Histological description of Snail Kite (Rostrhamus sociabilis) testicles. S. K. Cunha¹, J. M. Silva², A. L. S. Valente¹, A. S. Varela Junior², S. M. L. Costa¹, M. A. A. Coimbra¹, C. D. Corcini¹, and D. C. Bongalhardo*¹, ¹Federal University of Pelotais, RS, Brazil, ²Federal University of Rio Grande, RS, Brazil.

Birds of prey have an important role in maintaining the populations of small animals, helping to keep the ecological balance in the regions they inhabit. The Snail Kite (Rostrhamus sociabilis) is a bird from the Falconiforme order, Accipitridae family, which also comprises vultures, eagles and hawks. It is a diurnal bird found in the freshwater wetlands of Central and South America, Caribbean and southern United States. Although it is rated as Least Concern by Bird Life International, in Florida the species is locally endangered due to the water level control in the Everglades: the draining resulted in a decrease in the number of apple snails, the main element of its diet. The objective of this work was to study the testes of the Snail Kite, aiming to obtain knowledge about its reproductive cycle in southern Brazil. In October, one adult Snail Kite male was brought to the Wildlife Rehabilitation Center from the Federal University of Pelotas, presenting an exposed fracture in the distal portion of the humerus. The bird was euthanized one day after its arrival, and the testicles were collected for histology. The testes were placed in 10% buffered formalin; after fixation they were dehydrated in Paraplast Xtra, and sliced (5 μm) in rotating microtone. To observe all the structures, different cuts from the same testis were stained with one of 4 colorations: Harris Hematoxylin and Eosin (HE), which stains all the structures, different cuts from the same testis were stained with Acid Schiff + HE, which stains carbohydrates present in basal lamina, Mallory trichrome, which stains connec-
tures, as well as polygonal Sertoli cells, indicating intense seminifer-
sous tubules activity. These results show that in October, the bird was in the reproductive season, with the testes fully functional and producing sperm with normal morphology.

Key Words: birds of prey, seminiferous tubules, histology, testicles, sperm

368 The role of serotoninergic axis in reproductive failure associated to aging in broiler breeder roosters. N. Avital-Cohen* and I. Rozenboim, Hebrew University of Jerusalem, Rehovot Israel.

Decline in reproductive activities of aged roosters is a well known phe-
nomena. Serotonin modulate hypothalamic function, gonads function and incubation behavior. Serotonin elevation in the brain is associated with decreased reproductive performances manifested in aged roost-
ers. Seven serotonin receptors families are known with little informa-
tion in avian species. The aims of this study were: 1. Characterization of serotonin receptors in aged broiler breeder roosters, 2. the role of testosterone and estrogen interaction with serotoninergic axis in aged broiler breeder roosters. Exp. 1: 60 broiler breeder roosters at 64 wks of age were divided into 4 treated groups (n = 15): 1. LY227015-antag-
onist against 5-HT2B receptor (i.v. 0.3mg/kg once a week), 2. Meter-
goline- antagonist against 5-HT2C receptor (i.m. 3 mg/kg daily), 3. Ondansetron- antagonist against 5-HT3 receptor (i.m. 0.2 mg/kg daily) and 4. Untreated control. Exp. 2: 80 broiler breeders roosters at 66 wks of age were divided into 8 groups (n = 15) treated with: tamoxifen (TAM), testosterone (TES), TAM+TES, PCPA, TAM+PCPA, TES+ PCPA, TAM+TES+ PCPA and control. Semen qualities were weekly measured. Plasma steroid and prolactin level were measured every 2 wks. At the end of the experiments (66 wks in exp. 1 and 70 wks in exp. 2) roosters were euthanized and tissues were removed for mRNA genes expression of GnRH-I, VIP, LH-β, FSH-β, Prolactin, LH and Prolactin receptors. Treatment with 5-HT2B significantly improved semen quality. In addition, all antagonists groups increased gene expression of gonadal axis. Investigating the influence of TES, TAM and serotonin revealed that the combined treatment with TES, TAM and PCPA significantly increased semen quality, tests weight gonadal axis genes expression. The results suggest that reproductive failure associated with age is caused by an increase serotoninergic level via its 5-HT2B receptor. In addition aging is associated with a decrease in gonads function manifested by decrease in testosterone and increase in estrogen levels.

