choline for increasing liver betaine levels but in pigs that has not been studied previously. Individually penned Finnish Landrace and Yorkshire pigs (30 kg; n=70) were fed basal diet with no added betaine or choline, the basal diet supplemented with 250, 500 or 1000 mg/kg of betaine (Betafin® S1), or with a similar molar amount of choline (578, 1155 or 2310 mg/kg of choline chloride). The net energy content of the maize-soybean meal basal diet was diluted with oat hull meal (100 g/kg) and it contained 8.55 MJ/kg NE, 155 g/kg crude protein and 7.4, 4.4 and 4.3 g/kg digestible lysine, threonine and methionine+cystine, respectively. The pigs were on restricted diet, 1.5-3.0 kg feed/d. The experiment lasted 75 days. Daily weight gain improved linearly ($p \leq 0.01$) with increasing dietary betaine. The liver betaine level increased linearly with dietary betaine addition ($p \leq 0.05$). The additives had no significant effect ($p \geq 0.10$) on plasma homocysteine levels. Addition of betaine tended to improve the tensile strength of the proximal ileum linearly ($p=0.07$). Choline additions increased plasma carnitine linearly ($p \leq 0.01$) but had no effect on the pig performance, liver betaine or gut tensile strength. The results show that dietary betaine addition increased liver betaine and improved the daily weight gain of pigs on restricted diets with diluted energy concentration whereas choline addition increased plasma carnitine but had no effect on performance.

Betaine, mg/kg	0	250	500	1000	0	0	0	
Choline chloride, mg/kg	0	0	0	0	578	1155	2310	SEM
Daily weight gain, g	883	879	943	969	897	909	906	22.2
Liver betaine, mg/g	0.038	0.041	0.051	0.061	0.037	0.044	0.042	0.007
Plasma carnitine, mg/l	1.26	1.39	1.41	1.30	1.31	1.37	2.80	0.20
Gut tensile strength, kg	2.10	2.44	2.14	2.80	2.41	2.52	2.16	0.26

Key Words: Betaine, Choline, Pig

PSA Physiology: Cardiopulmonary, Immune, and Other Physiology

1620 Differences of autonomic nervous system activity in high and low body weight-selected chickens. A. Y. Kuo*[1], J. C. Lee[2], P. B. Siegel[1], and D. M. Denbow[1], [1]*Virginia Tech, Blacksburg,* [2]*VA-MD Regional Veterinary College, Blacksburg.*

This study was to investigate whether there are differences in the autonomic nervous system (ANS) function of chickens from lines selected or high (HWS) or low body-weight (LWS). The cardiovascular response to pharmacological agents was used as an indicator of ANS response. Ten birds from each line and sex were used. Catheters were introduced into the left brachial artery and vein, and connected to a MP100-BIOPAC system to record blood pressure (BP) and heart rate (HR). Birds were injected with phenylephrine, atropine, propranolol, and tetraethylammonium chloride (TEAC). Data were analyzed using ANOVA; significant differences imply $P \leq .05$. The LWS birds exhibited a greater increase in BP and less increase in HR than the HWS birds following atropine. The response to atropine showed a line and sex interaction, in which male birds had a greater increase in HR than females, and LWS females had a lower increase in HR than the HWS females. Injection of phenylephrine following pretreatment with atropine caused a baroreceptor reflex in which males showed a greater decrease in HR than females. In response to the beta-adrenergic receptor blocker propranolol, females displayed a greater decrease in BP than males, and LWS birds had a greater decrease in HR than HWS birds. In response to the autonomic ganglionic blocker TEAC, BP and HR were decreased equally in both lines. The percentage of adrenal and sympathetic impact on regulation of HR showed that LWS females required greater adrenal activity than the other birds. While change in the HR to BP ratio in response to phenylephrine was different between lines, the response was not different when phenylephrine was given following atropine. These results suggest that a higher parasympathetic nervous system tone is present in the HWS, and a higher sympathetic nervous system tone is present in the LWS than HWS birds. It is suggested that differences between the lines could be at the level of the adrenal gland.

Key Words: Autonomic nervous system, Blood pressure, Chickens

1621 Hemodynamic Responses of Broiler Pulmonary Vasculature to Intravenously Infused Serotonin. M. E. Chapman* and R. F. Wideman, *University of Arkansas, Fayetteville, AR, USA.*

Serotonin (5-hydroxytryptamine, 5HT) is a potent pulmonary vasoconstrictor actively accumulated by mammalian platelets and avian thrombocytes, and released into the plasma during platelet or thrombocyte aggregation. 5HT has been implicated in the mechanisms responsible for pulmonary hypertension in several human and animal studies. However, the role of 5HT in pulmonary hypertension syndrome (PHS, ascites) in broilers previously had not been evaluated. In the present study we evaluated the pulmonary hemodynamic responses of broilers to intravenous infusions of 5HT dissolved in 2.5% mannitol solution (carrier vehicle). Carrier vehicle infusion alone had no influence on any of the hemodynamic variables. 5HT infusion triggered rapid increases in pulmonary arterial pressure to approximately 50% above pre-infusion baseline values, accompanied by decreases in mean systemic arterial pressure and cardiac output. The peak pulmonary arterial pressure response occurred within approximately 70 s after the start of 5HT infusion, and remained elevated above baseline values over the course of a 10-minute infusion period. Pulmonary arterial pressure, mean systemic arterial pressure and cardiac output returned to pre-infusion baseline values upon cessation of 5HT infusion. Pulmonary hypertensive responses were associated with increased pulmonary vascular resistance (pulmonary vasoconstriction). The peak pulmonary arterial pressure attainable was inadequate to propel the normal cardiac output through the elevated pulmonary vascular resistance. Consequently, the impeded venous return to the left ventricle caused dependant reductions in stroke volume, cardiac output, and mean systemic arterial pressure. Reductions in cardiac output were associated with reductions in stroke volume but not heart rate. Any factor that reduces the pulmonary vascular capacity or increases the pulmonary vascular resistance theoretically can increase the incidence of PHS. The present study provides direct evidence that 5HT can trigger pulmonary vasoconstriction and pulmonary hypertension in broilers.

