T99 Differential expression of mitochondrial and extra-mitochondrial proteins in heart of low and high feed efficient broilers within a single male line. N. Tinsely*[1], M. Iqbal[1], N. R. Pumford[1], K. Lassiter[1], C. Ojano-Dirain[1], J. P. Higgins[1], W. Bottje[1], T. Wing[2], and M. Cooper[2], [1]*Department of Poultry Science, Center of Excellence for Poultry Science, University of Arkansas, Fayetteville,* [2]*Cobb-Vantress, Inc., Siloam Spring, AR.*

The objectives of this study were to determine the relationships of low or high feed efficiency (FE) with expression of mitochondrial and extra-mitochondrial proteins, and protein oxidation in heart muscle. Tissue homogenate was prepared from hearts of broilers with High (0.80 ± 0.01, n = 7) and Low FE (0.62 ± 0.02, n = 5). The levels of specific electron transport chain (ETC) immunoreactive proteins and protein oxidation (carbonyls) were analyzed using Western blots with a chemiluminescence detection system. A proteome from the pooled heart muscle homogenate in each group was obtained using two-dimensional electrophoresis (2DE). The intensity of each protein spot was quantified using Image Master 2D software. The expression of six mitochondrial proteins [CII 70 S (Complex II), ISP, cyt b, cyt c1 (Complex III), COX II (Complex IV)] and adenine nucleotide translocator 1 (ANT1) were higher in low FE heart mitochondria, but one protein [NAD6C (Complex I)] was higher in high FE birds, and there were no differences between groups in the expression of 18 other respiratory chain proteins. The levels of protein carbonyl (protein oxidation) were higher in the heart of low compared to high FE broilers. 2DE revealed several proteins that were differentially expressed between the low and high FE groups. These findings of differential expression of some mitochondrial and extramitochondrial proteins in Low FE tissues are similar to a recent report in breast muscle (Iqbal et al., 2004, Poult. Sci. 83:474-484) and may either be due to inherent genetic differences or represent a compensatory response to overcome the increased protein oxidation in low FE birds. Funded in part by USDA-NRI grant (#2001-03443).

Key Words: Feed Efficiency, Mitochondrial Proteins, Protein Oxidation

T100 Differential expression of mitochondrial and extra-mitochondrial proteins in lymphocytes of low and high feed efficient broilers within a single male line. K. Lassiter*[1], M. Iqbal[1], N. R. Pumford[1], C. Ojano-Dirain[1], N. Tinsley[1], W. Bottje[1], T. Wing[2], and M. Cooper[2], [1]*Department of Poultry Science, Center of Excellence for Poultry Science, University of Arkansas, Fayetteville,* [2]*Cobb-Vantress, Inc., Siloam Springs, AR.*

The objectives of this study were to establish relationships of a) mitochondrial and extramitochondrial protein expression, and b) protein oxidation (carbonyls) in lymphocytes with feed efficiency (FE) in broilers. Lymphocytes were isolated from a single line of male broilers with low (0.48 ± 0.02, n=8) and high (0.68 ± 0.01, n=7) FE. Protein bands were separated by electrophoresis (10% SDS-PAGE) and stained with Coomasie Blue. The band intensities were scanned with an Agfa (Arcus II) scanner and quantified using Scion software. Mitochondrial proteins, and oxidized protein (carbonyls) were analyzed using Western blots with chemiluminescence detection. A proteome from the pooled lymphocyte homogenate in each group was obtained using two-dimensional electrophoresis (2DE). The intensity of each protein spot was quantitated using Image Master 2D software. The expression of three mitochondrial proteins (core I, cyt c 1 [Complex III], and ATPase [Complex V]) were higher and lower for one protein (CII 30 [Complex II]) in High FE compared to Low FE lymphocytes, but there were no differences between groups for six other proteins. 2DE analysis showed a difference in the expression of several other proteins between groups. The levels of protein carbonyl were higher in High compared to Low FE birds. These results extend our reports in several tissues including breast muscle (Iqbal et al., 2004, Poult. Sci. 83:474-484) indicating that differences in protein expression may be involved in the phenotypic expression of feed efficiency. Supported in part by USDA-NRI grant (#2001-03443).

Key Words: Feed Efficiency, Lymphocytes, Mitochondrial Proteins

T101 Steady-state levels of mitochondrial phosphoproteins in broilers with and without pulmonary hypertension syndrome. C. R. Cisar*[1], J. M. Balog[1], J. O. Lay Jr.[2], N. B. Anthony[2], and A. M. Donoghue[1], [1]*Poultry Production & Product Safety, ARS, USDA, Fayetteville, AR,* [2]*University of Arkansas, Fayetteville.*

Pulmonary hypertension syndrome (PHS), also known as ascites syndrome, is a metabolic disease associated with the rapid growth rate of modern broilers. PHS symptoms include chronically elevated pulmonary blood pressure leading to right ventricular hypertrophy and eventually heart failure. Broilers resistant to PHS have elevated levels of several mitochondrial electron transport chain proteins in their right ventricles. Phosphorylation of mitochondrial proteins regulates respiratory activity and other mitochondrial functions. Therefore, we examined steady-state levels of mitochondrial phosphoproteins in broilers with and without PHS. Mitochondria were prepared from right ventricle cardiac muscle and mitochondrial proteins were separated by two-dimensional gel electrophoresis. Gels were stained with Pro-Q Diamond®, a fluorescent phosphoprotein-specific stain, and images were acquired using a laser scanner. Twenty putative phosphoproteins were detected. Four of these proteins appeared to be less phosphorylated in broilers without PHS than in broilers with PHS. Two proteins appeared to be more phosphorylated in broilers without PHS than in broilers with PHS. We are currently attempting to identify these six phosphoproteins from their peptide mass fingerprints as determined by MALDI-TOF mass spectrometry.

