chain reaction (RT-PCR). Viral RNA was extracted from the bursal tissue samples using Proteinase K digestion method. A 5 ug of the viral RNA was used for the synthesis of cDNA using RevertAid TM First Strand cDNA Synthesis Kit (MBI, Fermentas). The VP2 hypervariable region of the IBDV was amplified using specific primers. A single band of PCR product of the expected size was visualized on 1.5% agarose gel after ethidium bromide staining. Results indicated that PCR is a sensitive method for the rapid detection of IBDV.

Key Words: RT-PCR, IBDV, Pakistan

PSA-Physiology: Poultry Digestion and Metabolism

134 Compromised liver mitochondrial function and complex activity in low feed efficient broilers associated with higher oxidative stress and differential expression of proteins within a single male line. M. Iqbal*[1], N. R. Pumford[1], Z. X. Tang[1], K. Lassiter[1], W. Bottje[1], T. Wing[2], and M. Cooper[2], [1]*University of Arkansas, Fayetteville,* [2]*Cobb-Vantress, Inc., Siloam Springs, AR.*

Two experiments were carried out to determine the relationships of; a) mitochondrial function and activities of various complexes, b) production of reactive oxygen species (ROS) and its subsequent effect on protein oxidation, and c) protein expression in liver of male broilers with low and high feed efficiency (FE, n =5-8 per groups). Mitochondrial function and complex activities were measured polarographically and spectrophotometrically, respectively. Hydrogen peroxide (H_2O_2) was measured flourometrically, while oxidized protein (carbonyls) and immunoreactive mitochondrial proteins were analyzed using Western blots. Mitochondrial function (ETC coupling) and activities of all respiratory complexes (I, II, III, IV) were higher in high FE compared to low FE broilers. H_2O_2 and protein carbonyls were higher in the liver of low compared to high FE broilers. Whereas the expression of four immunoreactive proteins [NAD3 (Complex I), QPC (Complex III), COX II and COX IVb (Complex IV)] were higher in low FE liver mitochondria and two proteins [70S (Complex II) and alpha-ATPase (Complex V)] were higher in high FE birds, there were no differences between groups in the expression of 18 other respiratory chain proteins. SDS-PAGE revealed several proteins (ranging from 20 to 180 kDa) that were differentially expressed between the low and high FE groups. In conclusion, taken together with our previous findings in breast muscle (Iqbal et al., 2004, Poult. Sci. 83:474-484), the differential expression of certain mitochondrial proteins might be a compensatory response aimed to maintain the compromised respiratory chain activity and/or to overcome the increased protein oxidation in low FE birds. Funded in part by USDA-NRI grant (#2001-03443).

Key Words: Feed Efficiency, Mitochondrial Complex Activity, Oxidative Stress

135 Membrane potential and hydrogen peroxide production in duodenal mitochondria in broilers with low and high feed efficiency. C. Ojano-Dirain*[1], N. Tinsley[1], M. Iqbal[1], T. Wing[2], M. Cooper[2], and W. Bottje[1], [1]*University of Arkansas, Fayetteville,* [2]*Cobb Vantress Inc., Siloam Springs, AR.*

Increased hydrogen peroxide (H2O2) production was observed in duodenal mitochondria obtained from broilers with low feed efficiency (FE). As a decrease in mitochondrial membrane potential (MMP) due to stimulation of uncoupling of oxidative phosphorylation reduces reactive oxygen species (e.g., H2O2) production, this study was conducted to evaluate the effect of uncoupling on MMP and H2O2 production in duodenal mitochondria from broiler breeder males with low and high FE. Duodenal mitochondria were isolated from broilers with low (0.48 ± 0.02, n = 8) and high (0.68 ± 0.01, n = 7) FE. H2O2 production and membrane potential were measured fluorometrically using dichlorofluorescin and tetramethylrhodamine methyl ester probe, respectively, in the presence of different levels (0, 200, 400, 600, 800 and 1,000 nM) of an uncoupler, carbonylcyanide p-trifluoromethoxyphenylhydrazone (FCCP). The MMP was higher ($P < 0.05$) in the high FE mitochondria at 0 to 600 nM FCCP. A decrease in MMP was observed at 600 and 800 nM FCCP for the low and high FE groups, respectively. H2O2 generation was higher in the low FE mitochondria at all FCCP levels except at 200 nM. Adding 200 to 800 nM FCCP caused a decrease in H2O2 production in low but not in high FE mitochondria. The results indicate that FCCP-induced uncoupling lowered H2O2 production in low FE but not in high FE duodenal mitochondria and indicate that mitochondrial membrane potential may influence reactive oxygen species production in broilers with low FE. Supported in part by USDA-NRI #2001-03443.