Key Words: Serotonin, Broiler, Hypertension

1622 Pulmonary Wedge Pressures Confirm Pulmonary Hypertension in Broilers is Initiated by an Excessive Pulmonary Arterial Resistance. M. E. Chapman* and R. F. Wideman, *University of Arkansas, Fayetteville, AR, USA.*.

High retrograde pressure through the pulmonary venous system caused by failure of the left ventricle or left atrio-ventricular valve may result in the elevated pulmonary arterial pressure and right ventricular hypertrophy associated with pulmonary hypertension syndrome (PHS; ascites) in broilers. Unanaesthetized male broilers from an ascites-resistant line, the base population from which the resistant line was derived, and a separate unselected line were used to determine whether changes in wedge pressure are predictive of differences in the pulmonary arterial pressure of clinically healthy and pre-ascitic broilers. Venous, right atrial, right ventricular, pulmonary arterial, and wedge pressures were obtained by inserting a catheter into a wing vein and progressively advancing the catheter into a pulmonary branch artery until the catheter tip became wedged in and occluded the flow through a terminal artery. Mean right ventricular and pulmonary arterial pressures were lower in the resistant line than in the base population, but wedge pressures did not differ between the resistant, base, and unselected lines. Right:total ventricular weight ratios (RV:TV) and the percentage saturation of hemoglobin with oxygen in arterial blood ranged in value from 0.18 to 0.44 and 65 to 96%, respectively. Wedge pressure, however, remained similar when pre-ascitic broilers with high RV:TV values and low oximetry values were compared with clinically healthy broilers. In all birds, whether healthy or showing pre-ascitic characteristics, the wedge pressure was slightly

higher than the right atrial pressure, but substantially lower than pulmonary arterial pressure. These observations provide definitive proof that pulmonary hypertension is initiated as a consequence of excessive pulmonary arterial or arteriole resistance. Pulmonary venous pressure is estimated by measuring the pulmonary arterial wedge pressure, and high wedge pressures would be evident if pulmonary hypertension was caused by the elevated downstream resistances associated with left-sided heart failure.

Key Words: Ascites, Wedge Pressure, Broiler

1623 Cardiopulmonary and blood gas responses to cold exposure in broiler chickens. T.W. Odom*[1], M.A. Thompson[1], K.P. Floren[1], G.A. Ramirez[1], N. Puebla-Osorio[1], L.A. Martinez-Lemus[2], and J.S. Thomas[3], [1]*Department of Poultry Science, Texas Agricultural Experiment Station,* [2]*Deparments of Medical Physiology,* [3]*Veterinary Pathobiology, Texas A&M University, College Station, TX 77483.*

Cold exposure is a well-established cause of the development of pulmonary hypertension syndrome (PHS) in broiler chickens. Therefore, in this study we used cold exposure to induce PHS. Subsequently we measured venous blood acid-base balance and several cardiopulmonary parameters. Forty 1-day-old male broiler chickens, were equally divided in 2 environmental chambers and maintained at 25 C for one week. The temperature was not changed in one environmental chamber and the other environmental chamber was changed by a gradual step-down program until the chamber reached to 10 C and maintained at that point for an additional 3 weeks. Body weight (BW), heart rate (HR), right ventricular hypertrophy, venous blood gases, and plasma thrombin-antithrombin (TAT) complexes (a sensitive indicator of *in vivo* activation of the clotting cascade) were measured. Broiler chickens in the 10 C chamber had significantly ($P.\leq 0.05$) reduced BW and HR, increased total venous CO_2 and HCO_3^-, increased right ventricular hypertrophy, and increased plasma TAT complexes compared with the broiler chickens in 25 C chamber. This study suggests that cold exposure for 3 weeks results in an altered acid-base balance in the blood, a hypercoagulable state, and a cardiovascular dysfunctional circulation. Consequently, these results support that cold exposure produces stress on the cardiopulmonary system predisposing the broiler chicken to develop PHS.

Key Words: Broiler, Cold Stress, Blood Gas

1624 Thrombocyte aggregation does not correspond with nitric oxide and cardiovascular parameters in broiler chickens with pulmonary hypertension. A.R. Carpenter[1], L.A. Martinez-Lemus[2], J.S. Thomas[3], and T.W. Odom*[1], [1]*Department of Poultry Science, Texas Agricultural Experiment Station,* [2]*Deparments of Medical Physiology ,* [3]*Veterinary Pathobiology, Texas A&M University, College Station, TX 77843.*

Pulmonary hypertension syndrome (PHS) is a multifactoral disease that affects predominantly the fast-growing broiler chicken. Research in mammalian models has suggested that platelet activity, thrombosis, and reduced synthesis of nitric oxide (NO) may be involved in pulmonary hypertension. Therefore, this study was designed to investigate the relationship thrombocyte aggregation may have with NO, and other cardiovascular parameters in PHS. Broilers were reared either under hypobaric conditions (HC) to induce PHS or under normobaric conditions (NC) for five weeks. Aggregation was measured in both whole blood and in thrombocyte-rich plasma. Whole blood aggregation was measured in order to assess thrombocyte function in a milieu similar to that *in vivo*. Thrombocyte-rich plasma provided an environment, which allowed for a more clear representation of thrombocyte activity unaltered by other blood cells. Body weights and cardiovascular parameters were compared in both experiments, and NO produced by a right pulmonary artery ring was measured. Compared with NC broilers, HC broilers had increased hematocrit levels, right ventricular hypertrophy, and PHS mortality. Right pulmonary artery rings from HC broilers also produced significantly less NO than NC broilers at 4 weeks of age. There were no consistent differences in thrombocyte aggregation between the NC and HC broilers. Also, thrombocyte aggregation did not correspond with NO production by the right pulmonary artery ring or with PHS mortality. Thus, changes in thrombocyte aggregation may not be related with PHS in the broiler chickens.

