M135 Lysophosphatidic acid (LPA) stimulates activiation of ERK-1/2 and proliferation of C_2C_{12} cells but does not result in a significant increase in total DNA. J. M. Scheffler*, A. K. Batie, and S. J. Jones, *University of Nebraska*, *Lincoln*.

Lysophosphatidic acid (LPA) plays an important role in modulating cell survival, proliferation and apoptosis in a variety of cell lines. LPA's possible role in proliferation makes it a candidate to be a component used in defined cell culture media. Skeletal muscle expresses all four LPA receptors, however, the role LPA plays in skeletal muscle has not been clearly defined. This study investigated the effect of LPA on proliferating C_2C_{12} cells by a variety of means. Serum starved C_2C_{12} cells were treated with serum free Dulbecco's Modified Eagle Medium (DMEM) with either 5% fetal bovine serum or 50, 25, or 12.5 μm LPA. Total DNA, proliferation (thymidine incorporation), cell cycle analysis (flow cytometry), and ERK were measured. The FBS treatment stimulated ($P<.05$) an increase in DNA, LPA did not stimulate ($P<.05$) an increase in total DNA. During a 48 h period, there was an increase in [3H]thymidine incorporation after 24 h. Treatment with LPA did result ($P<.05$) in the phosphorylation of ERK-1/2 within 5 min, however, phosphorylation was not maintained to the extent seen with serum addition. Cell cycle analysis by flow cytometry revealed that LPA stimulated a transient increase in proliferation which abated by 24 h. These results indicate that LPA stimulates proliferation of C_2C_{12} cells and activation of ERK-1/2, however, these responses are transient. A significant increase in DNA could be detected after 48 h.

Key Words: LPA, ERK1/2, DNA

M136 Phospho-MAPK as a marker of myogenic satellite cell responsiveness to growth factors. D. C. McFarland* and J. E. Pesall, *South Dakota State University*, *Brookings*.

Previous studies identified variation in the mitogenic response of subpopulations of turkey myogenic satellite cells to growth factor stimuli. Further work determined that this was not due to differences in growth factor receptor numbers or affinity between the subpopulations. In order to determine if the differential response to growth factor stimuli was due to variation in the levels of activated intracellular signaling proteins, a Western blotting procedure was utilized to measure the levels of phospho-MAPK (phospho-ERK 1/2). Confluent cultures of turkey satellite cells were rinsed and administered serum/growth factor-free medium for 3 hrs. Following this, treatment media were applied to the cells for 3 min. Cells were then scraped from the wells, extracted, and the supernatant subjected to Western blotting with anti-phospho-ERK 1/2 antibodies and visualized by chemiluminescence. Initial measurements using serum mitogenic stimuli showed differences in phospho-MAPK levels between the clonal subpopulations, but the responses did not correlate with proliferation rates of the individual clones ($P > 0.05$). IGF-I alone did not increase phospho-MAPK levels compared to non-growth factor controls ($P > 0.05$), whereas fibroblast growth factor (FGF) did result in increased levels ($P < 0.05$). A synergistic response was seen in cells administered both IGF-I and FGF. When administered FGF and IGF-I, 2 of the slow growing clones exhibited lowest levels of phospho-MAPK ($P < 0.05$). One of the slow growing clones had similar levels of phospho-MAPK to the three fast growing clones ($P < 0.05$). Similar results were seen with cells administered FGF, IGF-I, platelet-derived growth factor BB and hepatocyte growth factor. The results suggest that variation in the responsiveness of some satellite cell subpopulations may be due to differences in phospho-MAPK generation.

Key Words: Muscle, Satellite Cells, Turkey

M137 Mapping the glucocorticoid responsive element of the growth hormone gene in chicken embryonic somatotrophs. K. A. Heuck* and T. E. Porter, *University of Maryland*, *College Park*.

