

to follow morphologically distinctive initial phases affecting first the connective tissue underlying the epithelium, then progressively invading the inner structures of the lamina propria. These initial changes are followed by the pathological process spreading throughout the entire lamina propria, as well as the adjacent epithelial cells, which showed all features of necrotic cell death. Upon electron microscopic examination of the mucosal epithelium, significant ultra-structural changes were apparent at a level of the connective tissue at the interface of lamina propria and mucosal epithelium, and in the lateral domain of enterocytes, but the overall structure of the apical domain of these cells (plasma membrane and microvilli) appeared intact, even in enterocytes that showed clear signs of advanced necrotic process. The birds challenged with *C. perfringens* experimentally showed histological and ultra-structural changes similar to those seen in NE specimens, but of lesser magnitude. Our findings suggest that, in addition to alpha toxin, other factors may be involved in the initiation of necrotic enteritis.

Key Words: clostridium perfringens, necrotic enteritis, morphological changes

68 Automatic counting with differential and selective media.

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The need to estimate bacterial diversity and identify certain organisms led to the development of chromatogenic media. These media are

expensive and only effective with certain groups of organisms. In order to determine if utilization of a computer-operated color colony counter could be "trained" to do the same thing using common differential and selective media, a series of tests were run. The primary focus was on coliform bacteria of concern in poultry production. The Spiral Biotechnology Color Q was used for the counts and the media selected included eosin methylene blue, brilliant green, McConkey, *Salmonella*-Shigella, Hektoen Enteric and Rambach.

First the counter was "trained" using known cultures on the various media to establish the color for each genus. Organisms chosen represented the target group as well as others. These included *Enterobacter aerogenes*, *Salmonella typhimurium*, *Streptococcus pneumoniae*, *Enterococcus faecalis*, *Proteus mirabilis* and *Escherichia coli*. Next mixed known cultures were inoculated on the various media and the system was allowed to do all counts according to the colors established in the training session.

The system is capable of differentiating among the colonies and thus speeding both the counting process and the selection of colonies for further testing resulting in significant savings of time, labor and expendables.

Key Words: bacteria, automatic counting, chromatogenic medium

Physiology, Endocrinology, and Reproduction: Endocrinology

69 Evaluation of the serotonin receptor blockers ketanserin and methiothepin on the pulmonary hypertensive responses of broilers to intravenously infused serotonin. M. E. Chapman* and R. F. Wideman, *University of Arkansas, Fayetteville.*

The pathogenesis of pulmonary hypertension remains incompletely understood. Many factors have been implicated, however there has been great interest in the potent pulmonary vasoconstrictor serotonin (5-HT) due to episodes of primary pulmonary hypertension in humans triggered by serotonergic appetite suppressant drugs. Pulmonary hypertensive patients have elevated blood 5-HT levels, and pulmonary vasoconstriction induced by 5-HT is believed to be mediated through 5-HT_{1B/1D} and 5-HT_{2A} receptors expressed by pulmonary smooth muscle cells. The vascular remodeling associated with pulmonary hypertension also appears to require the serotonin transporter (5-HTT). We investigated the roles of 5-HT receptor blockers on the development of pulmonary hypertension induced by infusing 5-HT i.v. in broilers. For this purpose, we treated broilers with the selective 5-HT_{2A} receptor antagonist ketanserin (5 mg/Kg BW) or with the nonselective 5-HT_{1/2} receptor antagonist methiothepin (3 mg/Kg BW). Receptor blockade was followed by infusion of 5-HT while recording pulmonary arterial pressure and pulmonary arterial blood flow. The results demonstrate that methiothepin but not ketanserin eliminated the 5-HT-induced pulmonary hypertensive responses in broilers. The 5-HT_{2A} receptor does not, therefore, appear to play a role in the 5-HT-induced pulmonary hypertensive responses in broilers. Methiothepin did not inhibit pulmonary vascular contractility per se, as the pulmonary hypertensive response to the thromboxane A₂ mimetic U44069 remained intact

in methiothepin-treated broilers. Methiothepin will be a useful tool for evaluating the role of 5-HT in the pathogenesis of pulmonary hypertension syndrome (PHS, ascites) as well as the onset of pulmonary hypertension triggered by inflammatory stimuli such as bacterial lipopolysaccharide.