Key Words: Broiler, Aggregation, Nitric Oxide

1625 Assessment of factor V, VII, and X activity, the key coagulant proteins of the tissue factor pathway in poultry plasma. A.E. Thomson*, E.J. Squires, and P.A. Gentry, *University of Guelph, Guelph Ontario Canada.*

Assay methods were developed for key componets of the tissue factor pathway of coagulation, namely Factor V, Factor VII and Factor X. Using these assays, plasma from healthly laying hens, roosters and broilers was shown to contain functional and equivalent amounts of each of these clotting factors. The plasma activity for Factor V, Factor VII, and Factor X can only be accurately determined when chicken tissue factor is used to initate the coagulation mechanism in poultry plasma. Neither human tissue factor nor rabbit tissue factor forms a fully functional enzyme reactive complex with chicken Factor VII. The overall Tissue Factor Pathway coagulation mechanism was evaluated in plasma from laying hens, roosters and broilers using the One-stage Prothrombin Time assay. As long a sufficient tissue factor was used, the overall clotting time results obtained with human recombinant tissue factor were not significantly different ($P > 0.05$) from those obtained with chicken tissue factor. We conclude that poultry plasma does possess a fully functional tissue factor coagulation mechanism, but homologous chicken tissue factor must be used for *in vitro* assays of the components of this pathway.

Key Words: Tissue Factor Pathway, Coagulant Proteins, One-Stage Prothrombin Time

1626 Establishing endocrine and behavioral indices for endocrine-disrupting chemicals in birds. M.A. Abdelnabi*, N. Thompson, and M.A. Ottinger, *University of Maryland, College Park, MD USA.*

Endocrine-disrupting chemicals (EDCs) would be expected to affect growth and reproduction. Japanese quail provide an avian model to establish endocrine and behavioral indices for EDC exposure. In these experiments, fertile eggs from the parents (P1) were injected at embryonic day 11 (E11) with estradiol benzoate (EB; 20 ug/egg), oil control (con), or uninjected. Chicks (F1) were raised to maturity and housed in pairs with all possible crosses (EB x con; EB x EB; and con x con) to assess fertility and productivity. A separate subset of Eb and control F1 males were photoregressed, then implanted with testosterone and tested for sexual behavior one week later. In addition, eggs were collected from EB and control F1 pairs, then incubated. At embryonic day 11, eggs from the EB pairs were divided into two groups; the first was injected with EB (F2a) while the rest of the eggs from the EB pairs were not treated (F2b) the eggs from control pairs were not treated (F2c). After hatch, chicks (F2) were raised exactly as F1 chicks had been raised. F1 males were demasculinized by the EB treatment and when paired with EB females, fertility was severely reduced (35% vs 85%). F1 females showed erratic egg production (55% vs 90%). F2 birds that were embryonically treated with EB (F2a) also showed reproductive impairment, similar to that observed in the EB treated F1s. Fertility and productivity were 90 and 83%; 86 and 72% and 57 and 32% for the control, non-injected and EB injected, respectively. Interestingly, there appeared to be a carry over effect in the F2 offspring. This study provides evidence for long-term consequences of embryonic estrogen exposure to estrogen and suggests that these measures are potential indices for EDC exposure. Supported by EPA #R826134010 (MAO) and NSF #IBN-9817024 (MAO).

Key Words: endocrine disrupting chemicals, embryonic development, sexual differentiation

1627 Immunological effects of genistein exposure in chicks. Alexander Peterson[1], Haitao Li[1], and Wallace Berry*[1], [1]*Auburn University Department of Poultry Science.*

Exposure of chicks to high levels of sex steroids causes involution of the thymus and bursa with subsequent impairment of immune function.

Domestic poultry are exposed from hatching onward to potentially estrogenic isoflavones in soy based diets. Our previous work has demonstrated that the soy isoflavone genistein is estrogenic in chickens. The purpose of this study was to determine whether genistein would exert effects on thymus and bursa in a manner similar to that of estrogen and testosterone Day old male chicks were assigned to 6 treatments as follows: chicks fed a typical soy based chick diet with daily subcutaneous injection of sesame oil vehicle (SV); soy-free diet and vehicle injection (V); daily injection of 0.5 mg diethylstilbestrol as a positive control (DES); 0.5 mg testosterone proprionate as a positive control (TP); 1.0 mg genistein injection (G1); and 10.0 mg genistein (G 10). At 12 days of age, the chicks were weighed and testes and livers were excised and weighed. DES treatment significantly decreased bursa and thymus weight and increased liver weight.TP also decreased bursa and thymus weight and increased liver and testes weight. G 10 treatment significantly reduced both bursa and thymus weight and increased liver weight. These results demonstrate that genistein has effects similar to the sex steroids on immune tissues in chickens and may influence immunological development in poultry species. (Supported by the Alabama Agricultural experiment Station and USDA AD-421 project S-289).

Key Words: Genistein, Thymus, Bursa

1628 Partial structural characterization of Bursal Anti-Steroidogenic Peptide (BASP) with structural homology to chicken histone H1. R.W. Moore*[1,2], D.Y. Caldwell[2], T.E. Porter[3], L.R. Berghman[2], F. Vandesande[4], J.A. Byrd[1], and B.M. Hargis[5], [1]*USDA-ARS-SPARC,* [2]*Texas A&M University,* [3]*University of Maryland,* [4]*University of Leuven, Belgium,* [5]*University of Arkansas.*

Previously, our laboratory has described a bursa of Fabricius-derived molecule that was named Bursal Anti-Steroidogenic Peptide (BASP) after its potent anti-steroidogenic activity. While the biological activity of BASP in several *in vitro* systems has been studied in our laboratory, until recently, molecular/structural characterization has remained elusive due lack of substantial amounts of highly purified biologically active material useful for conventional sequencing. Presently, endopeptidase digestion, fragment purification, and conventional N-terminus sequencing of each of the two predominant bands (24 and 30 kDa) from SDS PAGE of highly purified biologically-active BASP were performed and six low level sequences were determined. Each of these short fragment sequences showed homology with the known sequence of chicken histone H1. A single partial sequence homologous with the structure of chicken histone H1 was also obtained utilizing Q-TOF analysis. In addition, a unidirectional cDNA library constructed from bursa of Fabricius mRNA was screened with polyclonal antibody probes constructed against highly purified biologically active BASP. Six expression proteins were identified, and nucleotide sequencing of the lambda phage inserts revealed 4 unique partial nucleotide sequences from the 6 sequenced cDNA inserts. One of these nucleotide sequences (two overlapping cDNA insets) was 99% homologous with chicken histone H1. These observations may argue that BASP is either identical to, processed from, or shares homology with chicken histone H1. Purification of chicken histone H1 for bioassay has been initiated.