Key Words: Feed Efficiency, Duodenal Mitochondria, Membrane Potential and H2O2 Production

136 Evidence of protein oxidation in mitochondrial respiratory complexes in broilers with low and high feed efficiency. J. P. Higgins*[1], N. R. Pumford[1], M. Iqbal[1], T. Wing[1], M. Cooper[1], and W. G. Bottje[1], [1]*Department of Poultry Science, University of Arkansas, Fayetteville,* [2]*Cobb-Vantress, Inc., Siloam Springs, AR.*

We have observed increased mitochondrial radical production, increased protein oxidation and lower activities of respiratory chain complexes in broilers with low feed efficiency (FE) compared to broiler breeders with high FE. As radical-mediated protein damage could cause the reduction in complex activities, the purpose of this experiment was to determine if there is evidence of protein oxidation that is specifically associated with respiratory chain complexes. Mitochondria were isolated by differential centrifugation from breast muscle obtained from broiler breeder males identified as either low (0.62 ± 0.01, n = 8) or high (0.80 ± 0.01, n = 8) FE. Respiratory chain complexes were separated using a non-denaturing gradient polyacrylamide blue native gel. Protein carbonyl levels were measured with an immunochemical assay in which proteins are reacted with 2,4 dinitrophenylhydrazine to produce a corresponding hydrazone. The hydrazone is then detected by Western analysis using anti-dinitrophenyl antisera. Preliminary results indicate that Low FE mitochondria exhibited higher levels of protein carbonyls (indicating increased protein oxidation) in Complex III compared to levels in High FE mitochondria. These findings suggest that increased protein oxidation may be responsible in part for the lower respiratory chain activities observed in broilers with Low FE. Further research is being conducted to ascertain protein oxidation in other mitochondrial complexes and other mitochondrial proteins in relation to the phenotypic expression of feed efficiency in broilers. Supported in part by USDA-NRI (#2001-03443).

Key Words: Feed Efficiency, Protein Oxidation, Blue Native Gel Electrophoresis

137 Dietary phytates may noncompetitively inhibit intestinal mucosa phytase in broiler chicks. E. M. Onyango*[1], E. K. Asem[2], J. S. Sands[3], and O. Adeola[1], [1]*Department of Animal Sciences, Purdue University, West Lafayette, IN,* [2]*Basic Medical Sciences, Purdue University, West Lafayette, IN,* [3]*Danisco Animal Nutrition, Marlborough, Wiltshire, UK.*

The role of dietary phytate in the regulation of intestinal mucosa phytase was investigated in broiler chicks. One hundred and eight 7-d-old male broiler chicks were grouped by weight into six blocks of 3 cages with six birds per cage. Three chemically defined diets were randomly assigned to cages within each block. The three diets were a chemically defined casein diet; chemically defined casein diet plus sodium phytate (20 g/kg diet), and a chemically defined casein diet plus sodium phytate (20 g/kg diet) and microbial phytase (1000 U/kg diet). The chicks were fed experimental diets from 8 to 22 d of age. At the end of the study, chicks were weighed, killed, and duodenal mucosa and left tibiae removed. Tibiae were defatted and ashed overnight at 600 °C. Phytase activity in brush border vesicles prepared from the duodenal mucosa was determined by measuring the amount of inorganic phosphorus released from sodium phytate. Addition of phytate to the chemically defined diet reduced ($P < 0.05$) weight gain, feed intake, feed efficiency and percentage ash. However, addition of microbial phytase fully restored ($P < 0.05$) feed efficiency but weight gain was only partially restored ($P < 0.05$). Addition of phytate to the chemically defined diet reduced (P

< 0.05) the V_{max} of the intestinal mucosa phytase but the K_m of the enzyme was not affected. By comparison, the addition of microbial phytase to the diet did not restore the V_{max}. Phytate may noncompetitively inhibit intestinal mucosa phytase.