Key Words: Pulmonary Hypertension Syndrome, Mitochondria, Phosphoprotein

T102 Aorta pulse wave velocity is reduced by intravenous injections of L-arginine in female, but not male, white leghorn chickens. C. A. Ruiz-Feria*[1] and H. Nishimura[2], [1]*McGill University, Ste. Anne de Bellevue, QC, Canada,* [2]*University of Tennessee, Memphis.*

Chickens (males more so than females) have higher blood pressure that most mammals and develop neointimal lesions in the distal abdominal aorta (AbA). We found that L-arginine (Arg)reduced neointima plaque size in chicks (6-10 wks), but increased plaque size in older birds (12-16 wks), presumably due to endothelial damage in older birds. We evaluated the effect of an i.v. injection of Arg (160 mg/kg BW) on pulse wave velocity (PWV, m/s), mean arterial pressure (MAP, mm Hg) and pulse pressure (PP, systolic-diastolic BP, mm Hg) in 13-wk-old anesthetized male (n = 7) and female birds (n = 9). A Mikrotip® catheter with two intravascular pressure transducers was advanced to the AbA and pressure waves electronically recorded. PWV was calculated as the distance between the two transducers divided by the time delay at the foot of a pulse wave recorded at the two transducers. Data was analyzed using a one way repeated measure ANOVA, and significance declared at $P < 0.05$. PWV, MAP, and PP were not different between male and female chickens before Arg injection (control). In females, PWV increased 30 s after Arg (11.4 ± 0.9 Vs 13.2 ± 0.8), returned to control levels between 60 and 90 s, was lower than control levels after 120 s (8.0 ± 0.7), and returned to control levels after 11 min. PWV in males did not change with Arg. In females, MAP was lower 120 sec after Arg (87.5 ± 4.9) compared with control levels (100.0 ± 5.3), and remained lower up to 15 min after Arg. Again, no change was observed in males. PWV and MAP were lower in females than in males from 120 sec through 11 min after Arg. PP increased in both male (31.4 ± 3.1 and 43.5 ± 4.6, before and 120 s after Arg, respectively) and female (26.1 ± 2.4 and 43.3 ± 2.6, in the same order) chickens, but in males PP returned to control levels after 11 min whereas in females PP remained higher up to 15 min after Arg. Age-matched male and female chickens show a different response in aortic elasticity parameters to Arg, and this may be related to differences in endothelial integrity and capacity to synthesize nitric oxide.

Key Words: Pulse Wave Velocity, L-Arginine, Nitric Oxide

T103 Pulmonary and systemic hemodynamic responses to prostacyclin in broilers. R. F. Wideman* and M. E. Chapman, *University of Arkansas, Fayetteville*.

The vasodilator prostacyclin (PGI_2) reduces the resistance to pulmonary blood flow and attenuates pulmonary hypertension in mammals, however no information is available regarding the responsiveness of the avian pulmonary vasculature to PGI_2. Accordingly, in three experiments we evaluated the pulmonary vascular responses to PGI_2 in male broilers. In Experiment 1, infusing PGI_2 (10 μg/min) into clinically healthy broilers did not reduce their pulmonary vascular resistance (PVR) but did reduce the pulmonary arterial pressure (PAP) by lowering the cardiac output (CO). Within 4 min after stopping the PGI_2 infusion, the CO and PAP returned to pre-infusion levels. In Experiment 2, the responses to PGI_2 were evaluated after arachidonic acid (AA) had been infused to pre-constrict the pulmonary vasculature. The AA infusion (400 ug/min) consistently triggered dramatic, sustained pulmonary vasoconstriction (increased PVR) and pulmonary hypertension (increased PAP). Concurrent PGI_2 infusions did not reduce the PVR, but did reduce the PAP by lowering the CO. After the PGI_2 infusion was terminated, the PAP and CO returned to their previous (hypertensive) levels attributable to the ongoing AA infusion. In Experiment 3, PGI_2 was infused into clinically healthy (PAP $\leq$24 mmHg) or sub-clinically hypertensive (PAP $\geq$27 mmHg) broilers. Throughout this experiment broilers in the hypertensive group had higher PAP values than broilers in the healthy group. The PGI_2 infusion reduced the PAP in both groups, but did not reduce the PVR. Instead, the pulmonary hypotension in response to PGI_2 infusion was associated with a reduction in CO in both groups. In each of these experiments PGI_2 reduced the PAP by reducing the CO rather than by reducing the PVR. There was no evidence that PGI_2 acts as an effective pulmonary vasodilator in broilers regardless of whether their pulmonary vasculature was apparently normal (clinically healthy), had been pharmacologically pre-constricted (AA infusion), or initially exhibited the vasoconstriction that is typical of the pathogenesis of pulmonary hypertension syndrome in broilers.

Key Words: Broilers, Prostacyclin, Hemodynamics

T104 Pulmonary hypertensive responses of broilers to lipopolysaccharide: evaluation of source and dose, and impact of pre-existing pulmonary hypertension and cellulose micro-particle selection. M. E. Chapman*, W. Wang, and R. F. Wideman, *University of Arkansas, Fayetteville*.

Previous studies show that bacterial lipopolysaccharide (LPS) triggers pulmonary vasoconstriction leading to pulmonary hypertension (PHS) in broilers. The lungs of broilers are constantly challenged with LPS that can trigger thromboxane A_2- and serotonin-mediated pulmonary vasoconstriction. Among broilers from a single genetic line, some individuals respond to LPS with large increases in pulmonary arterial pressure (PAP), whereas others fail to exhibit a response to the same supra-maximal dose of LPS. It is possible that broilers are more likely to exhibit pulmonary hypertension when LPS elicits the production of more vasoconstrictors than vasodilators such as nitric oxide. In the present study we evaluated the impact of a variety of factors on the magnitude of the PAP response of male broilers to LPS, including: a) The role of the initial PAP (Low vs. High initial PAP); b) The source of the LPS (S. typhimurium vs. E. coli); c) The dose of LPS (0.02, 0.1, and 0.5 mg/kg BW); and d) The role of micro-particle selection for improved pulmonary vascular capacity (Cellulose survivors vs. Saline controls). Broilers with a low initial PAP did not differ in their pulmonary hypertensive response to LPS when compared with broilers having a high initial PAP. LPS from S. typhimurium elicited pulmonary hypertensive responses similar to those elicited by E. coli LPS. The results revealed that a dose of 0.1mg/KgBW LPS elicits a maximal pulmonary hypertensive response in broilers, and broilers selected for a robust pulmonary vascular capacity by micro-particle injection did not differ in their pulmonary hypertensive response to LPS when compared with saline injected controls. This confirms that the variable pulmonary hypertensive responses among broilers cannot be attributed to the source or dosage of LPS, or to differences in the baseline PAP or micro-particle selection prior to injecting LPS. This is consistent with the hypothesis that innate rather than acquired variability influences the chemical mediators released during the inflammatory cascade.

