

# Physiology and Endocrinology: Integrative Physiology and Endocrinology

**W290 Neuroendocrine regulation of rearing behavior in the native Thai hen.** O. Chaiyachet<sup>1</sup>, D. Chokchaloemwong<sup>1</sup>, N. Prakobsaeng<sup>1</sup>, N. Sartsoongnoen<sup>2</sup>, S. Kosonsiriluk<sup>3</sup>, I. Rozenboim<sup>4</sup>, M. E. El Halawani<sup>3</sup>, T. E. Porter<sup>5</sup>, and Y. Chaiseha\*<sup>1</sup>, <sup>1</sup>Suranaree University of Technology, Nakhon Ratchasima, Thailand, <sup>2</sup>Nakhon Ratchasima Rajabhat University, Nakhon Ratchasima, Thailand, <sup>3</sup>University of Minnesota, St. Paul, <sup>4</sup>The Hebrew University of Jerusalem, Rehovot, Israel, <sup>5</sup>University of Maryland, College Park.

Prolactin (PRL) circulating levels change during the avian reproductive cycle and vasoactive intestinal peptide (VIP) is the avian PRL releasing factor. This study investigated the changes in the VIP/PRL system of hens rearing their young as compared to hens deprived of rearing their chicks. The numbers of VIP-immunoreactive (VIP-ir) neurons in the nucleus infundibuli hypothalami (IN) and nucleus inferioris hypothalami (IH) of native Thai hens rearing chicks (R) were compared with those of non-rearing chicks (NR; n=4). Plasma PRL levels were determined by enzyme-linked immunosorbent assay. The numbers of VIP-ir neurons in the IN-IH on the day of hatch, and on days 4 (RD4), 7 (RD7), 10 (RD10), and 14 (RD14) of rearing were 59.4±5.9, 53.9±3.4, 30.3±1.0, 12.9±1.1, and 13.8±0.8 cells, respectively. When hens were not allowed to rear chicks, the number of VIP-ir neurons decreased as compared to their respective hens rearing chicks (P<0.05, NRD4=28.7±4.1; NRD7=15.3±6.6; NRD10=11.1±3.6; NRD14=14.8±2.5 cells). During the rearing period (week 1-5; n=6), PRL levels of R hens were compared (RW1 vs NRW1; 34.2±1.8 vs 27.4±1.7; P<0.05, RW2 vs NRW2; 37.8±7.8 vs 26.4±5.2; P<0.05, RW3 vs NRW3; 41.1±8.6 vs 18.5±1.5; P<0.05, RW4 vs NRW4; 36.3±9.6 vs 31.3±5.3, RW5 vs NRW5; 39.2±5.0 vs 27.9±4.3 ng/ml). These results indicate, for the first time, a role for the VIP/PRL system in the rearing behavior in gallinaceous avian species. It is concluded that the VIP/PRL system is not only a key regulator of incubation behavior as it is well established but it may also be involved in the regulation of rearing behavior in native Thai chicken. Supported by The Royal Golden Jubilee Ph.D. Program; #PHD/0230/2548(YC/OC).

**Key Words:** birds, rearing behavior, PRL

**W291 Cloning and characterization of chicken 5-hydroxytryptamine (5-HT) receptors 1A and 1B.** C. F. Wong\*, A. H. Y. Kwok, J. C. W. Ho, Y. Wang, and F. C. Leung, The University of Hong Kong, Hong Kong, HKSAR, China.

The aim of this study is to clone and characterize 5-hydroxytryptamine (5-HT; serotonin) receptors, HTR1A and HTR1B, from chicken. 5-HT is a monoamine neurotransmitter derived from tryptophan and is believed to be involved in a broad range of biological activity including mitogenesis of smooth muscle cells, psychological disorders like schizophrenia, anxiety and depression, and neuroendocrine functions like regulation of ACTH, glutamate and dopamine release, via interaction with a large family of G protein-coupled receptors. However, these receptors were not well studied in avian species. In this study, the full-length genes were cloned from chicken by PCR. HTR1A gene encodes a putative 413-amino acid protein, which shares 80%, 80% and 78% identity with human, chimpanzee and mouse homologues, respectively. Similarly, HTR1B gene encodes a putative 379-amino acid protein, which shares 82%, 82% and 86% identity with human, chimpanzee and mouse homologues respectively. Using a pGL3-CRE luciferase reporter system, activation of both HTR1A and HTR1B by 5-HT were found to inhibit forskolin-stimulated luciferase activity in CHO cells, suggesting

that both HTR1A and HTR1B are functionally coupled to Gi protein(s). Further characterization of HTR1A and HTR1B will shed light on the study of biological functions of 5-HT in chickens.