Key Words: Bursal Anti-Steroidogenic Peptide, Histone H1, Bursa of Fabricius

1629 Influence of broiler breeders age on villous and microvillous height in the embryo intestinal mucosa. Alex Maiorka[1], A.V. Fischer da Silva[1], E. Santin[1], L.O. Nakagui[1], and M. Macari[1], [1]*FCAV - UNESP.*

This study was carried out aiming to evaluate the effect of broiler breeders age (30 or 60 week) on embryo intestinal mucosa development. At 20th day of incubation, 8 embryos of each broiler breeder age were sacrificed and sample from different parts of the small ntestine were collected (duodenum, jejunum and ileum) and submitted to morphometric studies under light microscopy using an image analysis system. The parameters studied were villous height(micrometer), crypt depth (micrometer)and microvillous height (micrometer)using transmission electron microscopy. The experimental design was completely randomized with two treatments and eight repetitions, with the data submitted to analysis of variance. At light microscopy analysis it was observed that intestinal mucosa of embryos from 30-wk broiler breeders showed smaller villous height when compared to those from 60-wk broiler breeders (77 vs 102; 40 vs 45 and 31 vs 41 for duodenum, jejunum and ileum, respectively) and smaller crypt depth (44 vs 53 ; 31 vs 37 and 26 vs 31 for duodenum, jejunum and ileum, respectively). The results of the transmission electron microscopy analysis showed that the embryo from 30-wk broiler breeders had smaller jejunum microvillous height than that verified in jejunum of embryos from 60-wk broiler breeders (0.874 vs 1.090).These results indicate that broiler breeder age has an important role on intestinal mucosa embryo development. FAPESP Proc.98/11304-9

Key Words: Embryo, Intestinal mucosa development, Age of broiler breeders

1630 Effect of feed and/or water withdrawal on intestinal mucosa development in broiler chicks after hatching. Alex Maiorka*[1], Elizabeth Santin[1], Fabiano Dahlke[1], and Marcos Macari[1], [1]*FCAV-UNESP.*

This study aimed at evaluate the effect feed and/or water withdrawal on intestinal mucosa development in broiler chicks after hatching. In Trial I, 20 day-old broiler chicks, male, were used. After hatching, the birds were maintained in environmentally controlled room and divided in two groups and fed as follow: T1- water and feed ad libitum; T2 - water ad libitum and no feed. After 24 h of hatching samples from different segments of the small intestine were collected (duodenum, jejunum and ileum) which were submitted to morphometric studies using light microscopy using an image analysis system. The parameters studied were: villous weight and crypt depth. The results did not show difference between treatments in duodenum (P=0.434), jejunum (P = 0.699) and ileum (P = 0.060) for villous height and crypt depth in the duodenum (P = 0.434), jejunum (P = 0.825) and ileum (P = 0.246). In Trial II, 72 day-old broiler chicks, male, were divided in into 4 treatments and fed as follow: T1- water and feed ad libitum; T2 -water ad libitum and no feed; T3 - no water and feed ad libitum and, T4 - no water and no feed. After 24, 48 and 72 hours post- hatching, 6 birds of each treatment were sacrificed and sampled from different parts of the intestine were collected (duodenum, jejunum and ileum) and submitted to scanning electron microscope studies to evaluate the villous density. The chicks fasted of feed and/or water showed grater number of the villous/area in all intestine mucosa segments, irrespective of the fasting period, than those in the birds not fasted. The results of this study suggest that not only feed is relevant to villous development in the mucosa of broiler chicks after hatching, but water, as nutrient, also play a important role in this mechanism. The findings also revealed that 24 h fasting is deleterious to intestine mucosa integrity.FAPESP Proc. 98/11304-9.

Key Words: fasted, intestinal mucosa development, post hatching

1631 Expression of selected hepatic genes related to lipid metabolism in broiler breeders. M.P. Richards*[1], S.M. Poch[1], C.N. Coon[2], Y. Kirby[2], R.W. Rosebrough[1], C.M. Ashwell[1], and J.P. McMurtry[1], [1]*USDA, ARS, Beltsville, MD,* [2]*University of Arkansas, Fayetteville, AR.*

Cobb 500 broiler breeder pullets were fed according to Cobb Breeder Management Guide specifications until they reached 21 weeks of age. At this time, half the birds were switched to *ad libitum* feeding (AL) while the remaining birds continued to be fed according to Cobb guidelines (CF). At 22 weeks all birds were photostimulated and maintained throughout a laying cycle ending at 36 weeks. Samples of liver and abdominal fat pad were collected at the following times: 1) just prior to photostimulation, 2) after photostimulation through first egg, and 3) at peak egg production. Reverse transcription polymerase chain reaction in conjunction with capillary electrophoresis and laser-induced fluorescence detection (RT-PCR/CE-LIF) was used to quantify hepatic expression of specific genes relative to that of β-actin in total RNA samples. Expression of fatty acid synthase (FAS), malic enzyme (ME), and acetyl-CoA carboxylase (ACC) genes in AL hens declined from their highest levels just prior to photostimulation to reduced levels as the birds came into and maintained egg production. In contrast, the CF hens displayed a dramatic increase in the expression of these genes following photostimulation through first egg with a subsequent decline in expression levels as these birds reached peak egg production. Expression of fatty acid binding protein (FABP) and apolipoprotein B (ApoB) genes increased in both AL and CF breeders following photostimulation. This is consistent with increased hepatic lipid transfer capacity during egg production. Abdominal fat pad weights were significantly higher in the AL as compared to CF hens following photostimulation.

Hepatic leptin receptor gene expression did not differ significantly for AL as compared to CF hens at any time. In conclusion, the AL and CF feeding regimes produced significantly different effects on hepatic gene expression related to lipid metabolism in broiler breeders. By analyzing the expression of specific hepatic genes involved in lipogenesis and lipid transport as well as other metabolic pathways, it may be possible to assess the potential metabolic impact of changes in feed consumption and other management practices on growth, body composition and performance of broiler breeders.