Key Words: Ascites, Vasoconstriction, LPS

T105 Effect of selection of sires and dams for growth or egg production on embryonic cardiac physiology. V. L. Christensen*, D. T. Ort, J. L. Grimes, and M. J. Wineland, *College of Agriculture & Life Sciences, North Carolina State University, Raleigh*.

Poult cardiac tissue may appear normal at hatching, yet ventricular hypertrophy can increase the size of the heart and confers a round shape called spontaneous cardiomyopathy (SCM). The SCM might result from selection for economically important traits. The hypothesis was proposed that lines selected for increased egg production (Egg) or body weight (F) might affect embryo cardiac physiology. Lines of turkeys selected for either trait as well as their randombred control populations (R1 and R2, respectively) were studied. At sexual maturity, hens were assigned randomly to pens by line. Half of each line was mated to males of the same line whereas the remaining half was mated to males of the appropriate randombred strain in reciprocal crosses. Progeny resulting from the crosses were observed for heart rate and weight, cardiac glycogen and lactate concentrations and creatine kinase (CK) and lactate dehydrogenase (LDH) activities that are indicators of cardiac insufficiency. Data were analyzed comparing dam and sire effects of the Egg only with the R1 and the F only with R2. In the Egg/R1 comparison, Egg dams increased embryo heart rate compared to R1 and also depressed embryo heart weight compared to R1 dams. Sire effects were negligible. Tissue oxygen debt was indicated in poults from R1 dams and Egg sires at hatching. F sires and dams compared to R2 decreased embryonic heart rates and increased heart weights, but when observed relative to body mass, F sires increased heart weights. Selection for growth in dams decreased tissue oxygen debt as indicated by CK. Thus, selection for egg production has increased heart rates and depressed weights relative to body mass. F embryo tissue oxygen debt and reduced heart rates were also evident. Selection for growth also decreased poult tissue oxygen debt for 3 days posthatching. In conclusion, the data indicate that selection for both egg production and growth have affected embryo cardiac physiology divergently and may play roles in the increased incidence of SCM in turkeys.

Key Words: Turkey, Embryo, Heart

T106 Effect of excess dietary vitamin D supplementation as a management adjustment to reduce the effect of heat stress on three varieties of laying hens. D. J. Franco*, L. Robeson, and M. M. Beck, *University of Nebraska, Lincoln*.

Thirty-two hens of each strain (Brown, W98, W36) were randomly selected and allowed to acclimatize for two weeks at 22C. Sixteen birds from each strain received a commercial layer diet plus 22,000 IU/Kg feed of vitamin D_3; the rest of the birds were fed the commercial layer diet. Birds were exposed to heat stress (HS) at 35C for two weeks with two additional weeks at 22C to recover. Production parameters (PP), mortality rates (MR) and reproductive hormone levels (RHL) were measured for each phase. Acid base status (AB) and intestinal calcium uptake rate (CaT) data were obtained from samples collected before and during HS exposure. The data for PP and RHL were analyzed using the SAS program 1999 version 8.0 as a repeated measure ANOVA in a 3x3x2 factorial experiment with a level of significance of 0.1. The data for CaT, and AB were analyzed in a 3x2x2 factorial experiment with a level of significance of 0.05. Differences among means were obtained based on LSD test. The results showed less decrease in egg production (EP) during HS in all the varieties given the extra vitamin D_3 than birds receiving the regular diet. The rest of PP were negatively affected by HS with small differences among strains and diet. Blood pH and PO_2 levels increased, with a reduction in PCO_2 and HCO_3 levels in the three strains during HS. LH and estrogen blood levels were significantly lower during HS for the three strains, and progesterone levels showed a large increase after HS exposure. CaT was enhanced by vitamin D_3 during HS with the W36 and the W98 showing the higher rate compared to the Browns. Based on these results we can conclude that the addition of excess dietary vitamin D_3 in the diet did not reduce the effect of HS on egg weight, eggshell quality and other PP. However, the addition of this vitamin in the diet allowed the birds to maintain high EP and CaT. Even though vitamin D_3 improved CaT, utilization of calcium was not increased, likely because of the alkalosis noted, which was not affected by vitamin D_3.

Key Words: Laying Hens, Heat Stress, Vitamin D_3

T107 Heat stress supresses 3β-hydroxysteroid dehydrogenase activity differentially by strain in granulosa cells of laying hens. H. Taira* and M. M. Beck, *University of Nebraska, Lincoln*.

Heat stress (HS) is known to disrupt egg production and to suppress circulating progesterone (P_4), luteinizing hormone (LH), and estrogen. It has been shown that strain differences exist in response to HS (Franco et al., 2002, 2003). HS suppresses secretion of P_4 by granulosa cells (GC) in vitro, even when stimulated by addition of LH (Novero et al., 1990), and decreases activity of 3β-hydroxysteroid dehydrogenase (3β-HSD) in GC of laying hens (Alodan, 2001) and in testis of Japanese quail (Taira et al., 2003). In this study, two experiments were conducted to determine the effects of HS on estrogen receptor-α (ERα) localization and 3β-HSD activity. Comparisons were made among 3 types of Hy-Line hens (W-36, W-98 and Brown), with birds of each strain divided into 3 groups: thermoneutral (22C, 50% RH; TN), acute (A) HS (36C, 50% RH; 24h), and chronic (C) HS (36C, 50% RH; 2wk) conditions. Effect of HS on ERα staining in kidney, duodenum and shell gland by immunocytochemistry was inconclusive. Activity of 3β-HSD was determined by incubating GC in the presence of pregnenolone and nitroblue tetrazolium. To determine percentage of 3β-HSD active cells, a total of 200 cells per bird was counted. 3βHSD activity was suppressed by both AHS or CHS; AHS reduced 3β-HSD by 42.6% ($P<0.01$) and 36.2% ($P<0.01$), respectively compared to TN. Under CHS, 3β-HSD activity was lower in GC from Brown birds compared to W-98 ($P<0.01$); activity in cells from W-36 birds was intermediate. In addition, in Brown hens, CHS suppressed 3β-HSD to a greater extent than did AHS ($P<0.01$). The results of the 3β-HSD study suggest a possible mechanism by which the strain effects of HS may be mediated; earlier studies have shown that W-98 hens maintain production significantly better than either W-36 or Browns and that W-36 are always intermediate in response between W-98 and Browns. Because P_4 is the main ovarian ovulatory/oviposition hormone in birds, decreased activity of 3β-HSD by HS, particularly since it is differentially suppressed by strain, may be a logical point at which to investigate genomic aspects of the response.