Key Words: hypertension, ketanserin, methiothepin

70 Evaluation of the serotonin receptor blocker methiothepin in broilers injected intravenously lipopolysaccharide and microparticles. M. E. Chapman* and R. F. Wideman, *University of Arkansas, Fayetteville.*

There has been considerable interest in the role of serotonin (5-hydroxytryptamine, 5-HT) in the pathogenesis of pulmonary hypertension due to episodes of primary pulmonary hypertension (PPH) in humans linked to serotonergic appetite suppressant drugs. In this study, we investigated the impact of serotonin on the development of pulmonary hypertension induced by injecting bacterial lipopolysaccharide (LPS, endotoxin) and cellulose micro-particles intravenously using the non-selective 5-HT_{1/2} receptor antagonist methiothepin. In experiment 1 broilers selected for ascites susceptibility or resistance under conditions of hypobaric hypoxia were treated with methiothepin or saline followed by injection of LPS while recording PAP. In experiment 2 ascites-susceptible broilers were treated with methiothepin or saline followed by injection of cellulose micro-

particles while recording PAP. In experiment 3, an i.v. micro-particle injection dose shown to cause 50% mortality (LD50) was injected into ascites-susceptible and -resistant broilers subsequent to methiothepin or saline treatment. Injecting methiothepin reduced PAP below baseline values in ascites-susceptible and -resistant broilers suggesting a role for 5-HT in maintaining the basal tone of the pulmonary vasculature in broilers. Injecting micro-particles into the wing vein had no effect on the PAP in the broilers treated with methiothepin suggesting that 5-HT is an important mediator in the pulmonary hypertensive response of broilers to micro-particles. Furthermore, injecting an LD50 dose of micro-particles into ascites-susceptible and resistant broilers pretreated with methiothepin resulted in reduced mortality. Serotonin appears to play a less prominent role in the pulmonary hypertensive response of broilers to intravenously injected LPS indicating that other mediators within the innate response to inflammatory stimuli may also be involved. These results are consistent with our hypothesis that PHS ensues when vasoconstrictors such as 5-HT overwhelm the dilatory effects of vasodilators such as NO, thereby effectively reducing the pulmonary vascular capacity of PHS-susceptible broilers.

Key Words: hypertension, serotonin, methiothepin

71 Expression of gonadotropin inhibitory hormone receptor (GnIHR) in the chicken: Regulation by ovarian steroids. S. Maddineni*, O. M. Ocón-Grove, S. M. Krzysik-walker, G. L. Hendricks III, and R. Ramachandran, *The Pennsylvania State University, University Park.*

Gonadotropin inhibitory hormone (GnIH) has been found to inhibit gonadotropin secretion in several avian species. We recently cloned the complementary DNA encoding a G-protein coupled receptor that binds to GnIH (GnIHR). In the present study, we hypothesized that GnIH mRNA is expressed in the Leghorn chicken diencephalon and pituitary gland, and that its expression will be affected by estradiol and/or progesterone treatment. By RT-PCR, we detected GnIHR mRNA in the chicken diencephalon, anterior pituitary gland, total ovary, and ovarian granulosa cells but not in the liver. Using real-time quantitative PCR assays, we found that the quantity of GnIHR mRNA was the greatest in the diencephalon compared to that of the anterior pituitary gland and ovary. A polyclonal antisera raised against chicken GnIHR synthetic peptide detected GnIHR-immunoreactive cells in the anterior pituitary gland and in the granulosa cell layer of ovarian follicles. GnIHR mRNA quantity in total ovary was significantly greater in sexually immature chicken versus sexually mature hen ($P < 0.05$; $n = 6$). Sexually immature Leghorn chickens (16 wk old; $n = 8$) were injected with estradiol (E2) and/or progesterone (P4) to determine the influence of gonadal steroids on GnIHR mRNA quantity in the diencephalon, pituitary gland and total ovary. We found that GnIHR mRNA expression was significantly lower in total ovaries and pituitary glands of chickens treated with E2 or E2+P4 ($P < 0.05$) but E2 and/or P4 treatment did not alter GnIHR mRNA quantity in the diencephalon. We also found that GnIHR mRNA quantity was significantly lower in the total ovary of chickens treated with P4 alone ($P < 0.05$). We conclude that ovarian steroids influence GnIHR mRNA expression in the chicken anterior pituitary gland and ovary. GnIHR expressed in the chicken pituitary gland and ovary may possibly be involved in gonadotropin secretion and steroidogenesis, respectively.