Key Words: Gene Expression, Lipid Metabolism, Broiler Breeder

1632 Identification and expression of the turkey leptin receptor gene. M. P. Richards*, S. M. Poch, and C. M. Ashwell, *USDA, ARS, Beltsville, MD.*

Using reverse transcription polymerase chain reaction (RT-PCR) with total RNA isolated from whole brain tissue, we have sequenced 3976 bases of the turkey leptin receptor (Ob-R) gene corresponding to the complete coding sequence and portions of the 5'- and 3'-untranslated regions of the mRNA (GenBank accession no. AF321982). Turkey Ob-R showed greater than 90% sequence identity at both the nucleic acid and amino acid level with chicken Ob-R (GenBank accession nos. AB033383 and AF169827). The turkey Ob-R gene (long form) codes for a protein comprised of 1147 amino acids that displays a number of features similar to other leptin receptors including a signal peptide, a single trans-membrane domain and specific conserved motifs defining putative leptin binding and signal transduction regions of the protein. RT-PCR in conjunction with capillary electrophoresis and laser-induced fluorescence detection was used to quantify Ob-R expression relative to that of β-actin in total RNA samples isolated from various tissues. Ob-R expression was highest in brain, spleen and lung tissue obtained from 3 wk old poults with lower levels of expression in kidney, pancreas, duodenum, liver, fat, and breast muscle. Using a primer set specific for the long form of OB-R (Ob-RL), the highest levels of expression for this variant form were detected in brain, lung and spleen with lower levels in kidney, pancreas, fat and liver tissues. Ob-R expression in developing turkey embryos was highest in brain tissues and remained high throughout incubation (days 14-28). In contrast, expression of Ob-R in embryonic liver tissue peaked at day 16 and then declined toward hatching (day 28). Yolk sac expression of Ob-R declined from day 14 to day 20 and then increased toward hatching. These findings suggest differential regulation of Ob-R expression in turkeys during embryonic and post-hatch development. In conclusion, this is the first report of leptin receptor gene expression in turkeys.

Key Words: Leptin Receptor, Gene Expression, Turkey

1633 *Campylobacter* colonization of the crops of newly hatched chicks. R. L. Ziprin* and L. F. Kubena, *FFSRU/SPARC/ARS/USDA, College Station, TX/USA.*

It has been reported that up to sixty percent of the crops of market age birds contain *Campylobacter*. However, this number may not represent true colonization but only transient presence of the organism. In our present work we have studied colonization of crops in newly hatched chicks by wild-type and mutant strains of *C. jejuni*. Groups of 15 day-of-hatch Leghorn chicks were orally gavaged with 10^8 CFUs of the wild-type strain on day- of-hatch or with an equivalent number of mutant *C. jejuni*, which had been previously shown to be ineffective colonizers of the cecum. Three birds from each challenged group were killed by cervical dislocation on days 1,3,5,7, and 9 after inoculation. There was a 2-4 hour feed withdrawal period before sacrifice. Crops were aseptically removed, placed in individual plastic bags containing 5 ml of sterile water, and placed in a Stomacher device. The *C. jejuni* concentration in the resultant suspension was determined by standard plate count methods, using Campy-Cefex agar. We observed an initial rapid drop in *Campylobacter* concentrations present in the crop during the first 24 hrs after challenge, then a stable concentration of approximately 10^5 CFUs was established through days 7 to 9. Mutant strains lacking an ability to colonize cecum were found to persist in the crop. Concentrations of these mutant strains in the crop were usually below that of the wild-type, but were still between 10^3 to 10^5 CFUs. These results indicate that the bacterial factors necessary for colonization of the crop are not the same as those needed for colonization of the cecum.

Key Words: Campylobacter, Crop, Colonization

1634 Heterogeneity of Ryanodine Receptors in Turkeys. Wen Chiang* and Gale Strasburg, *Michigan State University.*

Pale, soft, exudative (PSE) meat has been a serious quality problem in the turkey industry. Because of the striking similarity of development of PSE turkey to that of PSE pork, there could be a genetic basis for some of the incidence of PSE turkey. A point mutation (R615C) in the SR calcium channel protein or ryanodine receptor (RYR1) is associated with porcine malignant hyperthermia, and results in economic losses to producers from animal death by stress, and to processors by abnormalities in meat quality resulting from rapid postmortem glycolysis. Over 20 mutations in the primary structure of mammalian RYR1 have been associated with Malignant Hyperthermia (MH) and Central Core Disease (CCD) in human. At least 9 of these mutations cluster in the region of amino acid residues 35 to 614. We hypothesized that one or more mutations exist in the region of the turkey alpha-RYR (homologous to mammalian RYR1) which give rise to this problem. RNA was purified from genetically unimproved turkeys and from a commercial line intensively selected for muscle growth. alpha-RYR cDNA was amplified through RT-PCR and was cloned into a plasmid vector. Analysis of the alpha-RYR cDNA covering amino acids 376 to 647 (human sequence) reveals sequence diversity in the unimproved turkey population. One group is homologous to the commercial line and to mammalian RYR sequences. Another unimproved group shows a 27 amino-acid-residue deletion (residue numbers: 417-443). Another unimproved group shows heterogeneity of cDNA variants. The variants are characterized by the presence or absence of 81 bp, 193 bp, and 329 bp sequences. The exclusion of the 81 bp sequence results in the absence of amino acids corresponding to Ser-416 to Ser-443. The exclusion of the 193 and 329 bp sequences leads to frame shifts that introduce internal stop codons. Avian muscle fibers also express an additional RYR isoform, beta-RYR, which is homologous to the mammalian non-muscle isoform. Our ryanodine binding studies indicate that there is functional diversity of beta isoforms within the commercial turkey population. These results suggest that mutations may exist in either isoform or in both, which could predispose turkeys to yielding of PSE meat.