**Key Words:** chicken, 5-hydroxytryptamine, 5-hydroxytryptamine receptor

**W292 Ergovaline and other ergopeptine alkaloids inhibit vesicular glutamate transporter (VGLUT)-mediated activity of bovine synaptic vesicles.** Y. Xue\*<sup>1</sup>, J. R. Strickland<sup>2</sup>, J. A. Boling<sup>1</sup>, and J. C. Matthews<sup>1</sup>, <sup>1</sup>University of Kentucky, Lexington, <sup>2</sup>USDA-ARS, Forage-Animal Production Research Unit, Lexington, KY.

L-Glutamate (Glu) is the major excitatory neurotransmitter responsible for the glutamatergic neurotransmission in the vertebrate central nervous system, including the gastrointestinal tract (GIT) of cattle. The vesicular Glu transporters VGLUT1 and VGLUT2 concentrate (50 mM) Glu ( $K_m = 1$  to 4 mM) into synaptic vesicles (SV) for subsequent release into the synaptic cleft of glutamatergic neurons. To test the hypothesis that ergopeptine alkaloids inhibit VGLUT activity of bovine cerebral SV, a SV Glu uptake (vacuolar H<sup>+</sup> ATPase-dependent) model was developed. The effect of ergopeptine alkaloids on VGLUT activity was tested on SV isolated from cerebral tissue of Angus-cross steers that were naive to ergot alkaloids. Data were analyzed by ANOVA using GLM. Immunoblot analysis (n = 8) validated the presence of VGLUT1, VGLUT2, synaptophysin 1, and vacuolar H<sup>+</sup> ATPase proteins in purified SV. Functional analysis of the mean (n = 3, ± SE) SV VGLUT activity (50 μM) was ATP- and vacuolar H<sup>+</sup> ATPase-dependent, and saturatable (apparent  $K_m = 1.53 ± 0.18$  mM). IC<sub>50</sub> experiments (n=3) revealed that VGLUT Glu uptake (50 μM) was inhibited ( $P ≤ 0.048$ ) by ergopeptine alkaloids: bromocriptine (2.83 ± 0.59 μM) < ergotamine (20.5 ± 2.77 μM) < ergocornine (114 ± 23.1 μM) < ergovaline (137 ± 6.55 μM). Subsequent ergovaline K<sub>i</sub> experiments (n = 3; Glu = 0.05, 0.10, 0.50, 1, 2, 4, 5 mM) demonstrated no change ( $P = 0.332$ ) in apparent  $K_m$ . However, the  $V_{max}$  of Glu uptake was decreased ( $P = 0.016$ ) when evaluated in the presence of 50, 100, and 200 μM ergovaline, suggesting that ergovaline inhibits SV VGLUT activity through a non-competitive mechanism. The findings of this study suggest cattle with fescue toxicosis may have a reduced glutamatergic neurotransmission capacity. Thus GIT functions, including feed intake and digestion, may be impaired in cattle consuming ergopeptine alkaloids.

**Key Words:** bovine, ergot alkaloids, fescue toxicosis, glutamate transporter, glutamatergic neurotransmission

**W293 Comparison of the somatotrophic axis of two precocial free-ranging ice seal species: harp (*Phoca groenlandica*) and hooded (*Cystophora cristata*).** C. E. Anderson\*<sup>1</sup>, J. P. Richmond<sup>1</sup>, J. M. Burns<sup>2</sup>, and S. A. Zinn<sup>1</sup>, <sup>1</sup>University of Connecticut, Storrs, <sup>2</sup>University of Alaska-Anchorage, Anchorage.

To determine if components of the somatotrophic axis in marine mammals are associated with differing life history and maternal investment strategies, blood samples and morphometrics were collected from 35 free-ranging North Atlantic ice seals [hooded seals (*Cystophora cristata*), n = 15; and harp seals (*Phoca groenlandica*), n = 20] ranging in age from newborn to adult. For each species, animals were categorized as nursing, late nursing, weaned, and adult based on size, umbilical status, and pelage. Within a species, there were 5 individuals per category except there were no hooded seal pups categorized as late nursing due