Key Words: GnIH, ovarian steroids, chicken

72 Pituitary procalcitonin gene expression is influenced by sexual maturation and ovarian steroids in the chicken. S. Maddineni, S. M. Krzysik-Walker, O. M. Ocón-Grove, S. M. Motch, G. L. Hendricks III, and R. Ramachandran*, *The Pennsylvania State University, University Park.*

Calcitonin is primarily produced by the thyroid C cells in mammals or by the ultimobranchial gland in chickens. However, calcitonin has been found to be expressed by the rat pituitary gland, where it functions as a paracrine factor causing decreased lactotroph proliferation and prolactin secretion. Calcitonin is derived from its precursor protein, procalcitonin. Procalcitonin gene expression in the avian pituitary gland has not been previously reported. We tested the hypotheses that procalcitonin mRNA is expressed in the chicken pituitary gland, and this expression will vary based on the reproductive status or in response to ovarian steroid administration. By RT-PCR, we detected robust expression of procalcitonin mRNA in the chicken pituitary gland. Pituitary-derived chicken procalcitonin mRNA sequence was identical to that expressed in the ultimobranchial gland. Quantitative real-time PCR assays revealed that sexually mature chicken pituitary glands contained less procalcitonin mRNA and greater prolactin mRNA quantities when compared with sexually immature chicken pituitary glands ($P < 0.05$). To determine the influence of ovarian steroids on procalcitonin mRNA expression, sexually immature Leghorn chickens (16 weeks old; $n = 8$) were injected with estradiol and/or progesterone. Estradiol treatment of sexually immature chickens led to a significant decrease in pituitary procalcitonin mRNA quantity ($P < 0.05$), while progesterone alone or in combination with estradiol did not change pituitary procalcitonin mRNA quantity relative to vehicle-treated chickens ($P > 0.05$). Our results suggest that pituitary procalcitonin mRNA expression is restored by progesterone from the suppressive effects of estradiol treatment. Further studies are required to elucidate the function of pituitary calcitonin on the secretion of pituitary hormones.

Key Words: procalcitonin, chicken, pituitary

73 Expression of 5-HT1A and 5-HT2C receptors in the hypothalamus during the turkey reproductive cycle: Colocalization with tyrosine hydroxylase. T. Bakken*¹, S. W. Kang¹, A. Thayananuphat¹, J. A. Proudman², and M. E. El Halawani¹, ¹*University of Minnesota, St. Paul,* ²*USDA, Beltsville, Maryland.*

Serotonin (5-HT) acts through dopamine (DA) and vasoactive intestinal peptide (VIP) to stimulate prolactin (PRL) release when infused into the third ventricle of the turkey brain. Dopamine also inhibits PRL release by antagonizing the stimulatory effect of VIP at the pituitary level. Recent studies have shown that a 5-HT2A/2C receptor agonist stimulates and a 5-HT1A receptor agonist inhibits PRL secretion. The stimulatory effect of 5-HT2A/2C receptor agonist appears to be mediated via DA cells in the nucleus preamillaris (PMN) and nucleus mamillaris lateralis (ML). The site of the inhibitory action of the 5-HT1A receptor agonist needs to be clarified. To clarify the involvement of 5-HT1A and 5-HT2C receptors in PRL regulation, in situ hybridization experiments were performed on tissue sections from the turkey hypothalamus. The nucleus preopticus medialis (POM), ML and PMN showed strong expression of 5-HT1A receptors, while 5-HT2C receptors were concentrated in the nucleus paraventricularis hypothalami (PVN), PMN and ML. The relative density of receptor expression in each hypothalamic region was compared between birds from different reproductive stages. A significant difference in 5-HT1A