Key Words: 3β-HSD, Granulosa Cells, Heat Stress

T108 Duration of follicular development increases with advancing duration of the reproductive period in broiler breeder hens. H.-K. Liu* and W. L. Bacon, *The Ohio State University, Wooster*.

Settable egg production of broiler breeder hens is increased by feed restriction, but declines as the duration of the reproductive period increases. We hypothesized that the decrease in egg production with advancing duration of the reproductive period is associated with a longer duration of follicular development. The objective of the current study was to document the duration of ovarian follicular development early (Early, at 3 to 6 wk of egg production) and late (Late, after egg production had declined by about 25% at 19 to 31 wk of egg production) in broiler hens that were full-fed during both growth and reproductive periods (FF), restricted-fed during growth but full-fed after photostimulation (RF), or restricted fed during both growth and reproduction periods (RR). Photostimulation was at 23 wk of age. Sudan IV (R; 100 mg/hen/d) and Sudan Black B (B; 50 mg/hen/d) were suspended in canola oil and 1.0 mL day fed alternatively to measure the duration of follicular development. The total egg production rate (74-84% and 52-56%, respectively) was not different between feeding treatments within Early and Late hens. The duration of ovarian follicular development (8.7-9.1 d and 9.8-10.2 d, respectively) was not different between feeding treatments within Early and Late hens. Early hens in each feeding treatment had a higher ($P<0.01$) total egg production rate but shorter ($P<0.005$) duration of follicular development in comparison to Late hens. We concluded that longer duration of ovarian follicular development in Late hens was associated with lower total egg production rate in broiler breeder hens.

Key Words: Follicle, Broiler, Feed Restriction

T109 Bone characteristics of laying hens as a function of age, diet and strain. J. Wardell*, N. Heywood, and M. M. Beck, *University of Nebraska, Lincoln*.

Many factors have been investigated, but none have been shown to function alone in the etiology of osteoporosis in the hen. The occurrence of medullary bone as the labile calcium reserve for shell formation complicates the picture. Trabecular bone is found in the middle of the ends of the long bones and in the vertebrae and contributes more to stress resistance. The partitioning of bone between medullary and trabecular components is of interest. Comparisons between younger (first egg) and older birds (70-72 wk) indicate that medullary bone formation and remodeling occur partly at the expense of trabecular bone (Whitehead, 1991). In this study, two strains of Hy-Line birds, W36 and W98, were started as chicks receiving NRC requirement diets or a diet supplemented with HyD®. Chicks were fed to achieve target body weight or 15% greater or less than target. Bones were sampled at 20 and 60 wk of age. The right tibia from two birds in each treatment was removed, cleaned, decalcified, cut into eight pieces (two halves of the proximal head, four pieces of the shaft, two halves of the distal end), embedded in paraffin, sectioned at 7μm, and stained with hematoxylin and eosin. Two slides per bird were used for histological examination and measurement of cortical bone diameter and percent trabecular bone. Cortical diameter (CD) was measured at one location on both sides of the bone; percent trabecular bone (%TB) was obtained by dividing TB area by total bone area. Measurements were made using Scion Image analysis program. Data were analyzed using SAS. Comparisons were age (A), strain (S), body weight (BW), and diet (D). For cortical bone, there were effects of age, SxBW, SxD, and AxSxBWxD. At 60 wk, CD was less than at 20 wk ($P=0.0001$). At 20 wk, addition of HyD® enhanced CD in W36 birds but not in W98; at 60 wk, there was no effect of D but W98 birds had greater CD than W36 birds ($P=0.0175$). For %TB, there were effects of A, S, BW, D, AxBW, SxBW, AxSxBW, SxD, BWxD, and AxSxBWxD. Considering the most complex interaction, it appeared that S, BW and A contributed most to %TB, and that there was relatively little effect of D.

Key Words: Osteoporosis, Trabecular Bone, Cortical Bone

T110 Yolk-related gene transcription by estrogen in the liver of Japanese quail. A. M. Hanafy[1,2], T. Sasanami[1], and M. Mori*[1], [1]*Shizuoka University, Shizouka, Japan,* [2]*United Graduate School of Agricultural Science, Gifu University, Gifu, Japan*.

The avian liver is known as a target tissue for estrogens. In the previous study, we have demonstrated that diethylstilbestrol stimulates the expression of ZP1, perivitelline membrane glycoprotein, in the liver of immature male quail, and ZP1 gene has been shown to be a sensitive biomarker for estrogenic compounds. In the present study, we compared the sensitivity of transcription of ZP1 with those of vitellogenin II (VTG II) and very-low-density apolipoprotein II (apoVLDL). Immature male quail were treated with single intraperitoneal injection of estrogenic compounds dissolved in corn oil. Total RNA extracted from liver was reverse-transcribed with oligo (dT) primers. The cDNA was subjected to real-time PCR performed with the gene-specific sets of primers (ZP1: AB061520, VTG II: AF499027, apoVLDL: S82591, GAPDH: Z19086) and TaqMan probes. Cycling conditions were 10 min at 95°C followed by 40 cycles of 15 sec at 95°C and 1 min at 60°C. The standard curves were almost liner between 10^9 to 10^5copies for all genes. Levels of mRNA of each gene were expressed in relation to house-keeping GAPDH mRNA levels. Expression of ZP1, VTG II, and apoVLDL genes are highly specific for mature female, and no significant expression was observed in the mature and immature male quail. Of the three female-specific genes measured, apoVLDL was the most sensitive for estrogenic compounds. Administration of ethinyl estradiol or diethylstilbestrol at 1 μg per 100 g body weight caused more than a ten-fold increase in the mRNAs. All three genes were induced by ethinyl estradiol or diethylstilbestrol at 100 μg per 100 g body weight, although the increase in the mRNA was prominent only one day after the administration. The present study shows that effects of estrogenic compounds vary in the induction of female-specific gene transcription, and apoVLDL was more responsive to the estrogenic compounds than ZP1 and VTG II as a biomarker gene. These results suggest that the expression of avian female-specific genes may be differentially regulated.