Key Words: Meat Quality, Ryanodine Receptor, PSE Meat

1635 Intestinal calcium uptake and reproductive hormones in three laying hen varieties after prolonged egg production. D. J. Franco*[1], K. K. Franzen[1], C. F. Toombs[1], and M. M. Beck[1], [1]*University of Nebraska.*

Hy-Line W-98, W-36, and Brown hens are among the most popular strains for commercial egg production around the world. Although hens of all three types lay approximately the same number of eggs/hen housed to 80 weeks, fewer eggs and more soft shelled or broken shell eggs are observed during prolonged egg production. Also, the increase in egg size with age brings, as a result, an insufficient calcium carbonate secretion, and consequently the thickness of the eggshell declines (R.J. Etches, 1996). As part of a larger study, hens of the three varieties were maintained under production longer than 100 weeks and hens of each strain were randomly selected for in vitro determination of calcium uptake (CaT) by duodenal cells. Blood samples were collected 4-6 hours prior to oviposition via brachial vein cannulation for plasma estrogen (E_2), luteinizing hormone (LH), and progesterone (P_4) determination. Hens were then euthanized by cervical dislocation and CaT determined. For W-36 hens, CaT was significantly greater than that found in Brown hens (P=0.042) while for W-98 there were no differences from W-36 or Brown strains (P=0.170 and 0.427), respectively. P_4 and LH concentrations were not significantly different in the three varieties of laying hens. E_2 concentration for Brown hens was significantly greater than that found in W-36 and W-98 hens (P=0.001 and 0.043, respectively), while for W-98 the E_2 concentration was significantly greater than that found in W-36 (P=0.083). Based on the higher CaT by duodenal cells and the lower plasma levels of E_2, these results suggest a higher efficacy of the W-36 hens in calcium regulation during the terminal phase of egg production (more than 100 weeks of age), compared to the Brown and W-98 hens. However, when specific gravity and thickness of shells were compared, the W-36 appeared to have no advantage over W-98 (P=0.460 and 0.684) or Brown (P=0.197 and 0.941) hens, respectively.

Key Words: Laying hens, Calcium transport, Reproductive hormones

1636 Estrogen receptor populations in various calcium regulating tissues in laying hens at three ages. K. K. Franzen*[1], D. Clopton[1], N. Caceres[2], G. Sarath[2], and M.M. Beck[1], [1]*Animal Sciences Dept., University of Nebraska,* [2]*Biochemistry Dept., University of Nebraska*.

Older hens in production lay larger but fewer eggs than younger birds and the incidence of soft and broken shells is greater in older hens than younger (Joyner et al., 1987). These changes are attributable at least in part to changing hormone profiles and diminished ability of the hen to process calcium at the duodenum (Al-Batshan et al., 1991; Hansen and Beck, 1997). However, in a recent study, duodenal cells from older birds ($\geq$71 wk) were able to take up ^{45}Ca as well as hens at 26-29 wk of age despite reduced estrogen (E_2) and progesterone (P_4) concentrations (Franzen and Beck, 2000). In further exploration of this relationship, a study was conducted with three ages of Hy-Line W-36 birds: pre-lay pullets (PL; 19 wk, 0% production) peak-production hens (PP; 29 wk, 93% production), and late stage hens (LS; 71 wk, 80% production). Hens from the PP and LS groups were palpated for presence of an egg in the shell gland, and a blood sample for E_2, P_4 and luteinizing hormone (LH) determinations was drawn from the brachial vein of all birds 4-6h prior to expected oviposition. Hens were then euthanized and tissues (kidney, duodenum, shell gland, hypothalamus) were removed for quantification of estrogen receptor-α (ERα) populations via immunocytochemical and Western blot analyses. Plasma E_2 and P_4 were both lower in LS birds than in either PL or PP birds ($P \leq 0.05$), but LH did not differ across ages. ERα staining was not different among age groups in shell gland; however, in both kidney and duodenum, PL birds had fewer receptors than LS birds ($P \leq 0.05$), with PP birds being intermediate. When comparisons were made within age, only LS hens had differences between tissues: ERα staining was greater in duodenum than in kidney or shell gland (P=0.0016, 0.0427) with the least amount of staining in the kidney ($P \leq 0.10$), the primary site of E_2 action on calcium regulatory processes. PL and PP birds had no differences in ERα occurrence in the various tissues.

Key Words: Estrogen receptors, Laying hen, Aging

1637 Frequency of Preovulatory Luteinizing Surges in Turkey Hens and Egg Production Rate. H.-K. Liu, D.W. Long, and W.L. Bacon, *The Ohio State University, Wooster OH*.

Whether the interval between preovulatory surges of luteinizing hormone (LH) was different between lines of turkey hens with either poor (RBC3 line, peak at 55%) or excellent (Egg line, peak at 85%) rates of egg production was examined. Twelve laying hens of each line were cannulated and bled hourly (1.5 mL) for 10 d at peak of production. The hens were photostimulated with constant light (24 h light: 0 h dark d^{-1}) to avoid diurnal masking of innate circadian rhythms. Cannula patency was lost during serial bleeding in 5 of the RBC3 line hens and 1 of the Egg line hens, so they were removed from the study. The mean interval between preovulatory LH surges for the RBC3 line hens was longer than for the Egg line hens (39.5 h and 26.0 h, respectively; $P < 0.001$) and had a higher coefficient of variation (21.9% and 7.2%, respectively). Intersequence LH surges were found in some of the RBC3 line hens (2 of 7 hens), but none were found in the Egg line hens (0 of 11 hens). All preovulatory progesterone (P_4) surges (144 total) were coupled with preovulatory LH surges, but not all LH-P_4 surges were coupled with ovipositions; uncoupled LH-P_4 surges are referred to as blind LH-P_4 surges. The incidence of blind LH-P_4 surges was not different between RBC3 and Egg line hens (23% and 9% of all surges, respectively; $P = 0.08$). The baseline concentration of LH between surges was higher in the Egg line hens than in the RBC3 line hens (2.39 and 1.82 ng mL-1, respectively $P < 0.001$), but the baseline and surge amplitude concentrations of P_4 were not different ($P > 0.25$) between lines, nor was the concentration of estradiol-17β . It was concluded that the longer interval between LH-P_4 surges was the major factor tested that was associated with the difference in egg production rate between the RBC3 and Egg line turkey hens. A higher incidence of blind LH surges may be a second contributing source of lower egg production in the RBC3 line in comparison to the Egg line turkey hens.

Key Words: turkey hen, luteinizing hormone, egg production rate

1638 Changes in morphology of granulosa cells in heat-stressed laying hens. M. A. Alodan*[1] and M. M. Beck[1], [1]*University of Nebraska*.