receptor expression was seen in the ML, PMN, and POM in birds of different stages, with photorefractory birds showing the highest and incubating birds the lowest expression. In the ML, 5-HT_{2C} receptors density was significantly higher in incubating birds than short day birds. In the ML and PMN, 5-HT_{1A} receptors were colocalized with tyrosine hydroxylase (TH) immunoreactive cells. The data presented, taken together with our previous findings showing that the two receptor subtypes are antagonistic to each other supports the notion that 5-HT, like DA, can both stimulate and inhibit PRL secretion, depending on the relative abundance of the two receptor subtypes.

Key Words: prolactin, 5-HT_{1A} receptor, 5-HT_{2C} receptor

74 Characterization of the serotonergic system in the brainstem during the turkey reproductive cycle. S. W. Kang*, T Bakken, and M. E. El Halawani, *University of Minnesota, St. Paul.*

The serotonergic (5-HTergic) system is an important regulator of the avian reproductive cycle. Our earlier studies indicated that serotonin (5-HT) played a pivotal role in the regulation of prolactin secretion. This regulatory effect of 5-HT was mediated via the DAergic system which expressed 5-HT receptors and with which 5-HT immunoreactive fibers were intermingled. However, little is known about the 5-HTergic system neuroanatomical distribution and its neuronal activity across the turkey reproductive cycle. These were investigated in the present study utilizing the expression of tryptophan hydroxylase 2 (TPH2) mRNA, the rate limiting enzyme in the 5-HT biosynthesis. We identified three 5-HTergic groups in the brainstem of the turkey using *in situ* hybridization technique for TPH2 expression including: (1) A ventro-rostral mesencephalic group located in the area ventralis of Tsai (AVT), dorsal to the nucleus interpeduncularis (IP). (2) A dorsal mesencephalo-pontine group located in the dorsal and ventral to the nucleus linearis caudalis (LC), and (3) A ventro-caudal ponto-medullary group, occupying the raphe nucleus (R). Significant differences in TPH2 expression across reproductive states were observed within the ventro-rostral mesencephalic and dorsal mesencephalo-pontine groups. TPH2 expression was markedly less in reproductively quiescent non photostimulated hens as compared with laying and incubating hens. Photorefractory hens displayed intermediate TPH2 expression. However, 5-HT neurons of raphe nucleus group in the ventro-caudal ponto-medullary region did not show significant alterations in TPH2 expression during the turkey reproductive cycle. These findings indicate that the variations in reproductive hormones expression and secretion observed across the turkey reproductive cycle may be explained, in part, by changes in neuronal serotonergic activity in the turkey brainstem. This is the first study to show alterations in neuronal 5-HT synthesis and the 5-HT neuronal group involved during the different reproductive stages in avian species.

Key Words: tryptophan hydroxylase 2 mRNA, serotonin, turkey brainstem

75 Dopamine and gonadotrophin releasing hormone-I neuronal activation following photostimulation in the turkey. A. Thayananuphat*, S. W. Kang¹, T. Bakken¹, J. R. Millam², and M. E. El Halawani¹, ¹*University of Minnesota, St Paul*, ²*University of California, Davis.*