to the 4-d lactation. Radioimmunoassays were used to quantify serum IGF-1 and GH, using bovine and human antisera, respectively. Data were analyzed with the Proc Mixed function of SAS and included species and age as independent variables. On average hooded seals gained  $24 \pm 11$  kg BW (6 kg/d) and  $3.4 \pm 0.2$  cm of blubber during the 4-d nursing period ( $P < 0.01$ ), without an increase in length ( $99.6 \pm 3$  cm;  $P = 0.92$ ). During the 12-d lactation harp seal pups increased BW  $34 \pm 1.5$  kg (8 to 42 kg; 2.8 kg/d), standard length  $25 \pm 3$  cm (76 to 101 cm), and blubber depth  $3.8 \pm 0.2$  cm ( $P < 0.01$ ). Concentrations of GH were similar in hooded seal neonates and late nursing pups ( $7.5$  and  $6.24 \pm 2.0$  ng/mL;  $P = 0.66$ ), but declined in harp seals for these periods ( $P = 0.01$ ;  $18.9$  to  $5.6 \pm 2.0$  ng/mL). Average IGF-1 was similar in neonates of both species ( $250 \pm 44$  ng/mL;  $P = 0.52$ ) and increased in both harp and hooded seals during nursing ( $434$  and  $345 \pm 44$  ng/mL;  $P = 0.01$ ). Coinciding with the post-weaning fast, harp seal IGF-1 rapidly declined from  $434$  to  $120 \pm 44$  ng/mL, whereas GH increased from  $5.6$  to  $12.4 \pm 2.0$  ng/mL ( $P < 0.01$ ). Adult hooded seals were larger ( $293 \pm 22$  vs.  $109 \pm 22$  kg) with greater IGF-1 ( $289$  vs.  $108 \pm 32$  ng/mL;  $P < 0.01$ ) than adult harp seals, but there were no differences in GH ( $5.2 \pm 1.2$  ng/mL;  $P > 0.10$ ). Concentrations of IGF-1 and GH reflect nutrient intake and growth rate during nursing and the post weaning fast in these precocial pinnipeds.

**Key Words:** pinnipeds, insulin-like growth factor-1, growth hormone

**W294 Effects of age and sex on hematologic and serum biochemical values of broiler chickens.** A. Viveros<sup>\*1</sup>, A. Brenes<sup>2</sup>, I. Arroyo<sup>1</sup>, M. Bascuñana<sup>1</sup>, A. Angosto<sup>1</sup>, and M. L. Fermin<sup>1</sup>, <sup>1</sup>*Facultad de Veterinaria, UCM, Madrid, Spain*, <sup>2</sup>*Instituto del FRIO-ICTAN, CSIC, Madrid, Spain*.

Due to lack of reference values for avian blood profiles, hematological and serum biochemical reference values may provide valuable information about the health status of broiler chickens. An experiment was conducted to study the effects of age and sex on hematological and serum biochemical values for 7 weeks of age. A total of 120, 1-d-old male and female broiler chickens (Cobb strain), were randomly assigned to 20 cages (6 chicks per cage). Blood samples were collected weekly by cardiac puncture and analyzed for red blood cell count (RBC), hemoglobin content (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and white blood cell count (WBC), heterophil (He), basophil (Ba), eosinophil (Eo), lymphocyte (Ly), monocyte (Mo) and thrombocyte counts. Serum biochemical analysis included total protein, albumin, glucose, triglycerides, cholesterol, bile acids, uric acid, amylase, CPK, LDH, AST, calcium, phosphorus, and sodium. This study showed that age affects the hematological and serum biochemical values of the male and female broiler chicks. With increasing of age, the erythrocytic parameters (except MCV and MCH) and leukocytic parameters (except lymphocyte) were increased ( $P < 0.001$ ), but MCV, MCH and absolute count of lymphocyte were decreased ( $P < 0.001$ ). Regarding serum biochemical values, glucose, total protein, albumin, triglycerides, cholesterol, bile acids, CPK, LDH, AST, and phosphorus concentrations were increased ( $P < 0.001$ ) and uric acid and amylase levels were decreased ( $P < 0.001$ ) during breeding period. In comparison to male broilers, female broilers had higher ( $P < 0.05$ ) RBC, Hb, and PCV levels ( $P < 0.05$ ), and lower ( $P < 0.001$ ) MCV, MCH, and heterophil values. Likewise, compared to male broilers, female broilers had higher ( $P < 0.01$ ) levels of total protein, albumin, triglycerides, amylase, CPK, and AST, and lower ( $P < 0.05$ ) levels of bile acids and uric acid. In conclusion, this study provided new infor-

mation about age and sex-related changes of hematological and serum biochemical values in broiler chickens.

**Key Words:** chickens, hematological values, serum biochemical parameters

**W295 Serum metabolite response of hens submitted to a second molt using soy hulls.** H. Mazzuco<sup>\*</sup>, L. S. Lopes, A. Coldebella, and V. S. Avila, *EMBRAPA Swine & Poultry, Concordia, SC, Brazil*.

