711 Starch digestion rate in the small intestine of broilers differs among feedstuffs. R.E. Weurding*[1], A. Veldman[1], W.A.G. Veen[1], M.W.A. Verstegen[2], and P.J. Van der Aar[1], [1]*Institute for Animal Nutrition 'De Schothorst', Lelystad, The Netherlands,* [2]*Wageningen University and Research Center, Wageningen, The Netherlands.*

A digestibility trial with 720 broilers was performed in which starch digestion of twelve diets, varying in starch source, was determined in three different segments of the small intestine as well as total starch digestion. The choice for the starch supplying feedstuffs was made considering known differences in starch accessibility, -structure and -composition. Based on digestibility coefficients and retention times in the different gut segments, in vivo starch digestion rate was calculated. Ileal starch digestion varied from 33% (potato starch) to 99% (tapioca). No starch degradation was observed in the hind gut. Dietary mean retention time in the small intestine varied from 136 min (barley diet) to 182 min (potato diet). Starch digestion rates varied from 0.5 /h (common beans) to 4.3 /h (tapioca). Ranking of feedstuffs according to total starch digestion (in increasing order) was: potato starch, legume seeds, cereal grains, tapioca. An in vitro technique which mimics passage through the gastrointestinal tract (GIT) of humans was adapted to mimic passage through the GIT of broilers. In vitro starch digestion was measured at different incubation times in the same diets as used in the in vivo digestion trial. From the starch digestion coefficients at the different incubation times, starch digestion rate was estimated. Correlations between in vivo and in vitro starch digestion data were calculated. In vitro starch digestion after 2 h incubation correlated well with starch digestion in the posterior jejunum ($r = 0.94$) and in vitro starch digestion after 4 h incubation correlated well with starch digestion in the posterior ileum ($r = 0.96$). In vitro starch digestion rate was lower, but showed a good correlation with in vivo starch digestion rate ($r = 0.87$). It was concluded that starch digestion rate in broilers varies among feedstuffs and can be predicted by the adapted in vitro method. The practical relevance of starch digestion rate will be studied in growth trials.

Key Words: Starch, Broilers, Digestion rate

712 Effect of colistin and aureomycin on intestinal microorganism and their relationship with the riboflavin metabolism of broilers. H. Y. Cai*[1], L. Wang[1], and G. H. Liu[1], [1]*Feed Research Institute, Chinese Academy of Agricultural Sciences, Beijing, P. R. China.*

To investigate the interrelationship between antibiotics, intestinal microorganisms and riboflavin metabolism in broilers, a total of 280 one day of age AA broilers were randomly allotted to 5 dietary treatments. Experimental diets were formulated to contain of two antibiotics at two different dosages (aureomycin: 50, 100 mg/kg; colistin: 10, 20 mg/kg) and the control without adding antibiotics. At 3 wk of age, four birds were taken at random from each of the treatment groups for the riboflavin (B2) analysis of cecal contents and determination of differential microbial counts. All data was statistically analyzed by Statistica 6.0 software and multiple comparisons conducted by DUNCAN. There was a gradual but significant ($P<0.05$) reduction in E. coli numbers with increasing levels of either colistin or aureomycin. Colistin supplementation at 20 mg/kg significantly ($P<0.05$) increased bifidobacterium numbers. Similarly, aureomycin supplementation at 50 mg/kg and 10 mg colistin/kg numerically increased bifidobacterium counts ($P>0.05$). The B2 concentrations in the broilers' cecal contents at 21 days of age were significantly higher ($P<0.05$) in birds receiving 20 mgcolistin/kg. Aureomycin supplementation at 100 mg/kg numerically increased the cecal level of B2 ($P>0.05$). The B2 concentrations in the colons of 21 day-old birds fed either 100 mg aureomycin/kg or 20 mg colistin/kg were numerically reduced ($P>0.05$) indicating a possible improvement of B2 absorption in the hindgut.

Key Words: Broilers, Antibiotics, Riboflavin

PSA Physiology: Reproduction

713 Use of the OptiBreed Sperm Quality Analyzer® for evaluating semen quality of turkey breeders. S. L. Neuman*[1], C. D. McDaniel[2], J. Radu[3], L. Frank[3], and P. Y. Hester[1], [1]*Purdue University,* [2]*Mississippi State University,* [3]*Alpharma, Inc.*.