Photoperiod plays a critical role in the regulation of turkey reproduction, but the precise neural pathways through which day length is detected,

measured, and transduced into a reproductive neuroendocrine response have yet to be elucidated. Dopamine (DA) has been shown to play a regulatory role in prolactin (PRL) and luteinizing hormone-follicle stimulating hormone (LH-FSH) secretion. However the neuro-anatomical interrelationship between DA and Gonadotrophin Releasing Hormone-I (GnRH-I)-LH/FSH and vasoactive intestinal peptide (VIP)-PRL systems in avian species is still unclear. In the present study, we used the photo-induced activation of c-fos mRNA expression and double *in situ* hybridization/immunocytochemistry (ISH/ICC) to identify the DAergic neural group or subgroup that became active following photostimulation. In order to establish reproductive neuroendocrine system activation, double ISH/ICC was also conducted on c-fos-GnRH-I and c-fos-VIP. In addition, we investigated the optimal length of light required to induce c-fos mRNA expression. C-fos mRNA expression was observed in DA neurons within the nucleus preamillaris (PMN) and in GnRH-I neurons in nucleus commissurae pallii (nCPA), nucleus preopticus medialis (POM), and nucleus paraventricularis hypothalami (PVN) of short day birds exposed to a 30 minute of light period at 14 hours after first light. No c-fos expression was observed in nucleus infundibularis (IN) where VIP neurons secreting the PRL-releasing factor are located. c-fos mRNA expression in the PMN was higher in hens exposed to a 30 min light period at 14 or 16 hours after first light than at 8 and 20 hours, or in short day non-treated controls. The results revealed several brain areas which were activated by light and suggested that DA neurons in the PMN might contribute to the initiation of avian sexual reproduction.

Key Words: dopamine, GnRH-I, photostimulation

76 Deiodinase II and the immediate early gene product c-fos in the hypothalamus of photostimulated turkey hens. J. R. Millam*¹, E. K. Hoye¹, and T. D. Siopes², ¹*University of California, Davis*, ²*North Carolina State University, Raleigh.*

Photo-induced expression of an immediate early gene product, c-fos, in photosensitive but not photorefractory turkey hens implicates cells in the tuberal hypothalamus in the development of photorefractoriness. Up-regulation of hypothalamic deiodinase II (Dio II) is one of the earliest steps in photosexual stimulation of Japanese quail; Dio II activation leads to retraction of glial end-feet thus giving GnRH I terminals access to the portal vasculature. We therefore asked whether c-fos expression, as a measure of neuronal activity, might be co-localized with Dio II in photostimulated turkey hens. Nicholas breeding hens were held on 8L:16D until 30 wk of age to assure a photosensitive condition, then photostimulated by exposure to 16L:8D for 2 d before sacrifice. Brains were briefly perfused, fixed overnight, cryoprotected, frozen, cryostat-sliced, and mounted on slides for staining. Immunohistochemical (IHC) expression of c-fos protein (rabbit polyclonal antibody, Santa Cruz #SC253) and *in situ* hybridization (ISH) expression of Dio II mRNA were prominent throughout the tuberal hypothalamus and in the ependymal layer surrounding the third ventricle. In double-label experiments, cells in the ependymal layer often appeared to contain both c-fos and Dio II. IHC staining of Dio II using a rabbit anti-human antibody, differing from chicken by one amino acid over a twenty amino acid sequence, labelled cells throughout the tuberal hypothalamus but not in the ependymal area. In contrast, photorefractory hens (ceasing egg-laying after extended exposure to 16L:8D) and held on 8L:16D for 2 wk – an insufficient time to reverse photorefractoriness – then sacrificed after 48 h of photostimulation, showed very sparse c-fos expression, though Dio

II staining was not appreciably different from photostimulated, photosensitive hens. While expression of Dio II either by IHC or ISH does not accurately estimate biologic activity, these results support a role for Dio II in photosexual stimulation of turkeys, but leave open the question of whether photorefractoriness might involve failure of Dio II activity.

Key Words: photostimulation, deiodinase, immediate early gene

77 The efficacy of intracerebroventricular injections of arginine vasotocin (AVT) and corticotropin releasing hormone (CRH) on plasma corticosterone levels in male and female broilers (*Gallus gallus*) and their respective distribution of AVT and CRH neurons. F. N. Madison*, A. Jurkevich, and W. J. Kuenzel, *University of Arkansas, Fayetteville*.

