and slow growing (SG) lines were reared with a dietary supplementation of vitamin E (500mg/kg) and subjected to acute oxidative stress (high thermal load-31°C/75%RH, 2h). FG birds exhibited a marked myopathic response, which was significantly reduced by vitamin E supplementation (p<0.05). It is suggested that vitamin E may exert protective actions in birds during spontaneous and stress induced myopathies. Its mechanism of action may involve inhibition of lipid peroxidation and/or PLA2 activity.

Key Words: Myopathy, Vitamin E, Lipid peroxidation, PLA2, Broiler

Physiology - Reproduction

181  Effect of electrolytic lesions of the lateral septal organ on gonadal development in male broiler chicks (Gallus domesticus). R. Thilakarathna and W. Kuenzel, University of Arkansas, Fayetteville, AR 72701.

Most species of birds in temperate zones respond to long photoperiods by showing recrudescence of gonads. Compelling evidence shows that non-retinal, non-pineal photoreceptors exist in the ventral forebrain. Within the ventral forebrain are specialized neurons found in the medial portion of a structure called the lateral septal organ (LSO). The LSO contains Vasointestinal polypeptide (VIP)-like-immunoreactive cerebrospinal fluid (CSF-) contacting neurons, which may act as receptors that respond to light. The objective of this study was to examine the effect of electrolytic lesioning of the LSO region on gonadal development in male broiler chicks (Gallus domesticus). Birds were placed in an environmentally controlled chamber on a short photoperiod (LD 8:16) and were given chick starter and water ad libitum until two weeks of age, at which time surgical procedures occurred. Birds were anesthetized, placed in a stereotaxic instrument and the target approached from the dorsal portion of the skull. An electrode was directed to brain loci determined by the stereotactic atlas of chick brain, and 1 mA current was passed for 12 seconds. All treatment groups had their respective sham-operated groups, where birds underwent the surgical procedure, but did not receive the current. After surgery, birds were kept on a long photoperiod (LD 16:8) and received chick starter ration mixed with 0.2% sulfaflumazine (SMZ), a compound known to advance sexual maturation in male chicks. Sham-operated groups either received chick starter, or chick starter mixed with 0.2% SMZ. At the end of three weeks, brains were perfused in situ with 4% paraformaldehyde and collected for histology, and testes were weighed. Standard histology and immunocytochemical techniques were used to confirm complete ablation of VIP-like immunoreactive neurons and the viability of GnrH neurons. In three separate experiments conducted to date, bilateral lesions directed to the LSO resulted in a significant decrease in testes weight, compared to the sham-operated controls (p<0.05). Results suggest that cerebrospinal fluid-contacting neurons in the LSO may be directly involved in photoreception and play a significant role in gonadal development in male chicks.

Key Words: Photoperiod, Lateral septal organ, Encephalic photoreceptor

182  Time-dependent c-fos expression as a neuronal activation marker following electrical stimulation in the turkey hypothalamus. S. W. Kang*, O. M. Youngren, and M. E. El Halawani, University of Minnesota, St. Paul, MN, USA.

It is well accepted that electrical stimulation (ES) within the medulla preoptic area (POA) of the turkey hypothalamus results in the secretion of prolactin (PRL) from the anterior pituitary gland. However, the neuronal connections activated by ES which lead to PRL release have not yet been adequately investigated. To clarify the pattern of neuronal connection, the time course of these responses, induced by unilateral ES in POA, was studied using c-fos activation as a marker for neuron activity. This study was designed to investigate the neuronal connection that projects from POA into the infundibular area (INF), and which is believed to be involved in the neuronal circuitry leading to PRL secretion. Unilateral ES (100 µA) was delivered into the POA for 5 min followed by a 15 min post-ES period in 6 birds, for 5 min followed by a 180 min post-ES period in 6 birds, and for 30 min followed by a 15 min post-ES period in 6 birds. Brain tissue was harvested at the end of the waiting period. Blood samples for PRL radioimmunoassay were collected before, during, and after ES. Birds that received no ES, but were not treated the same. The expression of c-fos was visualized using in situ hybridization. ES caused plasma PRL to increase from a baseline of 96.4 ± 10.3 ng/ml to 139.4 ± 15.6 ng/ml in birds stimulated for 5 min and to 336.2 ± 56.8 ng/ml in birds stimulated for 30 min. Unilateral expression of c-fos in the POA was observed following 5 min ES/15 min post ES and 30 min ES/15 min post ES. No significant c-fos expression was found following 5 min ES/180 min post ES. Unilateral c-fos expression was seen in the ventromedial nucleus of the hypothalamus (VMN) following 5 and 30 min ES, but none was seen in the control brains. This is the first demonstration that neurons in the VMN are active during the ES in the POA which initiates PRL secretion. USDA grant No. 00-35203-9157

