

Variable	NEG	RCT-500	RCT-750	RCT-1000	NG-500	POS
Analyzed phytase, units/kg	140	479	734	787	516	111
Body wt., g	1800c	1920b	1965ab	1962ab	1945b	2026a
Feed intake, g	2708c	2894b	2936b	2941b	2964b	2137a
F/G	1.506bc	1.508bc	1.497c	1.502bc	1.526ab	1.545a
Mortality, %	3.1	7.5	4.4	5.0	5.6	3.8
Tibia ash, % dry matter	49.7c	51.6b	51.6b	52.3ab	51.8b	53.1a

NEG, negative control; RCT, Ronozyme PTM CT; NG, Natuphos[®] G; POS, positive control; abcP<.05

Key Words: Ronozyme PTM CT, Natuphos[®] G, Floorpen broiler study

382 Reassessment of Trypsin Inhibitor Activity in Guar Meal. S.R. Conner*, A.L. Cartwright, and C.A. Bailey, Texas Agricultural Experiment Station.

Guar (*Cyamopsis tetragonoloba*) is a drought resistant annual summer legume grown primarily for its galatomannan polysaccharide gum. In the separation of the gum containing endosperm, two distinct fractions are obtained. The first fraction from the splitting of the guar bean has higher germ content and the second fraction removes the hull from the remaining gum containing endosperm. A third fraction results from a combination of the two fractions. Although guar meal can be used as a source of vegetable proteins in animal feeds, a major impediment to protein digestibility is commonly attributed to a trypsin inhibitor. Defatted raw soybeans, dehulled soybean meal and three guar fractions evolved from various stages of guar gum production were assayed for trypsin inhibitor activity using the American Oil Chemists' Society official method Ba 12-75. Results indicated that the trypsin inhibitor

activity of the raw guar beans, guar splits (gum), and the three guar meal fractions were substantially less than the trypsin inhibitor activity of heat treated dehulled soybean meal. Trypsin inhibitor activity is likely not the significant impediment to protein utilization in guar meal.

Key Words: Guar, Guar meal, Trypsin inhibitor

383 Immobilization of Keratinase-Streptavidin Fusion Protein for Keratinolysis. J.C.H. Shih* and J.-J. Wang, North Carolina State University, Raleigh, NC USA.

Keratinase, produced from *Bacillus licheniformis* PWD-1, is capable of hydrolyzing chicken feather keratin. Immobilized keratinase has many potential applications, including the conversion of ground feathers to soluble protein, peptides and amino acids. A biotechnology for keratinase immobilization has been developed. Fusion genes of keratinase (KER) and streptavidin (STP) were genetically constructed. Transformed *B. subtilis* and *E. coli* expressed and produced the bifunctional fusion protein, KER-STP. Isolation and immobilization were achieved by mixing a biotinylated solid matrix with the *B. subtilis* medium or the *E. coli* lysate. Since the fusion protein was produced intracellularly in the inclusion body of *E. coli*, the extraction and renaturation from the cell lysate were laborious and consequently caused a low yield. *B. subtilis* produced and secreted the fusion protein that can be readily immobilized from the medium. The properties of immobilized keratinase-streptavidin fusion protein were characterized. Heat stability, durability, and pH tolerance were found greatly improved. Kinetic parameters of immobilized keratinase, including V_{max}, K_m, and k_{cat} toward the peptide substrate were also determined and compared with the free enzyme. Hydrolysis of three different substrates, insoluble feather keratin, soluble α-casein and bovine serum albumin (BSA) were carried out and analyzed by HPLC. It is interesting to note that keratinolysis by both immobilized and free keratinase produces a characteristic soluble protein with a molecular weight of 18 kDa. (Patent application pending)

Key Words: feather keratin, keratinase, enzyme immobilization

PSA Physiology

384 Performance and Thermo Tolerance of Broilers as Affected by Genotype and Ambient Temperature. H. A. Al-Batshan* and E. O. Hussein, King Saud University, Riyadh, Saudi Arabia.