Stress is a common factor faced by birds in the poultry industry and it has been demonstrated that male birds seem more susceptible to stress than females. Little, however, is known about sex-related differences in the neuroendocrine control of the hypothalamo-pituitary adrenal axis (HPA) during the stress response in broilers. There is evidence showing that in addition to the well-known role of CRH, another hypothalamic neuropeptide, AVT stimulates the HPA in birds. The purpose of this study was to determine the efficacy of AVT and CRH administered intracerebroventricularly for releasing corticosterone (CORT) in undisturbed birds. Broiler chickens were fitted with a chronic cannula surgically placed in the lateral ventricle. Birds were housed individually in cages behind a one-way glass partition and unnecessary noise was avoided during the sampling period. Each bird in the study received a single 5.0 ml intracerebroventricular injection of either saline (SAL), AVT (100 pM), or CRH (100 pM). Blood was sampled remotely every 15 min for 2 h beginning from time of injection via a catheter implanted in the jugular vein and plasma CORT was determined by radioimmunoassay. Female birds injected with SAL had significantly higher plasma CORT levels than males injected with SAL 15 min post injection and maintained higher plasma CORT levels throughout the sampling period. Male birds injected with AVT had higher plasma CORT release than females injected with AVT, and levels were significantly higher at 1 h and 1 h 15 min post injection. Male birds injected with CRH had a significantly higher plasma CORT release 15 min post injection, which continued higher throughout the entire sampling period. Our lab is currently utilizing immunocytochemical techniques to quantitate AVT and CRH cells within the hypothalamus.

Key Words: sex differences, vasopressin, stress

78 Soy phytoestrogen effects on progesterone receptor, ovalbumin synthesis, and vitellogenin production in female broiler chicks. L. M. Stevenson*, S. H. Oates, A. L. Doernste, J. B. Hess, and W. D. Berry, *Auburn University, Auburn, Alabama*.

Developmentally inappropriate exposures to estrogenic compounds are known to alter morphology and function of the reproductive tract in various species. Chickens are continually exposed to the relatively potent estrogenic soy isoflavones through the diet. Previous experiments in this laboratory have demonstrated that the primary soy isoflavone genistein induces proliferation of the chick oviduct. However, information is lacking as to specific reproductive tract developmental effects of genistein exposure in chicks. Experiments were done to compare specific oviduct morphological and functional responses to genistein exposure with responses elicited by a classical

estrogen, diethylstilbestrol (DES). To avoid the effects of dietary soy isoflavones, the experimental diets were formulated with dried egg white, rather than the usual soybean meal, as a protein source. Day old female chicks were assigned to treatments: egg white based diet with daily oral gavage of corn oil vehicle (CV); 1mg diethylstilbestrol (DES); 2.0mg genistein (G2); 20mg genistein (G20); or 40mg genistein (G40). At 15 d of age, half the birds from each treatment received a single injection of 2mg progesterone in a corn oil vehicle to induce ovalbumin synthesis in the oviduct. The classical oviduct responses to estrogen, induction of progesterone receptor and initiation of ovalbumin synthesis, were examined by immunohistochemistry. At 16 d of age, DES treatments increased oviduct weight and percentage of final body weight as compared to all other treatments ($P \leq 0.05$). Immunohistochemistry of formalin fixed oviduct samples revealed that the DES, G20, and G40 treatments induced progesterone receptor and ovalbumin in the chick. A direct ELISA was performed to determine the amount of vitellogenin in plasma samples from the birds. There were no significant treatment effects on plasma vitellogenin. It was concluded that genistein, at the concentrations tested, acts as a weak estrogen in the chick oviduct.

Key Words: oviduct, phytoestrogen, development

79 Effect of sulfamethazine and photostimulation on gene expression of vasoactive intestinal polypeptide (VIP) and phosphodiesterase in the lateral septal organ (LSO) and pituitary gonadotropin content in the chick. H. Li*¹, J. A. Proudman², and W. J. Kuenzel¹, ¹*University of Arkansas, Fayetteville*, ²*USDA/ARS/BGPL, Beltsville, Maryland*.