Key Words: Turkey hypothalamus, Electrical stimulation, c-fos, In situ hybridization

183  WITHDRAWN. . .

184  Coexpression of dopamine or vasoactive intestinal peptide receptors with prolactin in the turkey pituitary. Y. D. Chaiseha*, O. M. Youngren, and M. E. El Halawani, 1 School of Biology, Institute of Science, Suranaree University of Technology, Thailand, 2 Department of Animal Science, University of Minnesota, St. Paul, MN.

Avian prolactin (PRL) secretion and PRL expression are regulated by vasoactive intestinal peptide (VIP) neurons residing in the infundibular nuclear complex (INF) of the hypothalamus. Dynorphin, serotonin, dopamine (DA), and VIP appear to stimulate PRL secretion along a common pathway, with VIP as the final mediator. Recent evidence indicates that DA and VIP receptors play a pivotal role in VIP and PRL secretion. The differential expression of DA and VIP receptors on anterior pituitary cells may regulate the prolactinemia observed during the turkey reproductive cycle. PRL mRNA gene expression was quantitated utilizing in situ hybridization histochemistry. Coexpression of D1D or D2 DA receptor mRNA or VIP receptor mRNA within pituitary cells expressing PRL mRNA was quantitated utilizing double in situ hybridization histochemistry. PRL mRNA, while found throughout the hypothalamus, was predominantly expressed within the pituitary. Pituitary PRL mRNA was 1.9-fold greater in laying hens and 6.5-fold greater in incubating hens than that of non-photostimulated hens. When hens shifted from incubation to photorefractoriness, pituitary PRL mRNA levels decreased to the same levels as that of non-photostimulated birds. Double in situ hybridization revealed that D1D and D2 DA receptor mRNA expression was more highly expressed than that of D1D receptor mRNA. The expression of D2 DA receptor mRNA on PRL-expressing cells was greatest in photorefractory and non-photostimulated birds, as compared to that of laying and incubating birds. The most dense VIP receptor mRNA expression was observed on cells expressing PRL mRNA in incubating birds, followed by laying birds, when compared to non-photostimulated birds. When hens stopped incubating and became photorefractory, PRL-VIP receptor coexpression became the same as that of non-photostimulated birds. The present findings clearly demonstrate that, in birds, DA and VIP receptors play a central role in VIP and PRL secretion, reinforces the evidence that VIP is the PRL-releasing factor, and suggests that PRL secretion is principally regulated by DA and VIP receptors at the pituitary level. USDA Grant No. 00-02157

Key Words: Dopamine receptor mRNA, Vasoactive intestinal peptide Receptor mRNA, Avian prolactin, Turkey pituitary, In situ hybridization

185  Tyrosine hydroxylase mRNA expression in the turkey hypothalamus. A. Thayanunphat*, S. W. Kang, K. Al-Zailaie, O. M. Youngren, and M. E. El Halawani, Department of Animal Science, University of Minnesota, St. Paul, MN.

Avian prolactin (PRL) secretion is regulated by vasoactive intestinal peptide (VIP) neurons residing in the infundibular nuclear complex (INF) of the hypothalamus. This VIPergic activity is modulated by stimulatory dopamine (DA) inputs acting through D1 DA receptors located in INF. At the level of the anterior pituitary DA activates inhibitory D2 DA receptors and subsequently prevents VIP from stimulating the
release of PRL. The neuroanatomical substrate for DA neurons in the turkey hypothalamus has been described utilizing tyrosine hydroxylase (TH) and DA-β-hydroxylase immunohistochemistry. To further characterize the distribution of DA neurons in the turkey hypothalamus, we investigated the distribution of neurons expressing TH mRNA in laying turkeys. TH mRNA expression was visualized by in situ hybridization histochemistry utilizing a TH riboprobe. A 372 bp TH cDNA was cloned from the turkey hypothalamus by reverse transcription polymerase chain reaction (GenBank TM accession No. AF 169720). The medial pre-optic area, suprachiasmatic area, paraventricular area, anterior medial hypothalamic area, ventromedial hypothalamic area, lateral hypothalamic area, dorsomedial thalamus, and the medial mammillary area were surveyed. The results showed that the expression of TH mRNA follows the same distribution pattern as that revealed by immunohistochemistry for TH protein.