Key Words: Liver, Estrogen, Vitellogenesis

T111 Identification of endocrine phenotypes of chicken pituitary cells expressing the vasotocin VT2 receptor subtype. A. Jurkevich*[1], L. R. Berghman[2], L. E. Cornett[3], and W. J. Kuenzel[1], [1]*Department of Poultry Science, University of Arkansas, Fayetteville,* [2]*Poultry Science Department, Texas A&M University, College Station,* [3]*University of Arkansas for Medical Sciences, Little Rock.*

Hypothalamic neurohormones of vasotocin/vasopressin family have long been shown influencing the endocrine and behavioral response to stress. The regulatory effects of these hormones are achieved by modulating neuronal processes in the brain and by controlling hormone release form the pituitary gland. Effects of vasotocin (VT) on pituitary hormones in the domestic fowl are mediated, at least in part, by the VT2 vasotocin receptor highly expressed in the pituitary. Immunohistochemical localization of VT2 receptor-expressing cells revealed that they are prevalent in the cephalic lobe of the anterior pituitary. The objective of this study was to identify the endocrine phenotypes of chicken pituitary cells containing immunoreactive VT2 receptor protein using a dual labeling immunohistochemical approach. The obtained results confirmed our previous finding that, at the subcellular level, the VT2 receptor is predominantly associated with the cell membrane. Dual immunofluorescent labeling demonstrated that virtually all corticotrophs are VT2 receptor-immunoreactive. The immunoreactive VT2 receptor was also found in a relatively small subpopulation of lactotrophs. Preliminary assays did not reveal extracellular membrane-associated VT2 receptor on gonadotrophs (visualized with the antibody to chicken LH β-subunit) and somatotrophs; however these findings are being verified on a larger amount of samples. Our results provide the first morphological evidence supporting the hypothesis that VT could be the primary ACTH secretagogue in birds acting directly on corticotrophs. Moreover, the data obtained suggest that the known stimulatory effects of VT on prolactin release in poultry species are likely to be mediated primarily by VT2 and perhaps other VT receptor subtypes.

Key Words: Vasotocin, Pituitary Gland, Stress

T112 Characterization and expression of the avian ghrelin gene. M. Richards*, S. Poch, and J. McMurtry, *USDA, ARS, Growth Biology Laboratory, Beltsville, MD.*

Ghrelin (GHR), a peptide hormone produced by the stomach in mammals, stimulates growth hormone (GH) release and food intake. Recently, GHR was identified and characterized in chicken proventriculus and shown to stimulate GH release but inhibit feed intake. The purpose of this work was to identify and characterize the GHR gene in Leghorn (LC) and broiler (BC) chickens and in turkeys (T). Using molecular cloning techniques we have sequenced cDNAs corresponding to LC and T GHR mRNAs. A total of 844 (LC) or 869 (T) bases including the complete coding regions (CDS), and the 5'- and 3'-untranslated regions (UTRs) were determined. Nucleotide sequence (CDS) predicted a 116 amino acid precursor protein for both LC and T that demonstrated complete conservation of an N-terminal active core (GSSF) including a serine (position 3) known to be a modification (acylation) site important for GHR bioactivity. Additional nucleotide sequence was found in the 5-UTRs of both LC and T cDNAs that was not present in BC. The T GHR gene, sequenced from genomic DNA templates, contained 5 exons and 4 introns, a structure similar to mammalian and chicken GHR genes. GHR was highly expressed in proventriculus with much lower levels of expression in other tissues such as pancreas, brain and intestine. RT-PCR was used to quantify GHR gene expression relative to 18S rRNA in 3 wk-old male BC. GHR expression in proventriculus increased in response to fasting but did not decline with subsequent refeeding. Plasma GHR, determined by RIA, did not change significantly in response to fasting or refeeding and did not reflect changes in proventriculus GHR gene expression. GHR expression declined in pancreas after a 48 hr fast and increased upon refeeding. Expression of the gene encoding the receptor for GHR (GH secretagogue receptor, GHS-R) was detected in pancreas possibly suggesting autocrine/paracrine effects. These results offer new insights into the avian GHR gene and the potential role of GHR in regulating feed intake and energy balance in poultry.

Key Words: Ghrelin, Gene Expression, Feed Intake

T113 The effect of dosimetric levels of dietary L-carnitine on semen traits of White Leghorns. Wei Zhai*[1], S. L. Neuman[2], C. D. McDaniel[3], M. A. Latour[1], and P. Y. Hester[1], [1]*Purdue University, West Lafayette, IN,* [2]*Astra Zeneca, Raleigh, NC,* [3]*Mississippi State University, Mississippi State.*

Feeding 500 ppm of dietary L-carnitine to young and aging White Leghorns for 5 wk improved sperm concentration and reduced sperm lipid peroxidation during the last half of supplementation (Neuman et al., 2002, Poultry Sci. 81:495). The current study examined the effect of feeding dosimetric levels of L-carnitine on semen traits of White Leghorns. An 8-wk trial was conducted with 48 White Leghorn roosters, 46 to 54 wk of age. Feed was formulated to contain to 0, 125, 250, and 500 mg of carnitine/kg of feed, but analyzed values were higher (3, 167, 357, and 674, respectively). Diets were fed ad libitum to 12 birds/treatment with the semen of two roosters pooled per rep per treatment to form 6 experimental units per treatment. Data were analyzed using ANOVA with repeated measurements using the mixed model procedure of SAS. Dietary carnitine did not affect feed consumption, body weight, and sperm viability. Sperm concentration of roosters fed 125 mg/kg of carnitine was significantly higher than the controls ($P < 0.05$). Dietary carnitine effects on semen volume varied over time (dietary treatment $\times$ age interaction, $P < 0.003$). Semen volume increased in roosters consuming 125 mg/kg at 6 wk as compared to 2 wk post treatment ($P < 0.002$). An increase in semen volume was noted in roosters consuming 500 mg/kg of carnitine at 6 ($P < 0.0002$), 7 ($P < 0.02$), and 8 ($P < 0.007$) wk when compared to 2 wk post-treatment. Circulating levels of free and total carnitine were elevated in roosters consuming 500 mg of carnitine/kg of feed as compared to controls ($P < 0.04$). It is concluded that White Leghorns consuming 125 mg/kg of carnitine for 8 weeks as compared to controls showed an increase in sperm concentration without affecting sperm viability or having a deleterious effect on semen volume. Research supported by the U. S. Poultry and Egg Association & Lonza Group Ltd.