This experiment was conducted to evaluate the effects of genotype (GT) and ambient temperature (AT) on subsequent performance and body core temperature (CT) of broiler chicks. A factorial arrangement of two GT (Hubbard and ISA) and two rearing AT (either under 33 C and then AT was reduced to 23 C by wk 4, or under constant AT of 33 C throughout the trial) was used in this study. Diurnal CT was monitored continually (2 min intervals) for 4 days using a biotelemetric system at six weeks of age. Results showed that weight gain and feed intake were significantly increased but feed:gain ratio was significantly decreased for Hubbard chicks compared to those of ISA chicks. High AT significantly reduced weight gain and feed intake. There was significant GT by AT interaction for weight gain, revealing that Hubbard chicks were more affected by high AT. Ambient temperature and GT significantly affected CT. Moreover, core body temperature of ISA chicks was significantly higher at the moderate ambient temperature but was significantly lower at the high ambient temperature than those of Hubbard chicks, which resulted in significant GT by AT interaction. The results of this study indicated that chicks with higher growth potential are more susceptible to heat stress.

Key Words: Broilers genotype, Ambient temperature, Diurnal body temperature

385 Changes in Growth and Function of Chick Small Intestine Epithelium Due to Heat Exposure Conditioning. Zehava Uni*¹, Orit Gal-Garber¹, Assaf Geyra¹, David Sklan¹, and Shlomo Yahav², ¹Faculty of Agriculture, Department of Animal Science, The Hebrew University of Jerusalem, Israel, ²Institute of Animal Sciences, ARO, The Vulcani Center, Bet-Dagan, Israel.

The effect of exposure to heat at 3 d of age on small intestine functionality and development was assayed by measuring villus size, proliferating enterocytes, and brush-border membrane enzyme expression and activity. Results showed that thermal conditioning caused an immediate effect characterized by lowered triiodothyron (T3) level, reduced feed intake, and depressed enterocyte proliferation and BBM enzyme activity. A second series of effects, observed 48 h posttreatment, was characterized by elevated T3, increased feed intake, increased enterocyte proliferation, and higher expression and activity of BBM enzymes. The association between ambient temperature, feed intake, growth rate, and plasma T3 levels was reflected in the structure and function of the intestinal tract. The results suggest that thermal conditioning at an early age influences T3 concentrations, which in turn alters the intestinal capacity to proliferate, grow and digest. However, these experiments were not able to separate between the effects due to feed intake from those due to thermal conditioning. These changes modulate the intestinal tract for compensatory growth commencing 48 h postthermal treatment.

Key Words: Heat, Small intestine, Chicks

386 Origin of thermal-load induced adaptations in intestinal hexose absorption: heat stress or reduced food intake? M.A. Mitchell*¹, R.R. Hunter¹, M. Moreto², C. Garriga², M. Mitjans², C. Amat², and J.M. Planas², ¹Roslin Institute, Roslin, Midlothian, UK, ²University of Barcelona, Barcelona, Spain.

Previous studies demonstrated increased expression of SGLT-1 transporters in the brush border membranes of enterocytes from chronically

heat stressed chickens. This adaptation may be attributable to the prolonged heat stress per se and/or to the reduced food intake associated with chronic exposure to elevated thermal loads. The present study attempts to dissociate the effects on intestinal function and structure of heat stress and inanition by means of paired feeding. Groups of 8, four week old, female broiler chickens were exposed to thermoneutral (TN; 20C, 50% Relative Humidity) or heat stress (HS; 30C, 70% R. H.) conditions or TN with paired feeding (PF) for 14 days. Food intake was determined daily. Pair fed birds received the amount of food consumed by the heat stress group on the previous day. Body weights and deep body temperatures were monitored throughout the experimental period. Blood samples were taken for hormone analysis (T4, T3, and glucagon). At the end of exposure birds were killed and jejunal segments removed for tissue morphology and assessment of the abundance of the glucose transporters SGLT-1 (brush border membrane - phloridzin binding) and GLUT-2 (basolateral membrane - cytochalasin B binding) and characterization of the transport kinetics of alpha-methyl-D-glucoside in brush border or basolateral membrane vesicles. HS increased phloridzin binding by 54% ($p < 0.05$) and V_{max} for the hexose transport by 55% ($p < 0.05$) in the brush border preparation with no effect upon K_m for active transport or diffusive uptake (K_d). PF had no effect upon binding or transport kinetics. No influence of either treatment (HS or PF) upon cytochalasin binding or hexose transport kinetics was observed at the basolateral membrane. Plasma T3 was reduced in heat stress but T4 and glucagon were unaffected by the treatments. The adaptations in intestinal absorptive function observed during prolonged exposure to elevated thermal loads result directly from the heat stress and not from reduced food intake. The physiological signals mediating this response may include alterations in endocrine and paracrine activity.