The OptiBreed Sperm Quality Analyzer® (SQA) measures overall sperm quality as a Sperm Quality Index (SQI). The SQI value generated by the SQA is indicative of turkey sperm concentration, motility, and viability (Neuman et al., Poultry Sci. 79:suppl.1:49) The objective of the current study was to monitor the quality of semen in a turkey breeder flock throughout its semen production cycle. A commercial secondary breeder flock of BUTA breeder toms was monitored for sperm quality at 4-wk intervals beginning at 32 wk of age. Individual ejaculates were collected from the same 200 male breeders each month for 7 months. The semen was diluted 50-fold in 0.85% saline prior to determining the SQI for each individual bird. The SQI values were measured within 5 to 10 minutes of collecting the ejaculates. Individual semen volume was also determined gravimetrically. A univariate procedure, conducted monthly on individual SQI values and semen volume, indicated that SQI values were not normally distributed with the exception of month 7 in which SQI values were normally distributed (Shapiro-Wilk $W = 0.9939$, $P = 0.65$ for month 7). Semen volume was normally distributed during months 1, 2, 6, and 7 ($P > 0.05$). The CV for SQI was highest during month 1 at 26%, but decreased during subsequent months and averaged 15%. The CV for monthly semen volume averaged 32%. An ANOVA, conducted on semen traits to evaluate time effects, indicated that mean SQI values were lowest during months 1 and 7 of semen production with peak values occurring during months 2 through 6. Semen volume was at its lowest during the first month of production with subsequent increases during months 2 through 7. Correlation for all months between semen volume and SQI values was low ($r = 0.17$, $P < 0.0001$). In conclusion, the SQA can be used as a tool to monitor semen quality of breeder toms as a flock ages.

Key Words: Sperm Quality Analyzer, Sperm Quality Index, Turkey breeder

714 Effects of Feeding Regimen and Strain on Fertility of Broiler Breeder Hens as Indicated by the Perivitelline Layer Sperm Penetration Assay. R. A. Renema*, F. E. Robinson, and G. M. Fasenko, *University of Alberta, Edmonton, AB., Canada.*

The fertility of broiler breeder hens can be reduced by overfeeding. Monitoring fertility is a key step in the early diagnosis of reproductive problems. The Perivitelline Layer (PL) Sperm Penetration Assay has been demonstrated to be an alternative, non-incubation method of fertility assessment. In this study, the PL Sperm Penetration Assay was used to assess fertility in broiler breeder hens of four strains either feed restricted (FR) or ad libitum (AL) fed from photostimulation. Four strains of pullets (Shaver Starbro, Cobb 500, Hubbard Hi-Y, Avian 24k) were reared on a common growth curve. Strains were assigned the anonymous labels W, X, Y, or Z. Forty pullets of mean BW from each strain were individually caged at 20 wk of age. Birds were photostimulated at 22 wk and either maintained under RF conditions, or switched to an AL regimen. At 45 wk of age, 15 hens/tmt were artificially inseminated with pooled semen, and eggs collected 2-9 d after insemination. Eggs were assessed for PL sperm hole number at 40X and 100X magnification and data summarized in 3, 3-d periods (2-4 d, 5-7 d, and 8-10 d). There were no strain differences in the number of PV sperm holes determined at 100X magnification. However, at 40X magnification, 146 PV holes were present in Strain W eggs 2-4 d after insemination compared to 72 and 80 in Strain X and Y eggs, respectively. Although PV hole numbers were low in Strain Y eggs during the 2-4 d period, they did not subsequently decline as rapidly as in other strains. There were 43.1% and 34.8% fewer PV sperm holes in AL eggs than in RF eggs during the 2-4 d post-insemination period when examined under 100X and 40X magnification, respectively. Despite differences in PV sperm holes due to feeding level, actual fertility was affected by AL feeding in a strain-dependent manner. However, as breeder strain influenced the PV hole number independently of actual fertility, the value of the PV hole method for strain comparison was reduced.