Key Words: L-carnitine, Semen Traits, Sperm Concentration

T114 Time course of thyroid hormone replacement and lipogenic enzyme gene expression in broilers. R. W. Rosebrough*, B. A. Russell, S. M. Poch, and M. P. Richards, *Growth Biology Laboratory, ARS, USDA, Beltsville, MD.*

The purpose of this experiment was to determine the possible relationship between certain indices of lipid metabolism and specific gene expression in chickens fed methimazole to produce a kind of artificial hypothyroidism. Male, broiler chickens growing from 7 to 28 days of age were fed diets containing 18% crude protein and either 0 or 1 g methimazole per kg of diet. At 28 days, these two groups were further subdivided into groups receiving 18% crude protein diets containing either 0 or 1 mg triiodothyronine (T3) per kg. Birds were sampled at 28, 30 and 33 days. Measurements taken included in vitro lipogenesis (IVL), malic enzyme (ME) activity, the expression of the genes for ME, fatty acid synthase (FAS) and acetyl coenzyme carboxylase (ACC). Hypothyroidism decreased IVL and ME at 28 d of age; however, T3 supplementation for 2 d restored both IVL and ME. Paradoxically, continuing T3 replenishment for an additional 3d decreased IVL without affecting ME activity. In contrast, supplemental T3 decreased IVL in euthyroid birds, regardless of the dosing interval, but had no effect on ME activity. Methimazole decreased plasma T3, T4, and uric acid. Although methimazole decreased ME gene expression, there was only a transitory relationship between enzyme activity and gene expression when plasma T3 was replenished with exogenous T3. These data may help to explain some of the apparent reported dichotomies in lipid metabolism elicited by changes in the thyroid state of animals. In addition, most metabolic changes in response to feeding T3 occurred within 2 to 5 d, suggesting that changes in intermediary metabolism preceded morphological changes. In conclusion, the thyroid state of the animal will determine responses to exogenous T3.

Key Words: Lipogenesis, Thyroid, Gene

T115 Evaluation of oils and polyethylene glycol as vehicles in a nine-day chick embryo xenobiotic assay. M. E. Persia*, T. Carro, and W. W. Saylor, *University of Delaware, Newark*.

The goal of this research was to evaluate procedures and vehicles that could be used in a xenobiotic metabolism assay utilizing an embryonating egg model. In four experiments, fertile Single-Comb White Leghorn eggs were incubated for 6 d and then injected with various oils or polyethylene glycol (PEG) to determine their effects on egg, embryo and shell weight and embryo mortality. Each treatment consisted of 10 replicate eggs sampled and analyzed on d 15. In Experiment 1, 500 μl of corn, soybean, sesame and mineral oil were compared to control eggs that did not receive injections. None of the oil treatments significantly affected egg, embryo or shell weight or embryo mortality. Autoclaved samples of corn and soybean oil were compared with non-autoclaved oil samples and controls in Experiment 2. No significant differences in weight or mortality were noted among the autoclaved, non-autoclaved and control eggs. In Experiment 3, PEG was injected into eggs at levels of 0, 250, 500, 750 and 1000 μl. Embryos in all eggs that received 1000 μl of PEG died by 9 d post injection. A linear increase in embryo mortality was seen with increasing PEG injection. Injection of eggs with 500 μl of PEG resulted in 50% survivability and will be utilized in subsequent experiments. Soybean oil and PEG were both tested at 500 μl in Experiment 4. Soybean oil did not differ from control values for any weight or embryo mortality, but 500 μl of PEG significantly reduced embryo weight and increased mortality (50%). The results of these experiments indicate that injecting fertile eggs with corn, soybean, sesame and mineral oils resulted in similar egg, embryo and shell weights and embryo mortality as control eggs, but PEG reduced embryo weight and increases mortality. Autoclaving oil samples did not alter egg, embryo or shell weight or affect mortality.

Key Words: Xenobiotic Metabolism, Embryo Model, Vehicles

T116 Bone density measurements in broiler chickens. A. Mitchell*[1] and R. Angel[2], [1] *USDA-ARS,* [2] *University of Maryland, College Park*.

The bone status of chickens can be measured by numerous techniques, providing a variety of parameters for evaluation. By the use of dual energy X-ray absorptiometry (DXA) it is possible to measure bone mineral content (BMC, g) and bone mineral density (BMD, g/cm^2) either in vivo or on excised bones. The purpose of this study was to evaluate the relationships between DXA measured BMC and BMD in broiler chickens in vivo and in the excised bones. In two separate studies, a total of 2244 broiler birds of diverse nutritional background were scanned by DXA. In one study consisting of 1542 birds (1922 to 4091 g body weight, BWT) DXA scans were analyzed for total body and in vivo tibia BMC and BMD. In a second study of 702 birds (470 to 4110 g BWT) DXA scans were analyzed for total body BMC and BMD, and the excised tibia and femur were scanned separately and analyzed for BMC and BMD. Linear regression analysis was used to compare total body to bone measurements. For tibiae analyzed in vivo, the correlation (R^2) between tibia BMC and total body BMC was 0.52 and the R^2 for BMD was 0.29. For excised bones, the R^2 with total body BMC was 0.65 for the tibiae and 0.56 for the femur, and the R^2 with total body BMD was 0.21 for the tibiae and 0.17 for the femur. The correlation between femur and tibia BMD was 0.58. Polynomial regression analysis was used to compare relationships among total body BWT, BMD and BMC. Combining the results of both studies, for the relationship between BWT and BMC the R^2 was 0.66, for BWT and BMD the R^2 was 0.13, and for BMC and BMD the R^2 was 0.58. In conclusion, the results of these studies indicate that in vivo and in vitro measurements of tibia BMC and BMD correlate similarly with total body measurements of BMC and BMD. In general, measurements of BMD, in the total body or in the individual bone showed low correlation with other parameters.

Key Words: Bone Mineral, Chickens, DXA

T117 Differentiation of chicken adipocytes *in vitro*. R. M. Morales*[1], M. S. Mayes[2], T. W. Huiatt[2], and C. G. Scanes[1,2], [1] *Program in Toxicology, Iowa State University, Ames,* [2] *Department of Animal Science, Iowa State University, Ames*.

Control of excess amounts of adipose tissue are important to the animal industry. It is well established that the differentiation of mammalian adipocyte is modulated hormonally. There is, however, little information on the hormonal control of differentiation of the chicken adipocyte. The present study examined the conditions necessary for differentiation of chicken preadipocytes in cell culture and, hence, the hormonal control of differentiation. Computer-assisted image analysis was used to analyze size of adipocytes and adipocyte clusters and the amount of lipid accumulation. Chicken preadipocytes cultured in serum free media for 3 and 5 days failed to show complete differentiation (e.g. clusters of at least five cells with abundant lipid droplets as stained with oil red O). Complete differentiation was allowed in the medium containing a combination of 1ug/ml insulin, 25ug/ml transferrin, 25ng/ml sodium selenite (ITS). The presence of triolein in the ITS media increased lipid accumulation as indicated by increased cell cross-sectional area of lipid droplets. The cocktail ITS also increased the size of clusters with <5 cells. A model to examine differentiation of chicken preadipocytes has been developed. The present data support the fundamental control of the differentiation of avian preadipocytes being similar to that in mammals.