Key Words: Broiler Chick, Intestinal Phytase, Microbial Phytase

138 Absorption of 2-hydroxy-4(methylthio) butanoic acid (HMTBA) (5-50mM) is equal to or greater than d,l-methionine (DLM) uptake in chicken intestinal slices. J. D. Richards*, C. Atwell, and J. J. Dibner, *Novus International, Inc., St. Louis, MO.*

The rates of intestinal HMTBA vs. DLM absorption have been controversial. DLM is absorbed mostly by active transport; HMTBA uptake is mainly by diffusion. *In vitro* membrane vesicle systems under-represent diffusion and, therefore, HMTBA uptake. Here, everted rings of chicken jejunum and ileum were used. Cobb broilers were grown on corn-soy diets supplemented with equimolar amounts of HMTBA (Alimet® feed supplement, Novus International, Inc.) and DLM (0.1% each). Birds (day 13-30) were sacrificed, and their intestines sliced into rings and everted. Sections were cultured at 37°C at pH 7 for 0.5 min, 1 min, 1.5 min, 2 min or 5 min in the presence of glucose; KCl or NaCl (to test Na-dependence); and 5, 10, 20 or 50mM 14C-HMTBA or 14C-DLM (both 2.5 μCi/100μmol). 3H-inulin (0.5 μCi/ml) was included (non-specific binding control). Slices were rinsed and scintillation counted. 3H-corrected HMTBA and DLM counts were calculated, and absorption of HMTBA or DLM per mg of tissue was determined (SAS, LSMEANS). No source by ion interactions existed, so KCl and NaCl data were combined. Absorption of each source was time and concentration dependent. In general, HMTBA uptake was equal to or greater ($P<.05$) than DLM uptake. The exception was at 1.5 minutes, where 5mM DLM absorption (ileum) was greater than 5mM HMTBA (jejunum) ($P<.05$). At 5 minutes, however, absorption of 5mM DLM (jejunum) was less than that of HMTBA or DLM absorption (ileum). No other significant differences existed at 5mM. At 10mM, absorption of HMTBA (ileum) was greater ($P<.05$) than that of DLM (jejunum or ileum), but there were no significant differences between HMTBA (jejunum) and DLM (either tissue). At 20mM and 50mM, absorption of HMTBA in both tissues was greater than or equal to DLM absorption at all time points. In contrast to data from *in vitro* membrane vesicles, this study provides further evidence that HMTBA availability is not limited by intestinal absorption.

Key Words: 2-Hydroxy-4(Methylthio) Butanoic Acid, Methionine, Absorption

139 Isolation and partial characterization of pancreatic alpha-amylase in turkey. E. Blair*, J. Firman, and S. Zhang, *University of Missouri, Columbia.*

White nicholas tom turkey α-amylase was purified by $(NH_4)_2SO_4$ fractionation, dialysis against a $C_2H_3O_2Na$ gradient, and β-cyclodextrin affinity chromatography from a aqueous pancreatic extract. The effects of pH and temperature on amylolytic activity were studied. The ideal pH for pancreatic amylase was determined to be approximately 5.9, with a conservative range of 5.7 to 6.2. The optimal range of temperature was determined to exist between 20 and 30 degrees Celsius. The homogenous mixture was subjected to SDS-PAGE to determine approximate molecular weight. A 10 kD protein marker was used as a standard. The approximate molecular weight of the amylase was determined to be 40 kD. Isoelectric focusing with a pH gradient of 5 to 8 was run to determine if any isozymes exist. From the IEF it was determined that at least 2 isoforms exist for pancreatic amylase in the turkey.