Key Words: Broiler breeder, Fertility, Genetic strain

715 Differences in in vitro sperm hydrolysis of the perivitelline layer between two commercial lines of turkeys. B. D. Fairchild* and V. L. Christensen, *North Carolina State Univeristy, Raleigh, NC USA.*

Sperm penetration of the ovum inner perivitelline layer (IPVL) is positively correlated with fertility. Higher sperm penetration of the IPVL is indicative of successful insemination and can be positively associated with filling of the sperm storage tubules in the uterovaginal region of the oviduct. The hypothesis tested was that in vitro sperm hydrolysis of the IPVL would differ between two commercial turkey lines at two different periods in production. The objectives were to determine strain, age, male and female influence on sperm hydrolysis of the IPVL. Two experiments were conducted. In Experiment 1 strain and hen age were examined. Two commercial strains (N & H) and two hen ages within the laying period (early period=1-4 weeks of lay and mid period=12-16 weeks of lay) were used as factors in a factorial design. In Experiment 2, Tom (HM and NM) and Hen (HF and NF) were arranged in a factorial design to examine male and female influence on sperm hydrolysis. Eggs were obtained at oviposition from non-fertilized hens. Perivitelline layer (PVL) was isolated from non-germinal disc regions of the egg, sectioned into $1cm^2$ pieces, then incubated with $25x10^6$ viable sperm cells. After incubation, all PVL sections were rinsed, fixed on a microscope slide and stained. Holes from sperm hydrolysis were then counted. In Experiment 1, young hens had significantly more hydrolyzed holes than older hens and H hens had more hydrolyzed holes than N hens. No interaction between age and commercial line was detected indicating that both lines performed similarly at both production periods examined. In Experiment 2, HM and HF had significantly more sperm hydrolyzed holes than NM and NF. The results of these two experiments suggest that strain, hen age, sire and hen have significant effects on sperm hydrolysis of the IPVL and that by crossing sires and hens of different lines no gain in sperm hydrolyzed holes was achieved in vitro.

Key Words: Turkey, Fertility, Sperm hydrolysis

716 Production of germline chimera by transferring gonadal germ cells (GGCs) collected from 7 or 9 day-old chick embryos. A. Tajima*[1], M. Ohara[2], T. Minematsu[1], T. Kuwana[3], and Y. Kanai[1], [1] *Institute of Agriculture and Forestry, University of Tsukuba ,* [2] *Poultry Division, Takikawa Agricultural Experiment Station,* [3] *Pathology Section, National Institute for Minamata Disease.*

Fertilized eggs of the genetically selected White Leghorn line (SH strain) produced at Takikawa Animal Experiment Station (Hokkaido, Japan) were transported to University of Tsukuba (Ibaraki, Japan) by a commercial courier. Eggs were incubated at 37.8 C for 7 or 9 days and the recovered gonads were dissociated mechanically. Dissociated gonadal cells containing 7-day-old or 9-day-old gonadal germ cells (7d-GGCs or 9d-GGCs, respectively) were suspended in freezing medium containing 10 % dimethyl sulphoxide (DMSO). The cell suspension was frozen at 1 C/min. until the temperature reached -80C; this was followed by immersing the cells into liquid nitrogen at -196 C then stored up to 3 months. Between twenty five to one hundred frozen/thawed GGCs were injected into the dorsal aorta of stage 14-15 (H&H) Rhode Island Red (RIR) embryo from which blood was drawn prior to germ cell injection. The injected embryos were incubated until hatched and hatched chicks were raised until sexually mature. Hatchability as well as male:female ratio were 78% (7/9) and 5:2, and 42% (5/12) and 3:2 for embryos transferred with 7d-GGCs and 9d-GGCs, respectively. Upon reaching sexual maturity, a progeny-test was performed for 4-19 weeks by mating recipient chicks with normal RIR of the opposite sex. Out of 5 male 7d-GGC recipient, one male produced chick derived from transferred GGCs and the ratio was 1.7 % (1/58). Out of 3 male 9d-GGC recipient chick, one male produced chick derived from transferred GGCs and the ratio was 3.9 % (8/197). None of the female recipients hens produced chick derived from transferred GGCs. Present results demonstrate that frozen/thawed male 7d-GGCs and male 9d-GGC are still capable of producing germ-line chimeras in chicken when injected into blood stream of 2-day-old recipient embryo.