Key Words: intestinal absorption, heat stress, chicken

387 Assessment of densitometry to measure bone mineral content and density in live birds as a tool for monitoring osteoporosis in laying hens. M.A. Schreiweis*¹, J.I. Orban², M.C. Ledur³, and P.Y. Hester¹, ¹Purdue University, W. Lafayette, IN, ²Southern University, Shreveport, LA, ³Embrapa Swine and Poultry Research Center, Concordia, SC, Brazil.

Osteoporosis, or loss of structural bone, may be induced by a high rate of lay, demand for calcium during eggshell formation, and limited exercise of birds in caged system. The objective of the current study was to determine the accuracy of bone densitometry as a non-invasive tool for monitoring bone integrity in an egg-laying strain of birds. Bone mineral content (BMC) and bone mineral density (BMD) of the right leg (tibia and fibula) and wing (humerus) bones were determined in 10 adult White Leghorn hens, 75 wk of age, using a Norland pDexa X-ray bone densitometer (Model # 476D014). The scans of respective bones were conducted in live, unanesthetized birds. Following live scans, birds were euthanized, and the humerus and tibia/fibula bones were excised and scanned in the same orientation as the previous live bird scan. The medullary tibia from live bone scans had significantly greater BMD ($0.249 \pm 0.022 \text{ g/cm}^2$) than the pneumatic humerus ($0.166 \pm 0.025 \text{ g/cm}^2$, $P < 0.0001$). The BMD and BMC of excised bones were strongly correlated with live scans ($r = 0.97$ and 0.99 , respectively). Variation in BMD among live scans of the 10 hens occurred as indicated by CV of 9% for the tibia and 15% for the humerus. In a subsequent experiment, live scans of 35 pedigree Hy-Line White Leghorn pullets, 15 wk of age, showed CV among birds of 7% for BMD in both the humerus and tibia. The BMD for tibia and humerus were 0.132 ± 0.010 and $0.110 \pm 0.008 \text{ g/cm}^2$, respectively ($P < 0.0001$). In Hy-Line birds 17 wk of age, the BMD and BMC of 10 excised bones did not differ from respective bones of live scans performed in situ ($r = 0.91$ and 0.99 , respectively). These preliminary data suggest that variation derived from densitometric scans of live birds may serve as a non-invasive tool for genetically selecting against osteoporosis.

Key Words: Bone density, Laying hen, Osteoporosis

388 Matrix metalloproteinases in turkey bile. N. C. Rath*, G. R. Huff, W. E. Huff, and J. M. Balog, *PPPSR/ARS/USDA, Fayetteville, AR*.

Matrix-metalloproteinases (MMPs), gelatinases, interstitial collagenases, stromelysins, and membrane-type MMPs, are endopeptidases

which breakdown extracellular matrix (ECM) components such as collagens and proteoglycans. The MMPs are involved in a variety of physiological functions such as tissue remodeling, angiogenesis, wound healing, and immune regulation. We found that turkey bile contains substantial gelatinase, collagenase, and stromelysin-like activities using gelatin, collagen, and casein zymography, and ³H labeled collagen degradation assays. The intact bile showed at least five major gelatinolytic bands corresponding to approximate MW 64 kDa, 60 kDa, 46 kDa, 40 kDa, and 36 kDa. Organomercurial compounds, p-aminophenylmercuric acetate and thimerosal, which facilitate transformation of proMMP to MMP, caused conversion of the 64 kDa band to the 60 kDa and the 46 kDa to the 40 kDa forms, respectively. The 64 kDa and the 60 kDa bands showed collagenolytic activities whereas the caseinolytic (stromelysin) activities appeared as diffuse bands corresponding to MW of approximately 60 kDa, 40 kDa, and 36 kDa. The bile enzymes showed both a time and a concentration dependent increase in the degradation of ³H-collagen which could be inhibited by EDTA, phenanthroline, and GM 6001 (the MMP inhibitors) but not by serine and cysteine protease inhibitors like phenylmethylsulfonyl fluoride and leupeptin. The 60 kDa and the 40 kDa gelatinase showed affinity adsorption to a gelatin-agarose matrix. The physiological functions of biliary MMPs are not clear. However, in most carnivorous and omnivorous birds there is a need to digest animal tissue proteins consisting of fair amounts of collagens and other ECM proteins in their native forms, therefore, MMPs may be involved in the digestion and denaturation of these proteins which may otherwise be resistant to conventional proteinases such as pepsin, trypsin, and chymotrypsin. These data provide evidence which suggests that biliary MMPs may be involved in the digestive process of birds.