**Key Words:** Tyrosine hydroxylase, Turkey hypothalamus, In situ hybridization, Avian prolactin

### 186 Age and photoperiod influences on the incidence of a polycystic ovarian follicle syndrome in laying turkey hens. W. Bacon* and H.-K. Liu, The Ohio State University. An arrest in laying associated with a polycystic ovarian follicle syndrome (PCOF) has been reported early in the egg production (EP) period in turkey hens photostimulated at 30 WOA with constant light (Poultry Sci. (2001) 80:1509). In comparison to normally laying hens, hens with the PCOF syndrome had ovaries that contained an increased number of yolky follicles, but their oviducts were of equal weight 2 to 3 wk after laying ceased. This initial report has been followed by 4 trials to examine effects of age at photostimulation and photostimulation with diurnal lighting of 14L:10D (14L) or constant lighting of 24L:0D (24L) on the incidence of the PCOF syndrome. Hens of the Egg line were given short-day photostimulation of 6L:18D at 16 WOA and then photostimulated with either 14L or 24L lighting at various ages between 26 to 70 WOA. EP was followed for 6 to 8 wk, and hens that stopped laying during this period were autopsied 2 to 3 wk later to determine incidence of the PCOF syndrome. At 26 WOA, the PCOF incidence was 80% with 24L lighting and 31% with 14L lighting (P<0.01). At 28 WOA, the PCOF incidence was 60% with 24L lighting and 27% with 14L lighting (P≤0.05). At 31, 34, and 41 WOA, there were no differences in incidence of the PCOF syndrome between the 24L and 14L treatments. With the 24L lighting treatment, the PCOF incidence at 26 and 28 WOA (80% and 60%) were greater than at 31 WOA (20%) and older ages (P<0.025). With the 14L lighting treatment, the PCOF incidence was not different among ages (26WOA, 31% to 41 WOA, 12%). We conclude that the incidence of the PCOF syndrome is greater when photosensitive turkey hens are photostimulated at relatively young ages (less than 31 WOA) with constant lighting.

**Key Words:** Photostimulation, Age, Polycystic ovarian follicle, Turkey

### 187 Genetic selection for growth, egg production or other traits; the resulting patterns of sperm penetration of the perivitelline layer at the germinal disc. R. K. Bramwell*, Center of Excellence for Poultry Science, University of Arkansas, Fayetteville, Arkansas. The process of fertilization requires that sperm bind and penetrate the outer vestments of the yolk material in order to gain access to the female pronuclei. The act of sperm penetration of the perivitelline layer must occur at the germinal disc region (GD) in order to properly fertilize the ovum. Various lines of poultry have been known to exhibit different capacities for sperm penetration at the GD, but sperm penetration away from the GD (nGD) have not been addressed. The purpose of this study is to evaluate the ratio of sperm penetration of the GD region to the nGD area in various lines of birds that have been subjected to vastly different genetic selection criteria. Hatching eggs were obtained from commercial broiler breeder flocks, and Brown and Bovan White egg-laying strains between 35 and 45 weeks of age. Sperm penetration of the GD area and nGD was determined for all eggs. Overall mean sperm penetration of the GD area was significantly different (50.0, 63.5 and 80.8) for the broiler breeders, Bovan Brown and Bovan White strains of birds, respectively. The ratio of GD:nGD sperm penetration was 13.68, 3.56 and 1.83 for the broiler breeders, Bovan Brown and Bovan White strains, respectively. Additionally, two lines of Japanese quail Coturnix coturnix, (P-line quail, 116 generations selected for growth, and a control line) were tested. The heavy P-line quail had mean sperm penetration values of 20.3 with a GD:nGD of 8.12 while the control line had mean value of 76.2 and a ratio of 1.29 GD holes for every nGD sperm hole. The numerical ratio in the P-line was actually more drastic as a few hens had nGD ratios of > 50:1 while many hens had few if any holes anywhere. Therefore, from this study it appears that selection pressure for growth has resulted in hens that have a greater propensity for sperm to bind and penetrate the ovum at the germinal disc region as opposed to other areas of the ovum.