Normal expression of growth hormone (GH) in the chicken pituitary occurs around embryonic day (e) 14 and can be induced earlier by the glucocorticoid (GC), corticosterone (CORT). Induction of GH gene expression by CORT can be blocked by the protein synthesis inhibitor, cycloheximide, indicating an indirect effect requiring translation of an intermediary protein(s). Furthermore, no consensus glucocorticoid response element (GRE) exists within 10 kilobase (kb) upstream and 5 kb downstream of the GH gene, only several imperfect GRE half sites. Therefore, the objective of this study was to identify the GC-responsive region of the GH gene. A previous report indicated that reporter constructs containing 1.7 kb of the chicken GH gene 5′-flanking region were minimally responsive to GC (two-fold) in rat pituitary cell line. In the present study, luciferase reporter constructs were tested containing 1.4 and 1.7 kb of the GH promoter. Chicken e11 pituitary cells were used to avoid effects of heterologous cell lines. Constructs were evaluated in 5 separate experiments, and luciferase activity compared by analysis of variance. Reporter activity of the 1.7 kb construct showed a mean twelve-fold increase over basal when treated with CORT. Deletion of the 1.7 kb region to 1.4 (-1467) kb resulted in ablation of reporter activity ($P<0.05$; $n=5$). Preliminary experiments with constructs with 1.6 (-1620) kb and 1.5 (-1544) kb of the GH promoter recapitulated the 1.7 kb response in reporter activity ($n=1$), indicating that a GRE resides in a region between 1.5 kb and 1.4 kb upstream of the chicken GH gene. Inspection of this region reveals potential binding sites for Ikaros, ELF2, HNF1 and CREB, which are known to be regulated by GC or involved in cell differentiation. We conclude that the chicken GH gene is responsive to GC without a classical GRE and that this response is most likely indirect due to other GC-responsive transcription factors binding upstream of the GH gene.

Key Words: Growth, Pituitary, Gene Expression

M138 Intestinal morphology and gene expression differences between broiler chicken lines selected for divergent growth rates. E. R. Feierstein*[1], E. R. Gilbert[2], M. E. Persia[1], E. A. Wong[2], W. W. Saylor[1], and C. J. Schmidt[1], [1]*University of Delaware*, *Newark*, [2]*Virginia Polytechnic Institute and State University*, *Blacksburg*.

Modern broilers have been selected for rapid growth, and a reasonable hypothesis is that this selection may have affected intestine development or function. The objective of this study was to compare intestinal gene expression patterns between two strains of broilers that exhibit different growth properties. For this study, a broiler strain Ross-708 (Allen Poultry Company) was compared with strain IL50, an inbred strain maintained at the University of Illinois that exhibits growth

properties similar to those seen in broilers in the early 1950's. At 35 d post hatch, the mass of the IL50 birds was 60% of the size of the Ross birds. Also, the intestine of the IL50 birds contributes 3.1 (± 0.1)% of the bird mass, while the intestine of the Ross line contributes 2.4 (± 0.1)%. The growth of the duodenum, jejunum and ileum was monitored during that period by sacrificing birds at weekly intervals and measuring the mass and length of the individual intestinal segments. The increase in length was complex, with both strains exhibiting a plateau in all three segments between d 7 and 14. During this period the segments continued to increase in mass. RNA was prepared from d 2 and 14 post-hatch duodenal segments and quantitative real time PCR used to determine the expression levels of various nutrient transporters. For example, ANOVA analysis indicates the mRNA encoding peptide transporter 1 (PepT1) mRNA decreased ($P < .05$) between d 2 to d 14. In addition, we have used microarrays to evaluate global patterns of gene expression in this segment. This study may be useful for identifying candidate genes that have played a role in improving production traits during the past 50 years.

Key Words: Poultry, Intestine, Gene

M139 Cloning of chicken ras-dva: Glucocorticoid regulation in the embryonic anterior pituitary. L. E. Ellestad*[1,2], S. A. Jenkins[1], and T. E. Porter[1,2], [1]*Department of Animal and Avian Sciences, University of Maryland*, *College Park*, [2]*Molecular and Cell Biology Program, University of Maryland*, *College Park*.

Understanding mechanisms involved in initiating pituitary growth hormone (GH) expression during embryogenesis should aid in developing strategies for improving growth in broiler chickens. Circulating corticosterone (CORT) increases GH production between embryonic day (e) 12 and e14 in the chicken through a mechanism involving a Ras protein. This study was aimed at characterizing chicken Ras-dva, a candidate gene that may mediate CORT induction of GH during development. Through random sequencing of a cDNA library, we identified and sequenced in its entirety a putative Ras-dva clone. Comparison of chicken Ras-dva with GenBank and with vertebrate genomes indicated that homologs are present only in non-mammalian vertebrates. Phylogenetic comparison of our nucleotide sequence (1276 bp) with that in fish and frogs indicated an overall identity of 52-62%, and the predicted amino acid sequence of chicken Ras-dva (208 aa) is 74-76% identical to Ras-dva in other non-mammalian vertebrates. Pituitary expression of Ras-dva in the chicken was confirmed with RT-PCR, and ontogenic analysis indicated that pituitary Ras-dva mRNA increases between e10 and e17 in a manner similar to GH ($P<0.05$; n=4). CORT treatment increased Ras-dva mRNA in e11 anterior pituitary cells in vitro ($P<0.05$; n=4). Although the increase was less than that in cells receiving CORT alone, Ras-dva mRNA was induced by CORT in the presence of a protein synthesis inhibitor ($P<0.05$; n=4), indicating that it may be a direct target of the glucocorticoid receptor (GR). Analysis of potential regulatory regions of the chicken Ras-dva gene identified three putative GR binding sites within 5 kb upstream and one within 4.2 kb downstream of its transcription start site. Six potential binding sites for the pituitary-specific factor Pit-1 were also identified within 5 kb upstream, indicating that Ras-dva may be expressed in Pit-1 expressing cells such as GH-producing somatotrophs. We conclude that Ras-dva is a glucocorticoid-induced gene in the chicken anterior pituitary gland that may play a role in initiating GH expression during embryonic development.