Key Words: Turkey, Bile, Matrix metalloproteinases

389 Development of the indicator amino acid oxidation technique for measuring amino acid requirements in chickens. H. Y. Tabiri*, R. O. Ball, R. Bertolo, and D. R. Korver, *University Of Alberta, Edmonton, AB, Canada*.

The indicator amino acid oxidation (IAAO) technique is being adapted for use in chickens as a rapid and sensitive method to determine amino acid (AA) requirements. During IAAO, Phe oxidation decreases, inversely to the change in protein synthesis, as limiting AA intake increases from deficient to adequate. In IAAO, the plateau in breath ¹⁴CO₂ during continuous infusion of labeled phenylalanine (Phe) is used to measure Phe oxidation. Plateau is reached when the ¹⁴CO₂ recovered from breath becomes constant as verified by linear regression. Bicarbonate retention factor (BRF) must be determined to correct for ¹⁴CO₂ that is not recovered in the breath. Metabolic chambers were constructed and calibrated to ensure 100% recovery of ¹⁴CO₂ liberated from NaH¹⁴CO₃. A surgical procedure was established to ensure long-term patency of jugular catheters. Male broiler breeders were surgically implanted with venous catheters, placed in the chambers and infused with NaH¹⁴CO₃. Breath ¹⁴CO₂ was recovered; BRF was 13.12% of infused dose. For the determination of Phe oxidation a bolus of ¹⁴C-Phe ranging from 4.5 to 7 $\mu\text{Ci/kg}$ was used to determine the correct priming dose for the Phe pool. A constant infusion dose was maintained at $3.5 \mu\text{Ci/kg}^{-1}$ for ratios of continuous infusion to priming doses of 1.29, 1.57 and 2.00. At a ratio of 1.57, breath ¹⁴CO₂ attained a plateau between 30 and 60 min after initiation of continuous infusion and remained constant for the next 3 hours. Phe oxidation at plateau was found to be 4.66% (uncorrected for BRF) and 5.27 % (corrected for BRF) of the infused dose. The % Phe dose oxidized is sufficient to be sensitive to changes in AA intake, making it suitable for the determination of AA requirements in chickens using the IAAO technique. This is the first study to our knowledge to determine BRF and Phe oxidation, from primed, constant infusion rate methodology, in chickens. The methods established in the present study will be used to estimate AA requirements in poultry.

Key Words: Indicator Amino Acid Oxidation Technique, Amino Acid Requirements, Bicarbonate Retention Factor

390 Dietary protein regulates in vitro lipogenesis and lipogenic gene expression in broilers. R. W. Rosebrough*, S. M. Poch, B. A. Russell, and M. P. Richards, *ARS, Beltsville, MD*.

The purpose of this experiment was to determine the possible relationship between certain indices of lipid metabolism and gene expression in chickens fed graded levels of dietary crude protein. Male, broiler chickens growing from 7 to 28 days of age were fed diets containing 12, 21 or

30% protein ad libitum. In addition, another group of birds was feed on a regimen consisting of a daily change in the dietary protein level (12 or 30%). This latter group was further subdivided such that one-half of the birds received each level of protein on a particular day. Birds were sampled from 28 to 30 days of age. Measurements taken included *in vitro* lipogenesis (IVL), hepatic enzyme activities and gene expression of these enzymes and thyroid hormones. *In vitro* lipogenesis was determined by incubating liver explants for 2 h at 37 C in Hanks salts containing 25 mM HEPES and 10 mM [2-¹⁴C]acetate. *In vitro* lipogenesis and ME activity were inversely related ($P < 0.01$) to dietary crude protein levels (12 to 30%) and by acute changes from 12 to 30%. In contrast, ME, FAS and AcCBX message expressions were constant over a dietary protein range of 12 to 21%, but were decreased ($P < 0.01$) by a 30% protein diet (acute or chronic feeding). Increasing dietary crude protein increased ($P < 0.01$) the activities of both AAT and ICD. Results of the present study demonstrate a continued role for protein in the regulation of metabolism in the broiler chicken. It should be pointed out, however, that metabolic regulation at the gene level only occurs with very high levels of dietary protein.