**Key Words:** Sperm penetration, Germinall disc

### 188 A novel method for improving the fertility of glycerol-exposed poultry semen. J. Long*1 and G. Kulkarni2, 1BGL, ANRI, BARC, ARS, USDA, 2ADOL, USDA. Semen cryopreservation is necessary to maintain valuable commercial and research poultry lines indefinitely. To date, glycerol is the most effective cryoprotectant for poultry sperm; however, the contraceptive effects of glycerol require complete removal of the cryoprotectant from thawed semen prior to artificial insemination. Objectives here were to investigate the effectiveness of an Accudenz TM density gradient for glycerol removal from poultry semen. For Study 1, highly inbred ADOL lines were used. Semen was pooled from 15 roosters, diluted 1:1 with Lake’s extender and then with 33% glycerol/Lake’s (vol/vol) to yield a final 11% concentration. Glycerolated semen was aliquoted as control, density gradient (AZ) or dialysis (DS) and processed accordingly to remove glycerol. Twenty hens/sperm treatment were inseminated weekly with 100x10⁶ sperm/ml for 6 weeks and egg fertility was assessed. All eggs from the control semen were infertile, and fertility rates in the DS treatment dropped steadily from 19.7±4.0 to 5.9±1.6% within the first 3 weeks. In contrast, fertility rates for the AZ treatment rose from 9.4±1.7% during week 1 to 36.4±5.7% during week 4. For Study 2, turkey semen from 6 toms was pooled and aliquoted for 3 treatments: control, AZ and AZ/G. Only semen in AZ/G was exposed to glycerol (11% final concentration). Ten hens/sperm treatment were inseminated weekly with 150x10⁶ sperm/ml for 6 weeks and egg fertility was assessed. Similar to Study 1, fertility rates in the AZ/G treatment steadily increased to 47.5±5.9% by 6 weeks. Interestingly, fertility for the AZ treatment increased steadily from 14.3±8.6 (week 1) to 100% (week 6). In summary, using an AZ density gradient improved the fertility of poultry semen both exposed and unexposed to glycerol. Further development of this protocol will enable successful line regeneration from poultry semen frozen with glycerol as the cryoprotectant.

**Key Words:** Poultry semen, Cryopreservation, Glycerol

### 189 Light-induced reduction of cytoplasmic free calcium in neurons proposed to be encephalic photoreceptors (EPRs) in chick brain. H. Li1*, M. Ferrari2, and W. Kuenzel1, 1University of Arkansas at Fayetteville, 2University of Missouri at Kansas City. It is established that photic stimuli associated with changing day length regulates seasonal cycles of reproduction in birds. The hypothesis of von Frisch proposed that the diencephalon houses nerve cells specifically sensitive to light, i.e. the brain contains EPRs. Support of his idea has been obtained in some avian species showing birds can develop gonads in the absence of eyes and pineal gland. Promising candidates for EPRs are vasoactive intestinal polypeptide (VIP)-containing cerebrospinal fluid (CSF)-contacting neurons in lateral septum (SL) and hypothalamus since the neurons successfully bind with an antibody for opsin, a highly conserved protein in rods and cones (Silver et al. 1988. Cell Tissue Res. 253:189). Later studies supplied more evidence by showing VIP-like-immunoreactive neurons directly contact GnRH-like-immunoreactive neurons in lateral septum-preoptic area of pigeon, and some subunits of transducin, photodiesterase, and cGMP-gated channel, important in the retinal photoreceptor-like phototransduction pathway, have been identified in the pigeon SL (Wada et al. 2000. J. Comp. Neurol. 428:138). Since past experiments indicated that bright light produces a decline of calcium within the retinal photoreceptor outer segment, we hypothesize that calcium concentration inside VIP-containing CSF-contacting neurons in SL should decrease in response to light stimuli due to a similar cascade involved in the signal transduction pathway. To address the question, we examined the response of some CSF-contacting neurons to photostimulation in chicks using Fluo-4, a fluorescent indicator of cytosolic free calcium. Results show that CSF-contacting neurons...
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190 Early brooding temperature considerations for bobwhite quail. J. P. Blake*, J. B. Hess, W. D. Berry, and N. W. Thornhill, Auburn University, AL.