Key Words: Chicken, Adipocyte, ITS

T118 Enhancing enterocyte enzymatic function by prebiotic feed supplementation. V. Davila[1], A. Diaz[2], A. Lopez-Mungia[3], M. P. Castaneda[1], and G. M. Nava*[1,4], [1] *CEIEPA, FMVZ-UNAM, Mexico City, Mexico,* [2] *Departamento de Nutricion Animal, FMVZ-UNAM, Mexico City, Mexico,* [3] *Instituto De Biotecnologia-UNAM, Mexico,* [4] *University of Arkansas, Fayetteville*.

Manipulation of the intestinal microflora with prebiotic products may affect the enterocyte physiology given that the intestinal mucosa maturation depends on beneficial bacterial colonization in neonatal animals. The objective of the present study was to evaluate the effect of inulin prebiotic administration on enterocyte enzymatic activity (maltase and sucrose) in the intestinal tract of neonatal broiler chickens. Arbor Acres chicks (1-day of hatch) were fed during 15 days with a standard diet (SD) or a standard diet supplemented with 0.2% of inulin prebiotic (SDI). At day 7 of age, intestinal samples (n=5) were taken and processed. Briefly, samples of duodenum and ileum were collected and intestinal mucosa was scraped in order to collect the enterocytes. Enterocyte homogenates (EH) were physically removed and brush border membrane vesicles (BBMV) were isolated. Enzymatic activity assays were performed using the EH and BBMV. Additionally, values of nucleotidase were analyzed. Inulin diet supplementation significantly ($p<0.05$) increased the concentration of intestinal maltase in the BBMV isolated from ileum at 7 days of age when compared to control diet (SD, 0.445 $\pm$ 0.04 and SDI, 0.608 $\pm$ 002 μg of disaccharide metabolized/mg of protein/minute). Additionally, inulin supplementation during 14 days significantly ($p<0.05$) increased the concentration of intestinal sucrose in the BBVM isolated from duodenum when compared to control diet (SD, 0.555 $\pm$ 0.053 and SDI, 0.816 $\pm$ 0.027 μg of disaccharide metabolized/mg of protein/minute). The low concentration of nucleotidase observed during this assay indicates that BBMV were isolated properly. Prebiotics stimulate the establishment of beneficial bacterial populations in the intestinal tract. It is possible that the early development of beneficial communities enhance the development of intestinal functions. The data from the present study support this hypothesis given that inulin prebiotic supplementation enhanced intestinal enzymatic activity in the BBMV section of the enterocyte.

Key Words: Prebiotic, Enterocyte, Inulin

T119 Developmental changes of plasma insulin, glucagon, IGF-I, IGF-II, thyroid hormones, and glucose concentration in chick embryo. J. Lu*[1], J. P. McMurtry[2], and C. N. Coon[1], [1] *University of Arkansas, Fayetteville,* [2] *USDA-ARS Growth Biology Laboratory, USDA-ARS, Beltsville, MD*.

Hatching eggs from a flock of Cobb 500 females were collected and incubated. Plasma samples were obtained daily from day 10 of embryogenesis (10E) through hatch. The developmental changes of plasma insulin, glucagon, insulin-like grow factor-I and -II (IGF-I, IGF-II), triiodothyronine (T3), thyroxine (T4), and glucose concentration were investigated at different ages of the chick embryos. A significant increase in plasma insulin levels was observed from 130pg/ml at 10E to 389pg/ml at 16E. The insulin levels showed two peaks during embryogenesis (460pg/ml at 17E; 488pg/ml at hatch). Plasma glucagon levels increased from 59pg/ml at 10E to 428pg/ml at hatch; however, there was a decrease

from 12E to 19E with the lowest level of 60pg/ml at 15E. The molar ratios of insulin to glucagon (I/G) from 14E to 17E ranged from 1.8 to 2.2 which were significantly greater than normal I/G ratio in the post-absorptive state in avian species (1.2-1.7). The results indicate that insulin is an important promoter of chick embryo growth by anabolic drive to promote protein deposition especially during the embryonic rapid growth period. There also was a significant increase in plasma glucose from 13E to 18E suggesting glucose is an important regulator of protein anabolism in the chick embryo via suppression of amino acid oxidation. Plasma IGF-1 and IGF-II levels increased from 10E to 14E and then IGF-1 slowly decreased until hatch, while IGF-II remained constant. IGF-II levels were about 10 fold greater than IGF-1 suggesting IGF-II is an important functionary for chick embryonic development. Plasma T3 and T4 levels showed a significant increase during the third week of incubation and reached a peak at 19-20 E. The findings are consistent with previous research showing a sharp rise in T3 and T4 activities when the embryo switches to lung respiration.

Key Words: Chick Embryo, Hormones, Developmental Change

T120 Developmental changes of hepatic enzyme activities involved in methionine metabolism for chick embryo. J. Lu* and C. N. Coon, *University of Arkansas*.

Developmental changes in hepatic methionine adenosyltransferase (EC 2.5.1.6, MAT), cystathionine β-synthase (EC 4.2.1.22, CBS), cystathionase (EC 4.4.1.1, C-ase) , 5-methyltetrahydrofolate-homocysteine S-methyltransferase (EC.2.1.1.13, MFM) and glycine N-methyltransferase (E.C.2.1.1.20 , GNMT) were determined in 11, 14, 17 and 19 day-old chick embryos and hatched chicks. Enzyme activities were expressed as nmole of product formed /min/mg of protein. The methionine-activating enzyme (MAT) activity was present at low level at day 11 of incubation but increased 8-fold to a maximum at day 19 and remained 6-fold at hatch. The MAT enzyme developmental pattern resembles the pattern observed in the rat fetus suggesting additional SAM (S-adenosyl-Methionine, sole product of MAT enzyme) is needed for rapid growth in the development of the chick embryo. Hepatic CBS activities did not change much during embryogenesis except the activity decreased to a low at day 19. High levels of hepatic C-ase were detected from day 11 through hatch. The developmental pattern of C-ase resembles the pattern observed in the rat fetus, although C-ase activity is absent from human fetal liver. Cystine can not be produced in premature human infants but chick embryos do not have this limitation. Hepatic MFMT activity was higher during embryogenesis than that at hatch suggesting remethylation of homocysteine through MFMT may be a priority for the chick embryo. Previous studies indicate MFMT activity in rat liver decreases with age. MFMT is one of two methionine-conserving enzymes in broilers and layers. Chick embryos have a higher GNMT activity at day 14 than that at day 17, 19 and hatch suggesting the enzyme is more important for regulating the intrahepatic ratio of SAM to SAH (S-adenosyl-homocystine) during the middle of the incubation period. GNMT is considered the main enzyme for maintaining methyl group availability for more than 200 methylation reactions in mammals and birds.