Key Words: Lipogenesis, Gene expression, Metabolism

391 Metformin decreases feed intake and induces hypoglycemia in broiler chicken. C.M. Ashwell^{*1} and J.P. McMurtry¹, ¹Growth Biology Laboratory, USDA-ARS, Beltsville, MD.

The bi-guanide metformin is used to treat non-insulin dependent diabetes in obese patients. In addition to having anti hyperglycemic effects, metformin is also anorectic and reduces body weight. These studies were performed to determine if metformin possesses similar properties in chickens. Metformin HCL was administered to 14-day old broiler chickens at either 300 or 600 mg/kg/day in the drinking water for 10 days while monitoring body weight and feed intake. No changes in water intake were observed, while feed intake and daily gains were only significantly reduced by the 600 mg/kg dose. After oral administration of a single dose of 300 mg/kg metformin HCL, feed intake was significantly reduced ($p = 0.011$) by 4 hours and remained suppressed for greater than 24 hours relative to controls. Plasma hormones and metabolites (glucose, lactate, insulin, glucagon, IGF-I, IGF-II, uric acid, NEFA, triglycerides, and cholesterol) were monitored at 1, 2, 3, 6, and 24 hours post treatment. Significant and acute decreases in blood glucose, insulin, and triglycerides were observed at 3 hours post treatment as compared to controls. Opposing acute increases in glucagon, IGF-II, and NEFA levels were also observed at 3 hours, followed by an increase in uric acid 6 hours post treatment. These observations suggest that metformin induces metabolic changes in birds, similar to that observed in mammals. Metformin may be useful in glucose metabolism studies by inducing hypoglycemia, a condition rarely observed in birds

Key Words: Feed Intake , Hypoglycemia, Broiler

392 *In ovo* and post-hatch administration of peptide YY (PYY) does not affect growth and feed conversion in Cobb X Cobb broiler chickens. B.A. Coles, J. Croom*, J. Brake, and L.R. Daniel, North Carolina State University, Raleigh, NC USA.

In ovo PYY administration at e18 increased 7 d but not 21 d or 42 d BW and adjusted feed conversion ratios (AFCR) in Ross male x Cobb female broilers (Coles et al., 1999). The present study was conducted to investigate whether post-hatch administration of PYY prolonged the positive effects of *in ovo* PYY administration on growth and feed conversion. On e18, Cobb x Cobb eggs were randomly divided into two treatment groups and 100ml of either 1.025% saline (control) or 1.025% saline plus 600 µg PYY/kg egg weight was injected into the air space. At hatch, birds (n = 1280) were sexed, wing-banded and randomly placed in 64 pens within a grower house (16 pens/house quarter, 4 reps/quarter, 20 birds/pen). Water and feed was offered *ad libitum*. Birds and feed were weighed on a pen basis at placement and 1, 2, 3, and 6 wk. At 1 wk, birds were weighed and administered either saline (control) or saline plus 600 µg PYY/kg BW s.c. No further injections were given beyond week 1. Growth data were statistically analyzed with treatment, sex and pen position (house block quadrant) as main effects using the GLM procedure of PC-SAS. At the completion of the study, 8 males per treatment were euthanized and intestinal length, weight and jejunal glucose uptake measured. There was a significant PYY x sex interaction in BW at hatch. Control females were heavier than females from PYY treated

eggs at hatch ($p = .04$, 39.65 g vs 39.05 g, respectively). This difference was not present at 1, 2, 3, and 6 wk. Both total intestinal length and intestinal length adjusted for BW were greater in PYY treated males at 6 wk as compared to controls ($p = .004$, .446 vs .380 per g BW, respectively). These data support previous observations of PYY's hypertrophic effect on the intestines. They do not confirm earlier reports of PYY's ability to enhance intestinal glucose absorption or post-hatch growth in poultry. These data suggest major genotypic effects on PYY's biological activity in broilers.