Over 20 million bobwhite quail are produced commercially to meet annual sporting needs. Limited information exists concerning their requirements for chick brooding to maintain livability and health. Three short-term (2-wks) studies evaluated brooding temperature requirements and effects of a feed supplement on early livability. In all experiments, birds were fed a 26% corn-soybean meal diet containing 2,811 kcal/kg ME. In Exp. 1, bobwhite quail were brooded at 90, 95, or 100 F (32.2, 35.0, or 37.8 C) for two weeks (n= 62 birds/pen with 2 reps/temp). For week 1, birds maintained at 95 F gained 9.65 g/bird compared to 90 and 100 F, which gained 9.11 and 9.10 g/bird, respectively (P<0.05). During week 2, birds maintained at 90 F gained 18.1 g/bird (P<0.05) compared to 95 and 100 F which gained 15.80 and 15.31 g/bird, respectively. Feed consumption for week 1 was greater (P<0.10) in birds brooded at 95 F (16.38 g/bird) versus 90 and 100 F being 13.82 and 14.38 g/bird, respectively. Feed consumption for week 2 was greater (P<0.05) in the 90 F versus the 100 F group (31.28 vs 26.96 g/bird), as the 95 F group remained intermediate (29.15 g/bird). It was concluded that bobwhite quail require a brooding temperature of 95 F during the first week and a brooding temperature of 90 F during the second week post hatch. In experiments 2 and 3, birds were brooded at either 85 or 95 F (29.4 or 35.0 C) and offered top-dressed feed supplements "Oasis" or "GroGel Plus" for three days post-hatch. Bird numbers were c.a. 115 birds/pen with 4 reps/temp in Exp. 2 and 85 birds/pen with 4 reps/temp in Exp. 3. The feed supplements failed to provide a significant improvement in growth, feed efficiency, or mortality during the first two weeks post hatch under conditions of this trial. Birds subjected to the 85 vs. 95 F brooding temperature exhibited decreased body weight gain (15.7%) and feed consumption (4.7%) during week 1 and a slight reduction in body weight gain (5.1%) during week 2. Results confirm that a brooding temperature of 85 F is below that required for the first two weeks post hatch.

Key Words: Bobwhite, Quail, Brooding

191 Changes in protein level for bobwhite quail. J. P. Blake*, J. B. Hess, and B. D. Bowers, Auburn University, AL.

Limited information exists concerning dietary protein requirements of bobwhite quail when reared for sporting purposes and producers could benefit from such information. In this experiment, 576 two-week-old Bobwhite quail were divided among 6 treatment groups and randomized into 8 replications with 12 birds/rep. A 26, 24 or 22% protein mash diet (2810 kcal ME/kg) was fed to 4 or 6 weeks of age followed by the introduction of a 20% protein mash diet (2810 kcal ME/kg) fed through 8 weeks of age. Birds and feed were weighed biweekly. Results indicate that there were no differences in bodyweight of birds subjected to the 22% protein regimes as compared to the 26 and 24% protein regimes. Preadipose bodyweight gains exhibited no distinct patterns based on protein level. However, during the 2-4 week period, bodyweight gains were slightly greater (3.8%) in the 26 and 24% protein fed birds as compared to the 22% protein level. During the 4-6 week period, bodyweight gain was significantly (P<0.05) lower in the group that received 22% protein from 2-4 weeks with a subsequent change to the 20% protein diet. This group consumed the lowest level of protein, but during the 6-8 week period exhibited the greatest bodyweight gain and feed consumption coinciding with compensatory growth. Feed efficiency appeared to be unaffected by feeding regime. Mortality was negligible during the course of this experiment, averaging less than 1% among treatments for the six-week experimental period. Results indicate that bobwhite quail exhibit compensatory gain as a response to a decrease in protein intake. Bobwhite quail appeared to be unaffected by major dietary changes in protein level under the conditions of this study and when maintained in a healthy environment.