Most birds living in the temperate zone use seasonal changes in day length as predictive environmental cues to initiate and terminate breeding. Long day illumination induces rapid gonadal development and stimulates gonadotropin-releasing hormone (GnRH), follicle stimulating hormone (FSH), luteinizing hormone (LH), and testosterone secretion in avian species. A group of non-retinal, non-pineal encephalic photoreceptors (EPRs) were hypothesized some time ago to sense external photoperiodic change. Two sites located in deep brain were suggested to house the potential EPRs, the LSO and the medial basal hypothalamus. Molecules identified in the putative EPRs of the LSO and considered as critical components in the possible phototransduction pathway included rhodopsin, rod-type cGMP-phosphodiesterase β -subunit, and VIP. Of interest is that the combination treatment of sulfamethazine (SMZ), a compound that stimulates gonadal development, together with long day exposure significantly increased plasma LH within 48 h. To elucidate whether and how SMZ and/or long day illumination affect the neuroendocrine system, a 2X2 factorial study was conducted in non-photostimulated or photostimulated and SMZ-fed or control diet-fed chickens. Pituitary content of prolactin (PRL) and gonadotropin were assayed and gene expression of rhodopsin, phosphodiesterase, and VIP in the LSO, as well as FSH and LH in the pituitary gland were quantitated using real time-PCR. SMZ treatment significantly increased pituitary content of LH at 16h, and decreased PRL at 12 h ($n=5$, $p < 0.05$). Peak of LH and FSH mRNA level occurred as early as 6 h in pituitary, while at the level of brain, VIP and phosphodiesterase increased in the LSO following SMZ feeding at 4h and 6 h, respectively. The current study indicated that SMZ functions at a level higher than the pituitary gland in the photoneuroendocrine system, and it might affect the phototransduction cascade in the EPRs.

Key Words: encephalic photoreceptors, photoneuroendocrine system, real time PCR

80 Immunohistochemical localization of dopamine neurons in the brain of the native Thai chicken (*Gallus domesticus*). N. Sartsoongnoen^{*1}, S. Kosonsiriluk¹, N. Prakobsaeng¹, A. Thayananuphat², T. Songserm³, M. E. El Halawani², and Y. Chaiseha¹, ¹*Suranaree University of Technology, Thailand*, ²*University of Minnesota, St. Paul*, ³*Kasetsart University, Thailand*.

Avian prolactin (PRL) secretion is regulated by vasoactive intestinal peptide (VIP). Dopamine (DA) acts centrally through D1 DA receptors to stimulate PRL secretion via an intact VIPergic system and activates D2 DA receptors and subsequently prevents VIP from stimulating the PRL release at the pituitary. The present study was aimed to characterize the distribution of the DAergic system in the native Thai chicken, a non-seasonally breeding tropical species, with paucity of information about its reproductive neuroendocrine regulation. An immunohistochemical method to localize tyrosine hydroxylase (TH) as a marker of the DAergic neuronal system was elucidated in the brain of laying hens. The results revealed that TH immunoreactive (TH-ir) cells and/or fibers were found throughout the brain and predominantly located within the diencephalon; nucleus preopticus medialis, nucleus paraventricularis magnocellularis, nucleus intramedialis, nucleus mamillaris medialis (MM), nucleus mamillaris lateralis (ML), and area ventralis (AVT). The greatest density was found within MM, ML, and AVT. The staining of TH-ir cells and fibers was also observed within nucleus accumbens, nucleus septalis lateralis, nucleus suprachiasmaticus, pars medialis, regio lateralis hypothalami, nucleus commissurae pallii, median eminence, etc. The distributions pattern of TH-ir observed in the present study is consistent with that reported previously in several avian species. It is suggested that DA may play a pivotal role in the regulation of reproduction activity in tropical species as in the case of temperate zone birds.