We know from previous experiments that heat stress (HS) reduces egg production in laying hens, in part at least through disruption of steroidogenesis. We also noted in several studies changes in the morphology of the granulosa cells (GC) at the level of lipid droplets (LD). It has been reported that HS increases lipid oxidation in the hen, and that normal lipid endocytosis is controlled by the amount of lipid in the cell. However, oxidized lipid receptors are not controlled, at least in human macrophages located in the endothelium of blood vessel walls, causing the formation of giant macrophage cells and leading to the formation of plaques in arteries. The aim of this study was to determine and describe the effect of HS on the morphology of LD in GC of the laying hen and the subsequent recovery to the pre-HS state. Two groups of hens were used; one group was subjected to 24C, 30%RH (control), the other to 36C, 60%RH for 24h (HS treatment). At the end of 24h, the F1 follicles were collected from both groups and the GC monolayers were isolated and fixed in 10% formalin for 24h. The monolayer was then stained using fat stain Oil Red O to localize LDs in the GC. In control GC, LDs were small and uniform in size and shape and they were randomly and evenly distributed in the cells. In contrast, the GC of the HS birds contained much larger LD of different sizes and shapes, and they were arranged neatly around the cell periphery. The percentage of cell area covered by LD in HS GC was significantly (P=0.000) greater than that of the control group. We hypothesize that, during HS, the reduction in activity of GC (steroid production) results in lipid accumulation in the cells. Alternatively, HS-induced lipid oxidation may lead to this accumulation via unregulated oxidized lipid receptors. Both of these are consistent with the recovery that occurs over time in these cells.

Key Words: Laying Hen, Granulosa cell, Lipid oxidation

1639 Active immunization against inhibin enhances reproductive measures in male broiler breeders. S. T. Pittman*, D. G. Satterlee, and G. G. Cadd, *Louisiana State University, Baton Rouge, LA/USA*.

Inoculation of female Coturnix and broiler breeder hens with an inhibin-based immunogen (MBP-cINA$_{521}$) accelerates puberty and enhances egg production. MBP-cINA$_{521}$ is a fusion protein consisting of the *E. coli* Maltose Binding Protein (MBP) and a fragment of the alpha-subunit of chicken inhibin (cINA$_{521}$). Herein, the effect of this immunogen on male broiler breeder development was evaluated. At 13 wk, male breeders (n = 288; Cobb) were randomly assigned into one of four treatment groups based on a primary inoculation of 0 (control), 1, 3, or 5 mg of MBP-cINA$_{521}$ per bird. Booster vaccinations (one-half the primary dose) were given at 18 wk. At 24, 28, and 39 wk, one-third of the birds in each treatment were weighed (BWT), blood sampled and sacrificed. Testes were weighed (TWT) and plasma testosterone (T) levels were determined by RIA. At 24 wk, birds given the 5-mg dose had greater ($P< 0.05$) BWT and TWT than controls. BWT and TWT responses in birds of the two lowest dosages were intermediate between, but not different from, those of the control or high dose groups. Plasma T levels were higher (> 2-fold; $P< 0.05$) in birds given 5 mg than in either the control or 1 mg groups. T levels were also appreciably elevated in the 3-mg group but not different from control T responses. No treatment differences were detected in BWT, TWT and T levels at 28 wk. However, by 39 wk, although BWT remained similar in all treatment groups, TWT was greater in males given the 1-mg dose (43 %, $P< 0.10$) than in controls. TWTs in the 3- and 5-mg groups were also numerically elevated (30 and 37 %, respectively) over control values. Additionally, age-related decline in TWT between 28 and 39 wk was markedly less in all MBP-cINA$_{521}$-treated birds than in controls. Plasma T at 39 wk in the 1-, 3- and 5-mg groups were, respectively, 115, 74, and 67 % greater than those found in the controls. Fighting reduced bird numbers and likely precluded the finding of statistical differences between plasma T means at 39 wk. Mortality was similar in all treatment groups. Collectively, our data suggest that inoculation of male broiler breeders with MBP-cINA$_{521}$ enhanced early measures of puberty and diminished the expected decline in TWT and T levels associated with aging.

Key Words: Broiler breeder, Inhibin, Testes

1640 Dual labeling immunofluorescent staining demonstrates the presence of a protease-inhibiting protein (ovoinhibitor) in the chicken pituitary. C. M. Oubre*[1], K. E. Clements[1], F. Vandesande[2], and L.R. Berghman[1], [1]*Texas A&M University,* [2]*University of Leuven, Belgium.*

Ovoinhibitor is a serine protease-inhibiting protein that specifically inhibits serine proteinases such as trypsin and chymotrypsin. During recent attempts to raise monoclonal antibodies (MABS) against chicken bursa of Fabricius proteins, one MAB was produced that specifically recognized chicken ovoinhibitor. This was the first demonstration of ovoinhibitor in an avian immune organ. Further immunocytochemical research revealed that in the pituitary and the brain of the chicken, some cells undeniably displayed immunoreactivity for ovoinhibitor. The present study was aimed at identifying the hypophysial hormone-producing cells that express ovoinhibitor immunoreactivity. Therefore, pituitary glands from 4-week old birds were fixed in phosphate-buffered paraformaldehyde (4 % w/v) and 30-μm vibratome sections were stained as floating sections in 12-well tissue culture plates. The mouse MAB against ovoinhibitor was used in conjunction with polyclonal antibodies against Luteinizing Hormone (LH), Growth Hormone (GH), Proopiomelanocortin (POMC) and Prolactin (PRL) and S-100, respectively. The mouse MAB was visualized with a rhodamine-conjugated secondary antibody, whereas the polyclonal rabbit antisera were detected with a biotinylated secondary antibody combined with FITC-conjugated streptavidin. This procedure stained ovoinhibitor-positive cells in red, while the pituitary hormone-positive cells were stained green; overlapping antigens were clearly visible in yellow. The results of these dual-staining experiments revealed partial co-localization of ovoinhibitor with GH, LH, and POMC, each time in a subset of the respective hormone producing cells. By contrast, no co-localization with PRL and S-100 could be demonstrated. Serine protease inhibitors from another family (the serpins) have recently been identified in rat brain and pituitary. In the latter, they have been suggested to play a role in the regulation of cell-extracellular matrix interactions .