Key Words: roosters, aging, fertility, serotonin, serotonin receptors

369 The use of reflectance spectroscopy for fertility detection in freshly laid egg and gender sorting in mid incubation period. I. Rozenboim*¹ and E. Ben Dor², ¹Hebrew University of Jerusalem, Rehovot, Israel, ²Tel Aviv University, Tel Aviv, Israel.

The development of chicken embryos involves a complex sequence of physiological and biochemical changes. Their early detection is of significant commercial value, but is limited to non-invasive methods. The current method for detection of egg fertility is candling conducted by 10 d of incubation and is followed by gender sorting after hatch. Reflectance spectroscopy is being used for detection of biochemical characteristics in the food, cosmetics, pharmacological industries and others. Recent progress in reflectance spectroscopy permits optical screening of the egg to yield both embryo and shell spectral signatures. A preliminary analysis, presented here, shows favorable classification results based on statistical approach and neural-networks algorithms using a limited population. Following a successful feasibility test of visual classification of this population, a monitored experiment on 450 White Leghorn eggs from a young flock (24 wks of age) was conducted. The eggs were measured on d 0, 1, 2 and 10 by reflectance spectrometry (ASD Fieldspec) with a dual-beam finger probe. The data set underwent PCA discrimination using the Unscrambler plat-
form and a neural-network classification model formulated for fertility and gender. Actual fertility was measured on the 10th day of incu-
bation, and actual sorting of gender was conducted at hatch. Actual and predicted results comparison indicated that prediction capability is over 95% for fertility tested on d 0 and 90% for gender detection on d 10. We conclude that the reflectance spectroscopy method is adequate for detection of fertility in freshly laid eggs, and that of gender at mid incubation period.

Key Words: embryo, fertility, gender, reflectance spectroscopy

370 Fasting reduces luteinizing hormone secretion and central galanin-like peptide expression but stimulates gonadotropin inhibitory hormone expression in the hypothalamus of the Pekin drake. E. Gerometta, S. Colton, E. Coombs, and G. S. Fraley*, Hope College, Holland, MI.

Pekin ducks are seasonal breeders that are also very sensitive to nutri-
tional status. It is known that fasting reduces reproductive hormone
secretion, however the neurobiology underlying this effect is not clear. The purpose of this study was to first confirm that fasting reduces plasma luteinizing hormone (LH) levels in male ducks. Second we set out to determine if the reduced LH levels were associated with changes in the hypothalamic expression of 2 peptides known to regulate feeding and reproduction, galanin-like peptide (GALP) and gonadotropin inhibitory hormone (GnIH). Groups of adult male Pekin ducks (~60 weeks of age) were either fed or fasted for 17, 24 or 48 h (n = 6 per group). At the end of each time point, ducks were euthanized by cervical dislocation and blood collected for plasma determination of LH levels. Brains were removed and processed for immunocytochemical detection of fos-, GALP-, and/or GnIH-immunoreactivity (ir). Circulating LH levels were significantly (P < 0.05) reduced after 24 h of fast. A significant increase (P < 0.01) in fos-ir was found in the hypothalamic paraventricular nucleus (PVN) in fasted drakes compared with fed controls. Many of the fos-ir neurons within the PVN also colocalized GnIH-ir. There was virtually a complete loss of GALP-ir with fed controls. Many of the fos-ir neurons within the PVN also colocalized GnIH-ir. There was virtually a complete loss of GALP-ir in the infundibular nucleus (INF) in fasted compared with fed ducks. These data suggest that GALP and GnIH neurons are important mediators between the feeding and reproductive systems in Pekin drakes.