Key Words: Germ line Chimeras, Gonadal Germ Cells, Conservation of Avian Genetic Resources

717 Luteinizing Hormone, Progesterone, and Estradiol-17β Concentrations, and distribution of Hierarchical Follicles in Normal and Arrested-laying Turkey Hens. H.-K. Liu, D.W. Long, and W.L. Bacon*, [1] *The Ohio State University, Wooster OH.*

Photosensitive turkey hens of a line selected for increased egg production (Egg line) were photostimulated with constant light (24 h light: 0 h $dark^{-1}$) at 30 wk of age. Egg laying became arrested in 6 of the 12 hens after only 2 to 3 wk of laying. Comparisons of ovarian and oviductal morphology and changes in plasma hormones [luteinizing hormone (LH), progesterone (P_4) and estradiol-17β(E_2)] over 10 d of hourly serial bleeding between the laying and arrested-laying hens were made. The number of hierarchical follicles (follicles $>$ 1.0 g) was much greater in the arrested-laying hens than in laying hens. Some of the arrested-laying hens presented a poly-cystic ovarian follicle condition, with a large number of follicles slightly heavier or much heavier in weight than F_1 follicles of the laying hens. The oviducts of the arrested-laying hens were fully developed and similar in weight to those of laying hens. The plasma concentration of LH in the arrested-laying hens was relatively low and without preovulatory surges, which were detected in the laying hens. The baseline concentration of LH in the laying hens and the interval between preovulatory surges were similar to previously reported values for laying hens of the Egg line. The concentration of P_4 in most of the arrested-laying hens was maintained at a relatively high level without preovulatory surges. The baseline and surge amplitude concentrations of P_4 in the laying hens and interval between surges were similar to previously reported values for laying hens of the Egg line. The concentrations of E_2 were not different between normal and arrested laying hens. In conclusion, the relatively high and surge-less concentration of P_4 in the plasma of the arrested-laying hens might block ovulations but not block entrance of follicles into the hierarchy nor alter their hierarchical growth, leading to an accumulation of numerous F_1 or larger sized follicles in the ovary. Further, some of the accumulated mature follicles may resume an abnormal growth leading to the formation of cystic ovarian follicles.

Key Words: Turkey hen, arrested-laying, progesterone

718 Profile of Plasma Hydroxyproline in Laying Hens During an Ovulatory Cycle. J. I. Orban*[1] and P. Y. Hester[2], [1] *Southern University at Shreveport, LA,* [2] *Purdue University, IN.*

Hydroxyproline is required for collagen formation and is associated with bone remodeling. In humans, estimation of hydroxyproline level in the blood and urine has become a useful method to assess bone turnover, especially in bone degradation. In laying hens, bone integrity depends on calcium need, which is in part associated with the rate and magnitude of bone remodeling. The objective of this study was to determine the level of free plasma hydroxyproline in laying hens in relation to plasma calcium level during the ovulatory cycle to assess when bone resorption occurs to provide additional calcium for eggshell calcification. Twenty-five laying Leghorn hens (12 hens, 25 wk-old and 13 hens, 60 wk-old) were bled at 0, 5, 10, 15 and 20 h post-oviposition. Blood plasma obtained was analyzed for free hydroxyproline level using a modified method of Dabev and Struck (1971, Biochem. Med. 5:17). Calcium was analyzed using a Sigma calcium kit (St. Louis, MO 63178). Data were analyzed using a one-way ANOVA with a split plot in time (h post-oviposition). Both the 25 and 60 wk-old hens showed a significant ($P < 0.0001$) increase in plasma levels of hydroxyproline at 15 h post-oviposition (8.8 ug/mL for 25 wk hens and 8.0 ug/mL for 60 wk hens) as compared to basal levels at 0, 5, 10, and 20 h post-oviposition (6.2, 6.4, 6.4, and 7.2 ug/mL for 25 wk hens and 6.3, 7.1, 6.6, and 5.7 ug/mL for 60 wk hens, respectively, pooled SEM = 0.4). Mean plasma calcium levels during the time period for both age groups were 36 (0 h), 39 (5 h) 34 (10h) 23 (15h) and 18 mg % (20 h), pooled SEM = 2. The significant ($P < 0.001$) drop in calcium level from 15 to 20 h during the ovulatory cycle indicated the period of active eggshell calcification during which the spike increase in hydroxyproline was observed. Results indicate that the 15 h post-oviposition spike increase in plasma hydroxyproline level could represent bone turnover or medullary bone resorption to release additional calcium for eggshell calcification.