Key Words: chicken, pituitary, ovoinhibitor

ASAS Nonruminant Nutrition: Vitamins, Minerals, and Energy

1641 The effect of genotype, parity and folic acid supplement on the expression of leptin, and its receptors in embryonic and endometrial tissues from pigs at 15 days of gestation. F. Guay[1], A. Gigure*[2], M.-F. Palin[2], C.L. Girard[2], J.J. Matte[2], and J.P. Laforest[1], [1]*Laval University, Department of Animal Science, Qc, Canada,* [2]*Dairy and Swine R & D Centre, Lennoxville, QC, Canada.*

We have previously demonstrated mRNA expression of leptin (*Lep*) and its receptors (*Lepr* and the long form *Lepr-L*) in endometrium and embryos at 25 days of gestation in sow. We have also shown that expression levels of *Lep* and *Lepr-L* could be influenced by dietary supplement of folic acid (B9). *Lep* and its receptors may therefore have a direct role in the embryo-maternal dialogue. The objectives of this study were to compare the mRNA expression of leptin (*Lep*) in backfat tissue, and its receptors (*Lepr* and *Lepr-L*) in embryonic and endometrial tissues of sows in relation to the genotype, parity, and B9 supplement. Eleven nulliparous Yorkshire-Landrace (GT), 12 multiparous Landrace (LD) and 10 multiparous Meishan-Landrace (ML) sows were randomly assigned to 2 dietary treatments: 0 ppm or 15 ppm of B9 given from the estrous preceding mating up to slaughter, on d15 of gestation. Embryonic and endometrial tissues were collected to evaluate the mRNA expression of *Lepr* and *Lepr-L* while expression levels of *Lep* were assessed in backfat tissue. Blood samples were taken at mating and at slaughter to determine the concentration of circulating leptin; backfat thickness was also recorded. The embryonic *Lepr-L* transcript was barely detectable. Endometrial *Lepr* and *Lepr-L* and embryonic *Lepr* expression were higher in LD than in GT and ML sows (P<.01). No B9 effect was seen on *Lep* and its receptors; breed and parity did not influence *Lep* in backfat. Plasma leptin and backfat thickness were higher in ML than in LD and GT sows (P<.01). Circulating *Lep* was correlated with backfat thickness (r=0.54, P<0.01) but not with backfat expression levels of *Lep*. These results suggest a developmental regulation of leptin receptors (*Lepr* and *Lepr-L*) in early pregnancy .The effect of B9 on *Lep* gene expression may occur between d15 and d25. Furthermore, in early gestation, the circulating leptin concentration is related to adiposity.

Key Words: Leptin, Sow, Gestation

1642 The effect of genetic type and parity and folic acid supplement on homocysteine metabolism from sows on day 15 of gestation. F. Guay*[1], A. Giguere[2], M.-F. Palin[2], C.L. Girard[2], J.J. Matte[2], and J.-P. Laforest[1], [1]*Laval University, Department of Animal Science, Qc Canada,* [2]*Dairy and Swine R & D Centre, AAC, Qc Canada.*

Supplements of folic acid (B9) have been shown to increase prolificacy in multiparous sows by increasing embryo survival. B9 supplement is also recognized to decrease the concentrations of homocysteine (HCY), which is known to be potentially teratogenic. The objective of this study was to compare the effects of B9 supplement on HCY metabolism in early gestating sows. Eleven nulliparous Yorkshire-Landrace (GT), 12 multiparous Landrace (LD) and 10 multiparous Meishan-Landrace (ML) sows were randomly assigned to 2 dietary treatments: 0 ppm or 15 ppm of B9. Supplements were given from the estrous preceding mating up to slaughter on d15 of gestation. The uterine flushing was collected to evaluate the total content of HCY in the uterine lumen. Blood samples were taken at mating (d0), d5, d10 and d15 of gestation to determine the concentrations of circulating HCY. B9 supplementation had no significant effect on the concentration of circulating HCY, but it decreased (P≤.06) HCY content of uterine flushing in LD (115 16 nmol vs 84 17 nmol) and GT sows (138 23 nmol vs 98 12 nmol) but not significantly in ML sows (117 30 nmol vs 102 30 nmol). ML (18.3 1.3 μM) and GT (16.6 1.0 μM) sows had lower (P≤.01) concentrations of circulating HCY than LD sows (24.5 1.3 μM). These results suggest that a decrease in the content of HCY in the uterine flushing may play an important role in the control of embryo survival by a supplement of B9.

Key Words: Folic acid, Homocysteine, Sow

1643 Phosphorus removal with and without phytase in finishing pigs. G.A. Apgar*[1], C.M. Peter[2], T.A. Guthrie[1], K.E. Griswold[1], and D.H. Baker[2], [1]*Southern Illinois University, Carbondale,* [2]*University of Illinois, Urbana.*

Two trials were conducted using 171 crossbred pigs (53.3 +/- 1.4 kg) to evaluate the effects of removing the inorganic P from finishing diets with or without phytase. The finisher period was broken into two 28-d phases, with the early phase formulated to contain 0.82% lysine and the late phase 0.65% lysine. Dietary treatments (Trt) were: 1) control (early = 0.49% total P, 0.19% available P (aP); late= 0.43% total P, 0.15% aP); 2) control without inorganic P (early= 0.36% total P, 0.07% aP; late= 0.33% total P, 0.06% aP); and 3) diet 2 + 500 U/kg phytase. Pigs were given ad-libitum access to feed and water. Average daily gain, ADFI and G:F were calculated every 2 wks. Pigs were scanned via ultrasound at 98.9 +/- 1.1 kg for 10^{th} rib backfat (BF) and loin eye area (LEA). One barrow and one gilt per pen were slaughtered (ave wt 108.4 +/- 1.4 kg) and metacarpals III and IV collected for bone ash determination. Data were analyzed using the GLM procedures of SAS (1999). The models included the effects of trial, dietary treatment, replicate and all pertinent interactions. There were no significant trial*treatment interactions. During both the early and late phases, pigs fed diet 3 had greater (P < 0.05) ADG than pigs fed diet 2. Pigs fed diet 3 also had greater (P < 0.05) daily feed intake during the late phase than pigs fed diet 2. Feed efficiency was not affected by dietary treatments. Loin eye area was greater for pigs fed Trt 1 (P < 0.05) than those fed Trt 2 and 3, or pigs fed Trt 3 (P < 0.05). Pigs fed Trt 1 tended to have greater (P = .072) ash content than pigs fed Trt 2 and 3. Removing P during the finishing phase did not alter BF, but reduced LEA and feed intake compared with animals fed a diet with supplemental P. Pigs fed supplemental phytase had improved ADG compared with non-supplemented pigs, but had lower LEA than control pigs.

Key Words: Pigs, Phytase, Finishing