**Key Words:** GALP, GnIH, seasonal breeder, hypothalamus, food intake

### 371 The maintenance of reproductive status in Pekin drakes requires both red and blue wavelengths of light: relationship to opsin-related proteins in the hypothalamus. G. S. Fraley*1 and W. J. Kuenzel2,3, 1Hope College, Holland, MI, 2University of Arkansas, Fayetteville, 3Center of Excellence for Poultry Science, Fayetteville, AR.

In birds, neither bilateral enucleation nor pinealectomy appear to affect seasonal changes in hypothalamic-pituitary-gonadal activation. Thus in birds there is compelling evidence that photoresponsiveness is mediated in part by neurons that express photosensitive pigments. These neurons have been referred to as deep brain photoreceptors (DBPs). Some success in identifying putative DBPs has come from using anti-opsin antibodies. Two of these opsin-related proteins, opsin and melanopsin, have been identified in avian brains. Pekin ducks are seasonal breeders and as such, very sensitive to artificial and natural light. The purpose of these studies was to determine if specific wavelengths of light are necessary to maintain plasma luteinizing hormone (LH) secretion and to determine the hypothalamic circuitry underlying this effect. First, drakes were exposed to full spectrum, white light or red (~625 nm) or blue (~450 nm) light and blood samples were taken at intervals around lights-on. We found that neither red nor blue wavelengths of light could maintain circulating LH levels compared with that of drakes housed under full spectrum white light. Second, drakes housed under white lights and brains processed for immunocytochemistry using an opsin (RET-P1) or melanopsin antibody showed opsin-ir in the lateral septal area (LS) and infundibular nuclei (INF), both loci colocalized with vasoactive intestinal polypeptide. Melanopsin-ir was observed in the premammillary nucleus (PMM) and colocalized with tyrosine hydroxylase. Immunoreactive fibers for both opsin- and melanopsin were observed throughout the septum and anterior diencephalon and found to be in close contact with gonadotropin releasing hormone cell bodies. Third, a significant (P < 0.01) increase in fos-ir was observed in all 3 nuclei (LS, INF and PMM) in drakes exposed to white light compared with dark conditions. These data suggest that multiple opsin-related peptides within the basal forebrain and diencephalon may be necessary to maintain photosensitivity in Pekin drakes.

**Key Words:** testicular development, brain, photosensitivity, luteinizing hormone

### 372 Genetic selection for parthenogenesis in virgin quail hens impact embryonic mortality and hatchability following mating. H. M. Parker*, A. S. Kiess, J. B. Wells, M. L. Robertson, and C. D. McDaniel, Mississippi State University, Mississippi State, MS.

Unfertilized bird eggs are capable of developing embryos by parthenogenesis, which can be controlled by genetic selection. However, it is unknown if genetic selection for parthenogenesis affects embryonic development and hatchability of fertilized eggs. Additionally, most parthenogenic development resembles early embryonic mortality in fertilized eggs during the first 2–3 d of incubation. Therefore, it is possible that many eggs classified as containing early dead embryos may actually be unfertilized eggs that contain parthenogens. The objective of this study was to determine if genetic selection, for parthenogenesis in virgin hens, would impact embryonic development and hatchability after mating. Based upon their ability to produce unfertilized eggs that exhibited at least 10% parthenogenesis, 307 virgin Chinese Painted quail hens were utilized across 5 generations of selection. In the first generation (P), random males were selected for mating. However, for subsequent generations (F1 to F5), males whose sisters or mothers exhibited parthenogenesis were mated to hens so that fertility, embryonic mortality, and hatchability could be evaluated. Early embryonic mortality was divided into 2 groups: ≤ 7mm (small early dead embryos, possible parthenogens) and >7 mm (large early dead embryos). After the F1 generation of selection, hatch of eggs set and hatch of fertile eggs decreased. The P generation had fewer possible parthenogens than did the F5 generation, yet both generations were similar for large early embryonic mortalities, middle deads, and cracked eggs. The P generation had more late embryonic mortality and contaminated eggs than the F5 generation. Fertility was not different across generation of selection, perhaps because many unfertilized eggs that exhibited parthenogenesis resembled early embryonic mortality and therefore were classified as early embryonic mortality. In conclusion, virgin quail hens that were selected for the parthenogenetic trait appear to have impaired hatchability and early embryonic development following mating.