Key Words: Peptide YY, Broilers, Growth

393 Mitochondrial Function and Feed Efficiency in Broilers. W. Bottje^{*1}, Z. Tang², M. Iqbal¹, D. Cawthon¹, T. Wing³, and M. Cooper³, ¹Dept. of Poultry Science, University of Arkansas, Fayetteville, AR 72701, ²Dept. of Veterinary Pathophysiology, South China Agricultural University, Guangzhou 510642, PRC, ³Cobb-Vantress Inc., Siloam Springs AR 72761..

The objective of this study was to determine relationships of mitochondrial function with feed efficiency (FE) in broiler males. Between 5 and 6 wk, FE was determined in 100 broiler breeder males. Birds with high (0.83 0.01, n = 7) and low (0.64 0.01, n = 7) FE were selected for mitochondrial studies. As feed intake did not differ ($P > 0.50$) between groups ($P > 0.50$), differences in FE were due to greater body weight gain ($P < 0.05$) in the high FE broilers. Mitochondria were isolated from individual birds by differential centrifugation. The respiratory control ratio (RCR, an index of respiratory chain coupling) was higher in breast and leg muscle mitochondria in the high FE group; due primarily to a lower state 4 (resting) respiration. Additionally, breast muscle mitochondrial hydrogen peroxide generation (an index of electron leak) was; a) higher in low FE breast muscle mitochondria, and b) magnified in low FE mitochondria by inhibition of electron transport by rotenone and antimycin A (at Complex I and III, respectively) indicating sites of electron leak from the respiratory chain. Complex I and II activities were higher in high FE than in low FE mitochondria. High FE mitochondria exhibited a much smaller ratio of Complex I to Complex II activities than did low FE mitochondria. Positive correlations were observed between feed efficiency and RCR and Complex activities. The results indicate that low FE was associated with a) lower RCR, b) higher electron leakage, and c) lower activities of respiratory chain Complex I and II activities compared to high FE mitochondria. The findings provide insight into cellular mechanisms associated with feed efficiency in broilers.

Key Words: Mitochondria, Feed Efficiency, Broilers

394 Cardiac energy metabolism slow and fast growing chickens. A.A. Olkowski* and H.L. Classen, University of Saskatchewan, Saskatoon, SK, Canada.

The present study compares the activity of cytosolic enzymes involved in energy synthesis, Creatine Kinase (CK) and Lactate Dehydrogenase (LDH), in left (LV) and right (RV) ventricular myocardium of fast growing broilers (susceptible to heart failure), and slow growing leghorns (resistant to heart disease). Within the category of broilers, comparisons are made between feed restricted, slow growing birds (low incidence of heart failure and ascites), fast growing birds fed ad libitum (high incidence of heart failure and ascites), and broilers with ascites. Overall, the activity profile of CK was lower ($P < 0.01$) in broilers in comparison to leghorns in both LV and RV, but the differences in the activity of the CK system among slow growing, fast growing, and ascitic broilers were only marginal. Total LDH activity was lower ($P < 0.01$) in slow growing broilers in comparison to leghorns. The total activity of LDH was 21, 15, and 9% higher in LV than in RV in leghorns, slow growing broilers, and fast growing broilers respectively, but not in ascitic broilers. There were no differences in total LDH activity in RV in fast growing and ascitic chickens in comparison to leghorns, but relative to leghorns the total LDH activity in LV was approximately 28% lower in slow growing broilers, and 20% lower in fast growing and ascitic broilers (both $P < 0.01$). This indicates that the metabolic demand for catalytic activity of LDH is higher in LV than in RV, and that broilers, in particular, may have insufficiency of LDH activity in LV. Considering that CK and LDH activities are generally lower in broilers in comparison to leghorns, it appears that the energy metabolism capacity may be insufficient in broilers. Inadequate activity of these enzymes may limit the ability of the cardiac muscle to increase its contractile performance during rapid

growth or in response to cold stress. From the present results it appears that, in comparison to leghorns (resistant to heart failure), fast growing broilers (susceptible to heart failure) in general have lower cardiac energy reserve.