Key Words: Chick Embryo, Enzyme Activities, Methionine Metabolism

Physiology and Endocrinology: Nutrition, Growth and Stress

T121 Effects of injecting transition cows with low doses of bovine somatotropin (bST) on milk yield, IGF-1, glucose and hepatic gene expression of gluconeogenic enzymes. M. Liboni*, M. S. Gulay, L. Badinga, M. J. Hayen, T. I. Belloso, C. J. Wilcox, and H. H. Head, *Department of Animal Sciences, University of Florida, Gainesville*.

Objectives were to evaluate effects of supplemental bST (0.4 mL, 10.2 mg/d, POSILAC®) during the prepartum and/or early postpartum periods on milk yield (MY), plasma concentrations of IGF-1 and glucose, and on steady state mRNA concentrations of hepatic gluconeogenic enzymes pyruvate carboxylase (PC) and phosphoenolpyruvate carboxykinase (PEPCK). Multiparous Holstein cows were assigned randomly to a 2x2 factorial arrangement of treatments (TRT) to give four groups (I=no bST, n=26; II=bST postpartum, n=25; III=bST prepartum, n=27; IV= bST prepartum and postpartum, n=25). Biweekly injections of bST were in left or right ischiorectal fossa beginning -21 d from expected calving through 70 DIM. Blood samples were collected from all cows thrice weekly throughout experiment, and liver biopsies were taken from 9 cows per TRT at -21, +2, +14 and +28 d from calving. Supplemental bST increased daily MY in all bST groups through 28 DIM ($P<0.019$) compared to TRT I; increases were 13.9, 12.7, and 26.6% for TRT II, III and IV, respectively. From 28 through 70 DIM, only TRT IV cows had greater daily milk yield than controls (18.7%, $P<0.019$). For IGF-1, significant increases were detected only during the postpartum period for cows on TRT II and IV (0-70 DIM, $P<0.045$); TRT group means were 107.0, 120.0, 102.8, and 132.6 ng/mL, respectively. No differences were detected in glucose concentrations ($P=0.52$). Hepatic levels of PC mRNA did not differ among TRT ($P=0.47$); PEPCK mRNA differed ($P<0.01$); mean levels were 165.9, 168.2, 162.7 and 161.2 arbitrary units, respectively. Results indicated that supplemental bST caused increased MY and postpartum plasma IGF-1 concentrations, but did not affect plasma glucose, or hepatic PC mRNA. Despite a small, but significant down-regulation of PEPCK mRNA for cows in TRT III and IV, these cows produced more milk than controls, and maintained similar plasma glucose concentrations.

Key Words: bST, Transition Period, Liver Gluconeogenesis

T122 Effect of insulin and growth hormone administration to mature miniature Brahman cattle on circulating concentrations of metabolic hormones and metabolites. C. C. Chase, Jr.*[1], D. G. Riley[1], T. H. Elsasser[2], L. J. Spicer[3], M. C. Lucy[4], S. W. Coleman[1], and T. A. Olson[5], [1]*USDA, ARS, Brooksville, FL,* [2]*USDA, ARS, Beltsville, MD,* [3]*Oklahoma State University, Stillwater,* [4]*University of Missouri, Columbia,* [5]*University of Florida, Gainesville*.

The objective of this study was to determine the effect of administration of GH, insulin (INS), and GH plus INS to mature miniature Brahman cows (n = 6; 9.7 ± 2.06 yr; 391 ± 48.6 kg) and bulls (n = 8; 9.4 ± 2.00 yr; 441 ± 54.0 kg) on plasma concentrations of metabolic hormones (GH, INS, IGF-I) and metabolites (glucose, urea nitrogen [PUN]). We hypothesized that IGF-I secretion could be enhanced by concomitant administration of exogenous GH and INS, but not by either hormone alone. Animals were allotted to a modified crossover design that included four treatments: control (CON), GH, insulin (INS), and GH+INS. At the start of the study, one-half of the animals were administered GH (POSILAC; 14-d slow release) and the other one-half served as controls (CON) for 7 d. Beginning on day 8 and for 7 d, insulin (Novolin L) was administered (0.125 IU/kg BW) twice daily (0700 and 1900) to all animals; hence the INS and GH+INS treatments. Animals were rested for 14 d and then were switched to the other treatment combination. Blood samples were collected at 12-h intervals during the study. Sex affected ($P < 0.05$) plasma concentrations of metabolic hormones but not ($P > 0.15$) blood metabolites. Compared to CON, GH treatment increased ($P < 0.01$) mean plasma concentrations of GH (11.1 vs 15.7 ± 0.94 ng/mL), INS (0.48 vs 1.00 ± 0.081 ng/mL), IGF-I (191.3 vs 319.3 ± 29.59 ng/mL), and glucose (73.9 vs 83.4 ± 2.12 mg/dL), but decreased ($P < 0.05$) PUN (14.2 vs 11.5 ± 0.75 mg/dL). Compared to INS, GH+INS treatment increased ($P < 0.05$) mean plasma concentrations of INS (0.71 vs 0.96 ± 0.081 ng/mL), IGF-I (228.7 vs 392.3 ± 29.74 ng/mL) and glucose (48.1 vs 66.70 ± 2.12 mg/dL), decreased ($P < 0.01$) PUN (13.6 vs 10.4 ± 0.76 mg/dL), and did not affect GH (13.5 vs 12.7 ± 0.95 ng/mL). In the miniature Brahman model, using mature animals, both GH and GH+INS treatments dramatically increased circulating concentrations of IGF-I.

Key Words: Miniature Cattle, GH, IGF-I