**Key Words:** parthenogenesis, hatchability, embryonic mortality, quail, genetic selection

### 373 Induced deep pectoral myopathy and broiler plasma creatine kinase. R. J. Lien*, S. F. Bilgili, and J. B. Hess, Auburn University, Auburn, AL.

The objectives were to determine if broiler plasma creatine kinase (CK) levels change due to deep pectoral myopathy (DPM) induced by encouraged wing flapping (EWF), if basal CK is related to susceptibility, and if CK after early EWF is related to susceptibility. In trial 1, 40 broilers were subjected to EWF at 51 d. Plasma CK was determined 3 d before and 1, 2, 4, 8 and 13 d after EWF. At 64 d, DPM was assessed. In trial 2, 120 broilers had basal CK determined at 45 d, were subjected to EWF at 49 d, and were sampled to determine CK at 52 d. At 56 d, DPM was assessed. In trial 3, 120 broilers were subjected to early EWF at 22 d, which is before DPM can be induced. At 24 d, CK was determined. At 38 d, DPM was induced by EWF, and DPM was assessed at 42 d. Plasma CK was determined using a Roche auto analyzer. Data were analyzed by ANOVA with P < 0.05. In trial 1, CK 3 d before EWF was 13,500 IU/L. One d post EWF, CK was 401,150
IU/L in broilers that developed DPM and greater ($P = 0.0033$) than the 62,333 IU/L in those that did not. Plasma CK returned to basal levels 8 d post EWF in broilers that developed DPM, and 2 d post EWF in those that did not. In trial 2, CK before EWF was 43,302 IU/L and did not differ ($P = 0.6735$) between broilers that developed DPM, and those that did not. After EWF, CK was 185,966 IU/L and greater ($P < 0.0001$) in broilers that developed DPM than the 69,573 IU/L in those that did not. In trial 3, basal CK at 24 d in birds not subjected to EWF was 3,636 IU/L. In birds that developed DPM due to subsequent EWF at 38 d, CK at 24 d (2 d after early EWF) was 6,411 IU/L and similar ($P = 0.1607$) to the basal level. However, in birds that did not develop DPM due to subsequent EWF at 38 d, CK at 24 d (2 d after early EWF) was 9,506 IU/L and greater than the basal level ($P = 0.0061$) and that of birds that developed DPM due to subsequent EWF ($P = 0.0148$). Results indicate CK is increased more in broilers that develop DPM than in those that do not, that basal CK does not appear to be related to susceptibility, and that a reduced CK response to early EWF may indicate susceptibility.

Key Words: broiler, deep pectoral myopathy, creatine kinase

374 Effect of linseed oil on egg yolk cholesterol and performance of laying hens. G. M. K. Mehaisen* 1, A. Abbas 1, A. M. H. Ahmed2, and A. Galal2, 1 Cairo University, Giza, Egypt, 2 Ain Shams University, Cairo, Egypt.