Key Words: broiler, energy metabolism, heart failure and ascites

395 Investigation of Proton Conductance in Liver Mitochondria of Broilers with Pulmonary Hypertension Syndrome (PHS). D. Cawthon*, M. Iqbal, J. Brand, and W. Bottje, *Department of Poultry Science, Univ. of Arkansas, Fayetteville AR 72701.*

We have observed mitochondrial dysfunction associated with PHS in broilers that includes increased oxygen radical production. Proton leak or conductance (the futile cycling of protons across the inner mitochondrial membrane) can account for at least 20% of mitochondrial O₂ consumption. The objective of this study was to determine if proton conductance differs in Control and PHS liver mitochondria. Liver mitochondria were isolated from broilers with PHS and from healthy Controls. PHS mitochondria had a lower respiratory control ratio than did Controls. Membrane potential and O₂ consumption were determined simultaneously by monitoring the distribution of the lipophilic cation (triphenylmethylphosphonium [TPMP]) with an ion-sensitive electrode and a Clark electrode, respectively (see Brand, 1995. Ch. 3 Bioenergetics IRL Press, Oxford). Mitochondria were treated with nigericin to dissipate the H⁺ gradient to enable membrane potential to be determined without concomitant changes in H⁺ concentration across the membrane. Proton conductance was assessed by following changes in TPMP⁺ as state 4 respiration was slowed with sequential additions of malonate. The results indicated that at any particular high membrane potential, the PHS mitochondria respired more slowly than Controls. This is the classic demonstration of a lower proton conductance in PHS mitochondria. The lower proton conductance may account for the increased oxygen radical production we have previously observed in PHS mitochondria. These findings help define the nature of mitochondrial dysfunction in broilers with PHS. (Supported by USDA-NRI #99-02123 to W. Bottje.)

Key Words: Pulmonary Hypertension Syndrome, Liver Mitochondria, Proton Conductance

396 Tissue and Mitochondrial Antioxidant Enzyme Activities in Broilers with Pulmonary Hypertension Syndrome (PHS). M. Iqbal*, D. Cawthon, R. Wideman, Jr., and W. Bottje, *Department of Poultry Science, Univ. of Arkansas, Fayetteville, AR 72701.*

Oxidative stress plays a role in the etiology of PHS in broilers. The objectives of this study were to determine if antioxidant enzyme activities a) are altered by PHS and/or high dietary vitamin E (VE), and b) differ in broilers genetically selected (S) or not selected (NS) for PHS resistance. In these studies, broilers were raised in cold temperatures to induce PHS. In Exp. 1, broilers were fed diets with 15 and 100 IU α -tocopherol (VE) per kg. Enzyme activities were determined spectrophotometrically using established procedures that were adapted for microtiter plate analyses. In Exp. 1, PHS lung mitochondria exhibited oxidative stress (lower α -tocopherol, reduced glutathione [GSH] and higher oxidized [GSSG] to reduced GSH ratio) that was associated with higher GSH peroxidase (GSHPx) and lower GSH reductase (GSHRed) activities compared to Controls. Liver γ -glutamyl cysteine synthetase (gGCS), the rate limiting enzyme in GSH synthesis was unaffected by

PHS or high VE. In liver, high dietary VE lowered GSHPx but had no effect on GSHRed or gGCS activities. In Exp. 2, GSHPx activity was higher and GSHRed lower in lung mitochondria obtained from NS compared to S broilers. The lower GSHRed activity corresponded to a higher GSSG/GSH ratio. Whereas there was no differences in liver GSHPx and GSHRed activities, liver gGCS activity was lower in livers obtained from NS compared to S broilers. The results indicate that activities of antioxidant enzymes are altered by PHS, presumably in response to the oxidative stress present in these birds. Modulation of antioxidant activities by high VE was observed in liver tissue. Higher gGCS in the liver birds genetically resistant to PHS could be important in overall antioxidant status of these birds. (Supported by USDA-NRI #99-02123 to W. Bottje.)