In our previous study, similar physiological responses were observed for some blood metabolites of hens submitted to alternative and conventional molting methods. The present research was conducted to assess selected blood serum metabolites in laying hens induced to a second molt at the end of their 2nd laying cycle. 450 Hy-Line W-36 hens housed two per cage (759 cm<sup>2</sup>/bird) were molted at 142 wks of age and assigned to 5 treatments in a randomized complete block design (9 replicates/10 hens). The treatments were: Full-fed (FF), Conventional molt (CM, 10 d fasting) and alternative molt diets consisting of 95%, 85% or 75% of soy-hulls. Hens consumed the molting diets (except CM group) during 10 d and then were all fed cracked corn for 8 d followed by a pullet developer diet for 10 d. The serum metabolites evaluated were total calcium (TCa), ionized calcium (iCa), glucose (GLU), cholesterol (CHO), triglycerides (TGL), high-density lipoproteins (HDL) and low-density lipoproteins (LDL). Samples of blood (5 mL) were taken from each hen and processed using a commercial kit on days 10 and 28 of molt, when birds were 143 and 146 wk-old, respectively. All data were subjected to ANOVA considering the effects of day of molt, treatment and interactions. Significant treatment  $\times$  day of molt effects were observed in CHO ( $P < 0.05$ ), HDL and LDL profiles ( $P < 0.05$  and  $P < 0.01$ , respectively). CHO were higher for CM hens at 10 d of molt but not at 28 d, when FF hens showed higher levels. Lower levels of HDL were found in FF hens at 28 d of molt compared to the molted hens and at 10 d of molt, LDL levels were higher for FF. Treatment effect ( $P < 0.01$ ) was observed for TCa and TGL. Highest levels of TCa and TGL at both days of molt were observed on FF hens. Day of molt influenced GLU and iCa ( $P < 0.0001$ ) and higher levels at 28 d than 10 d were observed for both variables. During a second molt, changes in the circulating levels of blood metabolites reflected the dietary effects and for some, followed related patterns observed on hens in their first molt.

**Key Words:** blood calcium, serum lipoproteins, third cycle

**W296 Pulmonary vascular pressure profiles in broilers selected for susceptibility to pulmonary hypertension syndrome: Age and gender comparisons.** R. F. Wideman<sup>\*</sup>, M. L. Eanes, K. R. Hamal, R. Klintworth, and N. B. Anthony, *University of Arkansas, Fayetteville*.

Broilers that are susceptible to pulmonary hypertension syndrome (PHS, ascites) have an elevated pulmonary arterial pressure (PAP) when compared with PHS-resistant broilers. Two distinctly different syndromes, pulmonary arterial hypertension (PAH) and pulmonary venous hypertension (PVH), both are associated with increases in PAP. Pulmonary arterial hypertension occurs when the right ventricle must elevate the PAP to overcome increased resistance to flow through restrictive pulmonary arterioles upstream from the pulmonary capillaries. In contrast, PVH is commonly caused by increased downstream (post-capillary) resistance. The sites of resistance to pulmonary blood flow are deduced by making contemporaneous measurements of the PAP and the wedge pressure (WP), and calculating the trans-pulmonary pressure gradient (TPG = PAP-WP). We obtained PAP, WP and TPG values from 8,12,16,20 and 24 wk old anesthetized male and female broilers from a PHS-susceptible line (IACUC Protocol #08036). Pressures were

recorded as a catheter inserted into a wing vein was advanced to the right atrium, right ventricle, main trunk of the pulmonary artery, and onward until the WP was obtained. Gender and age interactions were evaluated using the SigmaStat ANOVA package. To further characterize the relationship between PAP, WP and TPG the data also were pooled into three cohorts to compare vascular pressures in birds having the lowest PAP values (n = 52; range: 12 to 22.9 mmHg), intermediate PAP values (n = 63; range: 23 to 32.9 mmHg), and highest PAP values (n = 62; range: 33 to 62 mmHg). Within each of the age, gender and PAP

cohort comparisons, broilers with elevated PAP consistently exhibited the hemodynamic characteristics of PAH (elevated PAP and TPG combined with a normal WP) but not PVH (elevated PAP and WP combined with a normal or reduced TPG). Susceptibility to PHS can be attributed primarily to pulmonary arterial hypertension associated with increased pre-capillary (arteriole) resistance.

*Supported by NIH/NHLBI Grant 1R15HL092517 01.*

**Key Words:** broiler, pulmonary hypertension, ascites