This study aimed to investigate the effect of linseed oil as a natural source rich in omega-3 fatty acids on egg yolk cholesterol and performance of laying hens. A total of 180 commercial Hy-Line brown laying hens were randomly divided into 4 groups and were fed for 28 d on control diet and diets containing 2, 4 and 6% linseed oil. Egg production performance and feed consumption were recorded during 42 d of the study. Blood and egg samples were collected from laying hens at 14, 28 and 42 d of the experiment to measure the total protein, albumin, globulin, calcium and phosphorus in plasma as well as yolk cholesterol concentration in eggs. Results revealed that egg production performance was significantly improved by supplementation of linseed oil in the diets (egg number was 39.07 vs. 37.78 eggs/hen and egg mass was 2432.29 vs. 2358.13 g/hen for linseed groups vs. control group, respectively, $P < 0.05$). Feed consumption was significantly ($P < 0.05$) lower in linseed groups than in control group (110.78 vs. 121.05 g/hen/day), and consequently, the feed conversion ratio decreased (2.05 vs. 2.31). Plasma protein, albumin, globulin, calcium and phosphorus were not influenced by the linseed oil levels in the diet. Egg yolk cholesterol significantly decreased by linseed supplementation (11.57, 11.18 and 10.98 mg/g cholesterol in 2%, 4% and 6% linseed oil groups vs. 12.80 mg/g cholesterol in control group, $P < 0.05$). Therefore, the dietary supplementation of linseed oil in chicken diets as natural source of omega-3 fatty acids is healthier for egg consumers.

Key Words: laying hens, linseed oil, egg production, yolk cholesterol

375 The effect of breeder ages and egg sizes on yolk absorption and embryo development. A. Nangsuay*1, Y. Ruangpanit12, R. Meijerhof3, and S. Attammangkune1, 1 Kasetsart University, Kampheang Saen Campus, Nakhon Pathom, Thailand, 2 Poultry Research and Development Center, Suanvajyokkasit Animal R&D Institute, Nakhon Pathom, Thailand, 3 Poultry Performance Plus, Voorst, the Netherlands.

An experiment was conducted to study the effect of breeder age and egg size on yolk absorption and embryo development. Four thousand eight hundred Ross 308 hatching eggs were subjected to 4 treatments in a 2 x 2 factorial randomized complete block design using 2 breeder ages (29 and 53 weeks of age or young and old) and 2 egg sizes (57–61 g and 66–70 g or small and large), with 8 replicates per treatment. The results indicate that yolk weight increased with flock age, whereas an increase in egg size resulted in higher albumen content. A significant interaction between breeder age and egg size at d 7 was observed, resulting in higher yolk free body (YFB) weight of embryos originating from large eggs of the old flock than from the young flock. This interaction disappeared at later stages of incubation. Until 14 d of incubation, eggs of the old flock yielded embryos with bigger YFB than those of the young flock. At hatch, chicks of both age groups had comparable wet YFB weight, chick weight, wet and dry residual yolk (RY) weight and chick length. Dry YFB weight was higher for chicks originating from the old flock than from the young flock. Embryos and chicks of the large egg group had bigger YFB from d 14 to hatching than those originating from the small egg group. At hatch, these chicks were also heavier, longer and had higher wet and dry YFB and RY weight. Yolk absorption at d 18 and at hatch was higher for embryos and chicks originating from the old flock compared with those of the young flock, both in absolute values and as percentage of initial yolk weight. Yolk absorption at 18 d of incubation both in absolute values and as percentage of initial yolk weight as well as percentage of initial yolk weight at hatch, of embryos and chicks of the small eggs was higher than that of the large eggs. In our experiment, egg size was the determining factor for embryo development expressed as YFB weight, chick weight and chick length at hatch. Yolk availability and yolk absorption did not influence chick YFB weight, chick weight and chick length, but tended to influence dry YFB weight.

Key Words: breeder age, egg size, yolk absorption, embryo development

376 Effects of in ovo injection of carbohydrates on somatic characteristics of broiler embryos and hatchlings. W. Zhai*1, P. D. Gerard2, and E. D. Peebles1. 1 Mississippi State University, Mississippi State, MS, 2 Clemson University, Clemson, SC.