Key Words: Plasma hydroxyproline, Plasma calcium, Laying hen

719 Development of an ELISPOT assay for monitoring chicken Follicle-Stimulating Hormone (cFSH) release from individual dispersed pituitary cells. N. Puebla-Osorio*[1], J.A. Proudman[2], H.H.M. Gerets[3], F. Vandesande[3], and L.R. Berghman[1], [1] *Texas A&M University, College Station TX,* [2] *USDA-ARS Beltsville, MD,* [3] *University of Leuven, Belgium.*

From a methodological point of view, FSH and thyroid-stimulating hormone (TSH), are among the most difficult pituitary hormones to study in most vertebrate species. The amount of hormone in the pituitary tends to be very small and the glycoprotein hormones themselves are complex molecules consisting of two non-covalently linked glycosylated subunits, only one of which is hormone-specific. The present study describes the development of a new cFSH ELISPOT assay, a hybrid between a sandwich ELISA and an immunodot assay. Pituitary cells from laying White Leghorn hens were gently dispersed using trypsin (1 mg/ml). In the meantime a nitrocellulose (NC) -bottomed 96-well filtration plate (Millipore, Bedford, MA), was coated with 1 mg monoclonal anti-cFSH, and then blocked with 0.5 % of BSA. The single cell suspension was counted and 50 ml of cell suspension, densities ranging from 1000 to 100,000 cells/ml, were seeded onto the NC bottoms. The cells were left to incubate overnight in the presence or absence of 50 mM KCl in DMEM medium containing 1 mg/ml of BSA. After incubation, the cells were removed and the FSH that had been captured in the spots immediately surrounding the cells was further detected using rabbit anti-chicken FSHα. The latter was then identified with a biotinylated secondary antibody and alkaline phosphatase-conjugated streptavidin. Visualization of the spots was attained with a final incubation with the substrate system NBT/BCIP. The NC discs were then air dried for microscopic evaluation of the frequency and diameter of the spots. Dark purple spots were observed with the smallest cell number, the spot frequency being proportional to the cell density. No spots were observed in the absence of cells and detection antibody, respectively. Also, KCl-stimulated cells produced markedly more and bigger spots. As soon as the quantitative analysis of the results has been developed, this tool can be used to evaluate cFSH secretion from individual pituitary cells under different physiological circumstances.

Key Words: chicken, FSH, ELISPOT

720 Dopaminergic neurotransmission controlling PRL/VIP secretion in the turkey. O.M. Youngren[1], Y. Chaiseha[2], S.E. Whiting[1], and M.E. El Halawani*[1], [1] *University of Minnesota, St. Paul, MN,* [2] *School of Biology, Institute of Science, Suranaree University of Technology, Thailand.*

Vipergic neurons are the main element regulating avian PRL secretion. Our recent studies have demonstrated the modulation of VIPergic activity by stimulatory (via D_1 DA receptors) and inhibitory (via D_2 DA receptors) DAergic inputs. Dynorphin, serotonin (5-HT), DA, and VIP stimulate PRL secretion via a common hypothalamic pathway expressing κ opioid, 5-HTergic, DAergic, and VIPergic receptors, with the VIPergic system as the final mediator. Electrical stimulation (ES) within the turkey hypothalamus at the level of the medial preoptic nucleus (POM), the anterior hypothalamic nucleus (AM), the ventromedial hypothalamic nucleus (VMN), the infundibular nuclear complex (INF), and the median eminence (ME) results in the release of PRL. When the selective D_1 DA receptor antagonist SCH-23390 HCl was infused intraventricularly (icv) at the rate of 10 nmol/min, ES in POM, AM, and VMN was no longer able to increase PRL levels. Infusion of the D_1 DA antagonist did not prevent ES in ventral INF and ME from increasing PRL to the same level as that of controls. These results are interpreted to suggest that the D_1 DA receptors involved in PRL release lie caudal to VMN and dorsal to ventral INF. Electrical stimulation in POM, AM, or VMN appears to stimulate cells or fibers that release DA and this DA release is stimulatory for PRL secretion, whereas ES in INF and ME stimulates cells or fibers that release VIP directly to the pituitary. Microinjections of D_1 DA receptor agonist (SKF-38393 HCl, 50 ng, bilateral, n=5) into POM and VMN failed to produce any increase in PRL after 40 min, while microinjections in INF increased PRL from a baseline value of 40.8 $\pm$ 4.7 ng/ml to 75.8 $\pm$ 11.4 ng/ml after 15 min, reaching a high of 107.4 $\pm$ 25.5 ng/ml 30 min after injection. This suggests that the only D_1 DA receptors involved in PRL release are located in the INF, the same area where changes in VIP neurons are known to occur throughout the reproductive cycle. USDA Grant No. 00-02127

