birds on the enhanced diet than the standard. Blood vitamin E was numerically lower after LPS injection in birds receiving the industry standard (4.6 vs. 3.9 $\mu\text{g}/\text{mL}$; $P > 0.1$) and reduced diets (5.4 vs. 3.9 $\mu\text{g}/\text{mL}$; $P > 0.1$). Collectively, the results indicate that standard and enhanced vitamin E levels support growth performance, level of vitamin E modulates the expression of interferon-gamma, and enhanced levels of Natural Source Vitamin E may protect broilers against the reduction in gain that occurs with immune activation.

Key Words: natural source vitamin E, immunity, broiler