Key Words: Turkey, Amylase, Digestive Enzymes

140 Unique responses of pancreatic phospholipid metabolism of broiler hens in response to increased plasma glucose and lipid concentrations. S.-E. Chen*[1], R. L. Walzem[1], and J. P. McMurtry[2], [1]*Department of Poultry Science, Texas A&M University, College Station,* [2]*ARS Growth Biology Laboratory, Beltsville, MD.*

Higher caloric intake (twice the recommended 145 g/day/hen; T or free access to feed; F, n=15 for each) for 10 days in broiler hens (Cobb 500 Fast Feathering, age=35 wks) caused 500 grams body weight (BW) gain when compared with hens consuming recommended feed intakes (R, n=15) or with hens necropsied at the start of feeding trial as basal references (BR, n=15). Rapid BW gain was associated with an increase ($p \leq 0.05$) of relative liver (50%) and abdominal fat weights (30%), and elevated preprandial plasma insulin (45-60%), NEFA (40%), glucose (43%), VLDL-TG (27%), and total cholesterol (TC) concentrations (60-80%) but a decreased HDL-TC (45%) concentration, suggesting metabolic dysregulations similar to those of mammalian obesity and type-II diabetes. Higher caloric intake also increased ($p \leq 0.05$) relative pancreas weight (25-30%), pancreatic protein content (25%) and protein/DNA ratio (20%), and all classes of pancreatic phospholipids (average 35%), indicative of pancreatic hypertrophy. Notably, phosphatidylethanolamine increased 75% in concert with increased D9 desaturase activity (18:1n9/18:0, mol/mol) (15-30%) and a general enhancement of unsaturation in pancreatic PL fatty acids (12-35%) ($p \leq 0.05$) when compared with group R and PR hens. These observations indicate that specific changes in pancreatic lipid metabolism accompany the acceleration of endocrine/exocrine functions in response to food intake above the amount needed for optimal egg production. Hen pancreatic cells may mount specific metabolic responses to prevent cytotoxicity resulting from excessive glucose and saturated fatty acid availability.

Key Words: Pancreas Phospholipids, Hypertrophy, Lipotoxicity

141 Efficacy of injected gluconeogenic supplementation on the performance of broilers from young breeders. E. D. Peebles*[1], W. D. Berry[2], R. W. Keirs[3], L. W. Bennett[3], and P. D. Gerard[4], [1]*Department of Poultry Science, Mississippi State University, Mississippi State,* [2]*Department of Poultry Science, Auburn University, Auburn, AL,* [3]*College of Veterinary Medicine, Mississippi State University, Mississippi State,* [4]*Experimental Statistics Unit, Mississippi State University, Mississippi State.*

It has been reported that gluconeogenic supplementation using hydrolyzed casein augmented the early performance of broilers from a single young breeder flock at 29 wk of age. In an effort to further explore this concept and determine the efficacy of various other practical nutrient sources, injections (0.2 mL) containing physiological saline or a gluconeogenic energy source (casein hydrolysate, ovalbumin hydrolysate, or crude ovalbumin) were given subcutaneously (in the back of the neck) to 320 broiler chicks at hatch. Concentrations of gluconeogenic substances in solution were approximately 200 g / L. Biotin was added to injected crude ovalbumin solutions. Chicks were hatched from eggs that were obtained from a single young breeder flock at 27 weeks of age. At hatch, chicks were divided into 16 floor pens with 20 chicks in each of four replicate pens per treatment group, and were brooded under commercial conditions. Tissue condition at the site of injection, growth, feed consumption, feed conversion, water intake, livability, and liver weight were determined through 16 days post hatch. Treatment injection caused no local histological reaction. Body weight and relative liver weight at Day 16 were not affected by treatment. Furthermore, daily mortality, and average feed consumption, feed conversion, water consumption, and BW gain per bird over the entire 16 day period and at various shorter consecutive intervals were not affected by treatment. In this study, the use of injected casein hydrolysate, ovalbumin hydrolysate, or crude ovalbumin for gluconeogenic supplementation did not affect the performance of chicks from young parents that were provided adequate brooding conditions.

Key Words: Broiler, Chick, Gluconeogenesis