Increased carbohydrate injection volume has been shown to be positively related to broiler hatching BW but negatively related to hatchability. Also, yolk absorption has been found to be reduced by the provision of certain external supplemental carbohydrates to embryos at high volumes. However, a low injection volume (0.4 mL) has recently been found to be non-detrimental to hatchability. In the current study, effects of the in ovo injection of 0.4 mL of various carbohydrate solutions into the amnion on d 18 of incubation on the somatic characteristics of Ross x Ross 708 broiler embryos and hatchlings were investigated. Embryonated eggs were injected with the following carbohydrates dissolved in commercial diluent using an automated multiple-egg injector: 1) 6.25% glucose, 18.75% dextrin (G+D); 2) 6.25% sucrose, 18.75% dextrin (S+D); 3) 6.25% maltose, 18.75% dextrin (M+D); and 4) 25% dextrin. A non-injected control, and commercial (0.1 mL diluent-injected) and experimental (0.4 mL diluent-injected) treatment controls were also included. In comparison to non-injected controls, chick BW relative to set egg weight (SEW) was increased by the injection of 0.4 mL of diluent, G+D, S+D, or M+D, but was not affected by dextrin. In addition, d 19 embryo BW relative to SEW increased in all carbohydrate injection groups as compared with the non-injected control, and embryo yolk free BW (YFBW) relative to SEW increased in the G+D and dextrin groups as compared with the
non-injected and commercial control groups. Yolk sac moisture in the groups that received carbohydrate, except for the M+D group on d 19, was higher than that of the non-injected control group. Nevertheless, hatchling yolk sac weight and YFBW were not affected by any injection treatment. In conclusion, the injection of 0.4 mL of various carbohydrate solutions may improve chick BW and yolk water content without detrimentally affecting yolk nutrient absorption and deposition into embryo body tissues.

**Key Words:** BW, carbohydrate, embryogenesis, in ovo injection, yolk sac

377  **Aflatoxins and reproductive performance of two broiler breeder genotypes.** A. Scher¹, A. P. Rosa*¹, J. M. Santurio², A. Londero¹, and L. S. Boemo¹, ¹Poultry Laboratory, Universidade Federal de Santa Maria, RS, Brazil, ²Lapemi, Universidade Federal de Santa Maria, RS, Brazil.

The objective of this study was to determine the effect of aflatoxins (AFL) exposure on reproductive aspects of 2 broiler breeder genotypes. The experiment was carried out at The Federal University of Santa Maria – Brazil. 660 broiler breeder females and 60 males were submitted to intoxication with AFL (AFB1:86%, AFB2: 8.5%, AFG1:3.8%, AFG2: 1.7%) from the 24th to 64th week. To evaluate hatchability, hatchability of fertile eggs, fertility and embryo mortality were performed weekly incubations with all the hatching eggs produced in each week. The experimental design was in a CRD in factorial arrangement with 3 levels of AFL (0.0, 0.50 and 1.0 mg/kg diet) and 2 breeders’ strains (A and B), totalizing 6 treatments with 5 replicate pens of 22 females and 2 males each. The intoxication with aflatoxins did not affect the hatchability and fertility. However, hatchability of fertile eggs was depreciated from 93.36% to 92.14% and total embryo mortality was increased from 5.16% to 6.20% when the breeders received diets containing 1.0 mg AFL/kg. AFL did not affect embryo mortality in the early and middle stages of incubation, but in the period of d 15 to 21d of incubation, eggs from breeders intoxicated with 1.0 mg AFL/kg in the diet showed higher embryo mortality rates. The strain A showed the highest hatchability rate (84.15%) when compared with strain B (77.77%), fertility (91.17 vs. 83.12%) and embryo mortality (5.82 vs. 5.31%). However hatchability of fertile eggs was increased in the strain B breeders (93.57 to 92.29%). In the fertility study was observed an interaction between the AFL levels and the evaluated strains ($P = 0.0620$). The fertility of the strain A was not affected by the AFL intoxication. In the strain B was observed a reduction in the fertility rate of eggs when the birds fed diets containing 0.5 or 1.0mg AFL/Kg. The studied levels of AFL can negatively affect important reproductive characteristics of broiler breeders.

**Key Words:** mycotoxins, hatchability, strains