Key Words: bird, dopamine, immunohistochemistry

81 Distribution of cGnRH-I immunoreactive neurons and fibers in the brain of native Thai chicken (*Gallus domesticus*). N. Sartsoongnoen¹, S. Kosonsiriluk¹, S. W. Kang², J. R. Millam³, M. E. El Halawani², and Y. Chaiseha^{*1}, ¹*Suranaree University of Technology, Thailand*, ²*University of Minnesota, St. Paul*, ³*University of California, Davis*.

Avian reproduction is primarily regulated by gonadotropin releasing hormone-I (GnRH-I) which is synthesized by neurosecretory cells in the hypothalamus. This decapeptide stimulates the synthesis and release of pituitary gonadotropins. However, the data of the neuroendocrine regulation of native Thai chicken, a non-seasonally breeding tropical species are limited. The distribution of GnRH-I neurons has been reported in many temperate zone species. The GnRH-I neuronal system needs to be clarified in the native Thai chicken. The distribution of GnRH-I neurons of native Thai chicken brain was elucidated utilizing immunohistochemical technique. The results revealed that cGnRH-I-immunoreactive (cGnRH-I-ir)-cells

and fibers were found mainly in the hypothalamus; nucleus preopticus medialis (POM), nucleus septalis lateralis, nucleus septalis medialis, nucleus accumbens, nucleus paraventricularis magnocellularis, nucleus periventricularis hypothalami, nucleus suprachiasmaticus, pars medialis, regio lateralis hypothalami, nucleus commissurae pallii (nCPa), nucleus habenularis medialis, nucleus habenularis lateralis, nucleus subhabenularis lateralis, and nucleus mesencephalicus nervi trigemini. The most abundance of cGnRH-I-ir neurons was found within the POM and nCPa. cGnRH-I-ir-fibers were mainly bilaterally located along the third ventricle with more abundance around the organum vasculosum lamina terminalis and very dense fibers were observed in the external layer of the median eminence as has been reported for other avian species. This present study confirms a pivotal role in the control of avian reproduction of non-seasonally breeding tropical species.

Key Words: bird, GnRH, immunohistochemistry

82 Distribution of vasoactive intestinal peptide-expressing neurons in the brain of the native Thai chicken (*Gallus domesticus*). S. Kosonsiriluk^{*1}, N. Sartsoongnoen¹, N. Prakobsaeng¹, T. Bakken², T. Songserm³, M. E. El Halawani², and Y. Chaiseha¹, ¹*Suranaree University of Technology, Thailand*, ²*University of Minnesota, St. Paul*, ³*Kasetsart University, Thailand*.

Avian prolactin (PRL) secretion is under stimulatory control by vasoactive intestinal peptide (VIP), the PRL-releasing factor, residing in nucleus infundibuli hypothalami (IN). The release and expression of VIP/PRL system is photo-periodically regulated by gonad stimulatory photoperiod in temperate zone birds. The distribution of VIP-immunoreactivity (VIP-ir) in several brain areas of these species has been reported. This study was designed to characterize the distribution of VIP system in the native Thai chicken brain, a non-photoperiodic species. The differential VIP expression may give us insight into the mechanism(s) underlying the regulation of the reproductive cycle in this species. Immunohistochemistry technique with quantitative analysis was performed throughout the brain of laying hens. The results revealed that VIP-ir cells and fibers were found throughout the brain and predominantly located within the diencephalon; nucleus preopticus medialis, nucleus anterior medialis hypothalami, regio lateralis hypothalami, nucleus paraventricularis magnocellularis, nucleus ventromedialis hypothalami, IN, and median eminence. The greatest staining was found within the IN. VIP-ir neurons were also observed within nucleus accumbens, cerebellum, nucleus septalis lateralis, nucleus commissurae pallii, etc. The pattern of VIP-ir distributions in this study is similar to that reported previously in several avian species. The abundance of VIP neuronal network in hypothalamus of the native Thai chicken suggests of its importance in the regulation of reproductive behavior in equatorial birds.

Key Words: bird, immunohistochemistry, VIP