Key Words: Bobwhite, Quail, Protein

192 The effects of feeding different dietary formulations on growth criteria in ring necked pheasants. G. S. Davis1*, K. E. Anderson1, C. R. Parkhurst1, and L. R. Minear2, 1North Carolina State University, Raleigh, NC/USA, 2Southern States Cooperative, Richmond, VA/USA.

Ring necked pheasants exhibit aggressive and cannibalistic behavior resulting in mortality or poor feather quality thereby reducing their desirability to hunting pressure operators and hunters. Producers proclaim that high dietary protein levels and vegetative cover in flight pens reduce cannibalism, mortality, and enhance feather development. One hundred ninety two one-day-old pheasants were divided equally into sixteen 9.3 sq. m floor pens. Four dietary treatments consisted of: (1) 28% protein starter, 24% developer, 20% conditioner; (2) 26% starter, 23% developer, 19% conditioner; (3) Combination of diets 1 and 2; (4) one diet at 22.5% protein level. The starter diet was fed until 5 wk of age, the developer until 10 wk, and conditioner until 17 wk. There were 4 replications per treatment and each replicate pen housed one of two different hiding barriers, bales of straw or a PVC framed-barrier covered with burlap cloth. Mortality was measured daily, and BW and feed consumption were measured weekly. Feather quality was measured at 5, 10, and 17 wk of age. In addition, at 18 wk of age, flight ability was measured at 2 different hunting preserves. To study aggressive and cannibalistic behavior, the pens were periodically time-lapsed video taped. There were no differences among the treatments in mortality. Pheasants in treatments 1 and 3 had significantly (p<0.05) heavier BW, pheasants in treatment 4 exhibited lower BW with BW of treatment 2 being intermediate to the others until 15 wk of age. BW among all treatments was similar from 15 to 17 wk of age. Feather quality of pheasants in treatments 1 and 3 was significantly (p<0.05) better than treatments 2 and 4 at 5 wk of age. However, feather quality of all treatments was similar at 10 and 17 wk of age. Flight ability was not different among the treatments, however, hens tended to fly better than cocks. Minimal aggressive and cannibalistic behavior was observed throughout the entire study. Pheasants in pens with bales of straw tended to exhibit less aggression and appeared to cope with personal space invasion by flock mates. It was concluded that pheasants can exhibit compensatory growth and feather development when fed lower levels of protein; and providing barriers in pens can reduce cannibalism and aggression.

Key Words: Ring necked pheasants, Dietary protein, Feather quality

193 The second cycle behavioral response and fearfulness of commercial laying hen strains to alternative molting programs. K. E. Anderson1*, D. Joyce2, 1North Carolina State University, Raleigh, NC, 2North Carolina Department of Agriculture and Consumer Services, Salisbury, NC.

Animal rights groups are pressuring government and the food service industry to force an end to the husbandry practice of molting, which they deem as cruel. Molt initiators that are known stressors, that trigger the weight loss and cessation of egg production, are the targets. Therefore, a behavioral study was conducted to examine four alternative molt programs impact on 2nd cycle behavior patterns; Not molted (NM); Non-Fasted Molt (NF); 5 d Fast Feed Restrict Molt (SF); 13 d Fast Molt (FM). This represents factorial design of 3 strains x 4 programs; a total of 24 replicates containing 24 hens per replicate at a density of 413 cm²/hen. Hens were observed using a modified scanning technique at lights on, mid-day, and just prior to lights off on two consecutive days every 8 weeks. Behavioral observations were conducted throughout the second cycle. The hens from each strain developed different (P<0.05) behavior patterns as they progressed through the different production phases. The frequency of the independent act (those involving a single hen) of standing, which did not change. The frequency of social behaviors (behaviors that require the interaction of two hens) responded to molting in a similar manner. Aggression, and feather pecking frequencies were different (P<0.05) between the strains, however, the pattern of change was similar for each strain. The FM hens Aggression and Avoid and Escape frequencies increased (P<0.05) post-fast and returned to pre-molt levels.