Key Words: Turkey VIP, Avian Prolactin, Dopamine Receptor

721 Expression of D_1 and D_2 dopamine receptors in the hypothalamus and pituitary during the turkey reproductive cycle. Y. Chaiseha*[1], O.M. Youngren[2], S.A. Schnell[3], and M.E. El Halawani[2], [1] *School of Biology, Institute of Science, Suranaree University of Technology, Thailand,* [2] *University of Minnesota, St. Paul, MN,* [3] *University of Minnesota, Minneapolis, MN.*

The regulation of avian prolactin (PRL) secretion and PRL gene expression is influenced by hypothalamic vasoactive intestinal peptide (VIP), the PRL releasing factor in avian species. Recent evidence indicates that D_1 and D_2 dopamine (DA) receptors play a pivotal role in VIP and PRL secretion. The differential expression of DA receptors on hypothalamic VIP neurons and anterior pituitary cells may affect the degree of prolactinemia observed during the turkey reproductive cycle. The relative expression of D_1 and D_2 DA receptor subtype mRNAs was quantitated using in situ hybridization histochemistry (ISH). The expression of D_1 DA receptor mRNA in the hypothalamus was found to be 6.8-fold greater than that of D_2 DA receptor mRNA. Higher D_1 DA receptor mRNA content was found in the anterior hypothalamus (3.6-fold), the ventromedial nucleus (2.0-fold), the infundibular nuclear complex (INF; 1.9-fold), and the medial preoptic nucleus (1.5-fold) of laying hens as compared to that of non-photostimulated hens. The levels seen in laying hens remained essentially the same in incubating hens, except for the INF area where levels increased 52%. During the photorefractory stage, the D_1 DA receptor mRNA was at its lowest level in all areas tested. No differences were observed in hypothalamic D_2 DA receptor mRNA abundance throughout the reproductive cycle. However, a marked reduction in pituitary D_2 DA receptor mRNA was observed in incubating hens. Pituitary D_1 DA receptor mRNA remained unchanged during the incubating phase. These results clearly demonstrate that the expression of stimulatory D_1 DA receptor mRNA in the hypothalamus increases in hyperprolactinemic incubating hens, whereas inhibitory D_2 DA receptor mRNA increases in the pituitary of hypoprolactinemic photorefractory hens. USDA Grant No. 00-02127

Key Words: In situ hybridization, Dopamine Receptor mRNA, Turkey Vasoactive intestinal peptide

722 Regulation of Prolactin Gene Expression by Vasoactive Intestinal Peptide and Dopamine: Role of Ca^{2+} Signaling. A. A. Al-Kahtane*[1], D. Deepak[2], M Kannan[2], and M El Halawane[1], [1] *University of minnesota - Department of Animal Sciences,* [2] *University of minnesota - Department of Veterinary Pathobiology.*

It is well documented that vasoactive intestinal peptide (VIP) is the prolactin (PRL) releasing factor in avian species. Our previous study shows that dopamine (DA), through D-2 DA receptors on pituitary cells, inhibits the stimulatory effects of VIP on PRL secretion, PRL-mRNA steady state level, PRL transcription rate and PRL-mRNA half-life. In this study we examined: 1) the effects of VIP and DA D_2 receptor agonist (R(-)-propylnorapomorphine hydrochloride) on intracellular Ca^{2+} $[Ca^{2+}]i$ in turkey primary pituitary cells, and 2) the involvement of extracellular Ca^{2+} in the regulation of PRL gene expression by VIP and DA. Fura-2 (Molecular Probes) was used as the fluorescent calcium indicator to measure $[Ca^{2+}]i$ in turkey pituitary cells. Reverse transcription-polymerase chain reaction (RT-PCR) technique was used to determine PRL-mRNA levels. Measurement of $[Ca^{2+}]i$ by Fura-2 indicated that VIP gradually increased $[Ca^{2+}]i$ with all doses used (10^{-9} to 10^{-5}M). Preincubating the pituitary cells with DA D_2 receptor agonist (10^{-10}M) for 10 min. inhibited the stimulatory effect of VIP on $[Ca^{2+}]i$. Blocking Ca^{2+} influx by verapamil, an L-type Ca^{2+} channel blocker, diminished the stimulatory effect of VIP on PRL-mRNA level. In contrast, Bay K8644, an L-type Ca^{2+} channel agonist, mimiced the stimulatory effect of VIP on PRL-mRNA content. These results show clearly that: 1) the influx of extracellular Ca^{2+} is required for the stimulatory effect of VIP on PRL gene expression and PRL secretion in avian species, and 2) DA, via D_2 DA receptors, antagonizes the stimulatory effects of VIP on PRL-mRNA and PRL secretion at least by inhibiting the influx of extracellular Ca^{2+} in pituitary cells. This demonstrated the involvement of the Ca^{2+} signaling pathway in the dual regulation of PRL secretion and PRL gene expression by DA (inhibitory) and VIP (stimulatory) at the pituitary level. Supported by USDA grant #00-02127.