Key Words: Pulmonary Hypertension Syndrome, Antioxidant Enzymes, Broilers

397 Effects of dietary sodium chloride, sodium sesquicarbonate, or ammonium chloride, in various combinations and levels, on ascites susceptibility of young broiler chickens in a cool environment at simulated high altitude (17% oxygen). R. G. Teeter*¹, J. H. Swartzlander¹, A. Beker¹, D. M. Hooge², and K. R. Cummings, ¹Oklahoma State University, Stillwater, OK, ²Hooge Consulting Service, Inc., Eagle Mountain, UT, ³Church & Dwight Company, Inc., Princeton, NJ.

Day old, vaccinated Cobb 500 chicks were evaluated in two metabolic chamber studies, 240 chicks per trial (24 chambers of 10 birds each), to 21 days old under moderate cool stress and 17% oxygen atmosphere (nitrogen administration). Ten practical corn-soy-meal blend diets were formulated to contain various levels of sodium chloride (NaCl) or sodium sesquicarbonate (SS; 30.4% Na) singly, or in combination, or ammonium chloride (AC; 66% Cl) and SS in place of NaCl. The SS has equimolar Na bicarbonate and Na carbonate. Analyzed Na levels were 0.19, 0.28, and 0.37% in combinations with Cl levels of 0.11, 0.25, and 0.39% (from previous regular pen trial, using same basal diet, in which 0.23% NaCl + 0.3% SS gave best broiler results with ionophore, coccidial challenge, and built-up litter; see Hooge *et al.*, 2001, IPF abstract 14, Atlanta). Dietary electrolyte balances, Na+K-Cl in mEq/100 g, ranged from 20.8 to 36.5. Significance was at P<0.05 using a completely randomized design. Body weights overall were below industry norms due to oxygen deficiency. The SS treatments without NaCl appeared deficient in Cl; for example, the 0.3% SS diet with 0.19% Na and 0.11% Cl was lower in body weight at wk 1 and 2 than all or most other treatments, but adapted somewhat by wk 3. Heaviest 3 wk body weight was with 0.46% NaCl and lightest was with 0.23% NaCl + 0.3% SS. No differences in feed conversion ratio were detected. Right ventricle weight and ascites heart index (right ventricle/total heart) were not affected, but ascites score (0=least to 3=most severe; P=0.054) at wk 1 was highest for 0.3% SS + 0.42% AC, with 0.23% NaCl intermediate, and 0.3% SS lowest. The 0.23% NaCl control had lowest oxygen consumption, heat production, and carbon dioxide exhaled, but third highest 3 wk weight. Hematocrit was negatively correlated ($r^2=-0.53$) with oxygen consumption or heat production. This confirmed a relationship previously found between lower oxygen consumption, lower heat production, higher hematocrit, and higher ascites score. Under simulated high altitude and moderate cool stress, young broilers had best 3 wk weight (541.7 g) and worst ascites score (0.74) with 0.28% Na and 0.39% Cl from 0.46% NaCl, which could be moderated to good weight (488.7 g) and ascites score (0.52) with 0.37% Na and 0.39% Cl from 0.46% NaCl + 0.3% SS.

Key Words: Ascites, Salt, Sodium sesquicarbonate

Nitrogen, Phosphorus, and Sulfur Interfaces Between Beef Cattle Production and the Environment

398 Federal environmental policy directions for animal agriculture. J.S. Jonker*, *AAAS Environmental Fellow - US EPA, Washington, DC USA.*

The animal agriculture industry has undergone dramatic changes in the past 20 years. The trend toward fewer but larger operations, coupled with greater emphasis on more intensive production methods and specialization, has concentrated more animal manure within some geographic areas. This increase in geographic concentration has led to an increasing concern over environmental impacts of animal agriculture. According to the 1998 *National Water Quality Inventory* which re-

ported on assessments for 32 percent of the U.S. waters, 27,751 river and stream miles and 99,936 lake acres were impacted by animal feeding operations. A recent USDA analysis of 1997 county-level manure production relative to crop uptake in the United States estimated 73 counties had excess manure N and 160 counties had excess manure P. The number of counties with excess manure nutrients has significantly increased since 1982. Some manure could be exported to cropland in other nearby counties, but manure bulk will limit the distance manure can be transported economically. For many of these counties with excess manure nutrients, alternative uses for excess manure will need to be ex-