Key Words: VIP, Dopamine, Calcium

723 Characterization of the VIP response element (VRE) in turkey prolactin promoter. S.W. Kang*[1], S. You[2], E.A. Wong[3], T. Bakken[1], and M.E. El Halawani[1], [1]*Dept. of Animal Science, Univ. of Minnesota,* [2]*Dept. of Animal Scinece and Technology, Seoul National University,* [3]*Virginia Polytechnic Institute and State University.*

We showed previously that vasoactive intestinal peptide (VIP) increases prolactin (PRL) gene expression and secretion in turkey primary pituitary cells. We have now used 5'- flanking deletions and mutations of the turkey PRL promoter fused to the luciferase (Luc) reporter gene in transient transfection assays to further characterize sequences involved in stimulation of PRL gene expression by VIP. Promoter activities were determined by quantitative RT-PCR of Luc mRNA. The deletion analysis of turkey PRL promoter (tPRLP) indicated that the VIP-stimulated tPRLP activity was controlled by three major positive regulatory regions and two negative regions. From the -127 to -14 Luc construct, where the 7-8 fold increase of promoter activity by VIP occured, we did deletion assay with -92/-14 and -60/-14 Luc constructs for investigating the minimal VRE of the promoter. The 35-base pair (bp) segment (position -127 to -93) deletion induced complete suppression of VIP-stimulated promoter activity, suggesting that the nucleotides between position -127 and -93 in the tPRL promoter are essential for the VIP-stimulated promoter activity. A putative Pit-1 binding site was found in the middle of the 35-bp segment and the significance of this element (12 bp) was tested by Decoy assay, deletion, and mutation analysis. The result of the present study demonstrated that VRE (12bp) in the proximal prolactin promoter is an important cis-element for the VIP-stimulated PRL gene expression in turkey primary pituitary cells.
USDA grant No. 00-02127 Key Words: Prolactin Promoter, Turkey VIP, Pituitary

Key Words: Prolactin Promoter, Turkey VIP, Pituitary

724 Met-enkephalin directly regulates the GnRH-I system in Japanese quail. MA Ottinger*[1], N Thompson[1], and P Micevych[2], [1]*University of Maryland,* [2]*UCLA Center of Health Sciences.*

Studies in our laboratory have shown that met-enkepalin (ENK) is a powerful regulator of the GnRH-I system. We have demonstrated a dose dependent, ENK -inhibition of GnRH-I release in vitro (Chen and Ottinger, 1999; Poult Sci abstr. #293). Other studies have suggested that ENK-inhibition of GnRH-I release may be directly on GnRH neurons and indirectly, through interneurons. The current study was conducted to examine the anatomical relationship of ENK immunoreactive to the GnRH-I cell bodies and processes. Double-labeling immunocytochemistry revealed close anatomical proximity of ENK and sexual dimorphismin the GnRH-I system as well as in ENK innervation. Confocal microscopy was used to determine if the ENK preferring delta opioid receptors occur on GnRH-I neurons, thereby providing a means for direct opiate regulation of the GnRH-I neuron. Fixed brains from adult males and females (n=6/group) were sectioned (20 m) and double stained using antibodies specific for GnRH-I (kindly provided by Dr. S. Wray, NIH) or d-opioid receptor (Diasorin, Inc). Many, but not all, GnRH-I positive cells were also immunoreactive for delta-opioid receptor. Further, the distribution of double labeled cell bodies tended to be in the rostral preoptic-septal region. GnRH-I axonal projections to the median eminence were also double labeled. These data provide evidence for colocalization of opiate receptor in the GnRH-I neuron, indicating a mechanism for direct opioid peptide regulation of the GnRH-I neuron.
Supported by NRI #92-37203 (MAO) and NS 39495 (PM).

Key Words: GnRH-I, Avian Reproduction, Opioid Peptides

725 Localization of neurons projecting to the infundibular nuclear complex and the median eminence in the turkey hypothalamus. K Al-Zailaie, O Youngren, and M EL Halawani, *Dept. of Animal Science, University of Minnesota.*

The release of prolactin (PRL) from avian anterior pituitary is under stimulatory control by vasoactive intestinal peptide (VIP), which functions as the PRL-releasing factor. VIP is mobilized from neurons concentrated within the hypothalamic infundibular nuclear complex (INF) that project to the external layer of the median eminence (ME). Dopamine (DA) acts centrally through D1 DA receptors to stimulate turkey PRL secretion and requires an intact VIPergic system in order to do so. The mechanisms of dopaminergic action upon VIP neurons are unclear. We have previously demonstrated the distribution of dopaminergic neurons in the turkey hypothalamus. It is not known if these DA neurons project directly to VIP neurons or if transsynaptic regulation is involved. To address this question, we have used the lipophilic fluorescent tracer Dil to determine the location of neurons which project to INF and ME. Birds were injected in INF and ME with the tracer and allowed to survive for 5 days, after which the brains were perfused, processed, and examined for retrograde transport. Following application of Dil to the INF, labeled neurons were detected in four distinct regions: preoptic area (POA), paraventricular nucleus (PVN), preopticus dorsolateralis (PD) and lateral septum (LS). After injecting Dil into ME, labeled neurons were detected in three distinct regions: INF, PVN, and preoptic medialis. The projections described above could provide anatomical substrates for neuroendocrine regulation of PRL. The identification of these labeled neurons is currently under investigation. USDA Grant No. 00-02127

Key Words: DA regulation of VIP, Turkey PRL, Dil

Reciprocation Sessions on Meat Science

726 The MARC beef carcass image analysis system. S. D. Shackelford*, T. L. Wheeler, and M. Koohmaraie, *U.S. Meat Animal Research Center.*

At present, beef carcass value is a function of USDA quality grade, a subjective estimate of meat palatability, and USDA yield grade, a subjective estimate of carcass composition. Although "expert" calculated USDA yield grade is a relatively accurate predictor of carcass composition, producers continue to distrust use of yield grade in pricing formulas because application of yield grades is subjective and a high level of error can occur when carcasses are evaluated at rates of 300 to 400 head per hour. In 1997, we developed a system to predict beef carcass cutability based on image analysis of the 12^{th} rib cross-section which was removed from carcasses for tenderness classification. This method provided a more accurate prediction of beef carcass cutability than did "expert" calculated USDA yield grade (R^2 = 0.89 vs 0.77). The Agricultural Research Service entered into a cooperative research and development agreement with IBP, Inc. to adopt this technology for application directly to beef carcasses. On-line testing has shown that this system provides a much more accurate (R^2 = 0.89 vs 0.61) prediction of boneless closely-trimmed, "user-friendly" beef carcass yields than is currently achieved by on-line USDA graders. In fact, the MARC image analysis system provides a more accurate prediction of beef carcass yields than does expert calculated yield grade (R^2 = 0.75). The ability of this system to predict "expert" calculated USDA yield grade was evaluated using 182 steer and 219 heifer carcasses that encompassed the typical range for carcass weight (227 to 455 kg), adjusted preliminary yield grade (2.2 to 4.7), ribeye area (57 to 132 cm^2), and calculated yield grade (-0.3 to 5.1). Combining image analysis variables with hot carcass weight explained 88% of the variation in "expert" calculated USDA yield grade. Implementation of this technology should allow beef packers to more accurately determine differences among carcasses in cut-out value. Thus, this system should be a key component of value-based beef price discovery systems.

Key Words: Beef, Cutability, Prediction

727 Development of Instructional Materials for CD-rom and the Internet, the Beef Myology and Muscle Profiling project. S.J. Jones* and R.L. Roeber, *University of Nebraska-Lincoln.*

With the improvement of computers to handle graphic images and search through large databases rapidly, it is now possible to develop a CD-ROM or web page that will serve as a resource for the muscular anatomy of the beef animal. With funding from the National Cattlemen's Beef Association, a CD-rom was developed on beef myology and muscle profiling. Six different sections were developed to view the muscular and skeletal anatomy of the beef carcass; they included cross-sections, lateral views, sub-primal views, skeletal views and muscle and bone descriptions by