Key Words: Growth Hormone, Somatotroph, Corticosterone

M140 Identification of potential feed efficiency biomarkers. C. P. Ojano-Dirain*[1], N. R. Pumford[1], T. Wing[2], M. Cooper[2], J. Lay[3], R. Liyanage[3], and W. G. Bottje[1], [1]*Center of Excellence for Poultry Science, University of Arkansas*, *Fayetteville*, [2]*Cobb-Vantress, Inc.*, *Siloam Springs, AR*, [3]*State Wide Mass Spectrometry Laboratory, University of Arkansas*, *Fayetteville*.

Our previous research has reported that certain mitochondrial proteins were differentially expressed in breast muscle, liver and duodenum in broilers with high or low phenotypic expression of feed efficiency (FE, gain to feed). The objective of this study was to identify proteins that are expressed differently in readily accessible tissue, such as lymphocytes or plasma, from broilers with high or low FE. In Experiment 1, lymphocytes were isolated from whole blood of broilers with a continuum of FE (n = 25 to 48) using density gradient Histopaque-1077. Results of Western blot assay showed a significant correlation ($P<0.05$) between FE and the levels of vinculin and cytochrome c in lymphocytes. Using the level of these two potential biomarkers, we found that we could identify the bottom 30% of birds in terms of FE with ~90% accuracy. In Experiment 2, plasma was separated by 2D electrophoresis and four separate gels from each group of high or low FE broilers were analyzed for significant differences. Ten proteins from the plasma proteome were expressed differently ($P<0.05$) between the high and low FE broilers. Of these 10 proteins, 4 have been identified using MALDI-TOF MS, namely transthyretin, histone deacetylase 1 and 2, adipophilin and albumin. Although the mechanism behind the association of plasma proteins to FE is yet unknown, results of this study indicates that some mitochondrial and extramitochondrial proteins are potential candidates for developing a biomarker assay that could aid in identifying low FE birds for exclusion from the breeding line.

Key Words: Feed Efficiency, Broilers, Potential Biomarkers

M141 Physiological function of butoxybutyl alcohol a novel compound in broilers. S. Inada*, A. Ohtsuka, and K. Hayashi, *Kagoshima University*, *Kagoshima city , Korimoto, Japan*.

We have shown that sweet potato shochu distillery by-product (SDBP) contains growth promoting factor. Butoxybutyl alcohol (BBA) is a new compound which may be responsible for the growth promotion. The ether extract of SDBP was first fractionated by a Sephadex column (Sephadex LH-20), purified by HPLC (high performance liquid chromatography) and the chemical structure was determined by NMR (nuclear magnetic resonance) and MS (mass spectrometry). In the present study, chemical synthesis of BBA was carried out using butanol, butyric aldehyde and *p*-toluenesulfonic acid as the catalyst. After the fractionation by a Sephadex column, BBA was verified by HPLC. Then physiological function of BBA was investigated *in vivo* (broiler) and *in vitro* (chick muscle cells). Birds (Cobb strain, male) were raised under 24 ±1° C in wire bottom cages for 20 d from 15 d of age. Feed (CP 23%; 3200 kcal ME/kg) and water were given *ad libitum* during the experimental period. BBA was mixed into the feed at the level of 70 ppm. Body weight was recorded every 3 d and feed intake was recorded daily. At 35 d of age, all the birds were killed and dissected to measure muscle and organs weights. Blood was collected to measure GOT (glutamate oxaloacetate transaminase) as an index of liver function. In the case of *in vitro* experiment, BBA was added to the culture medium at the levels of 1, 10 and 100 ppm, and cells were cultivated for seven days. BBA increased feed intake ($P<.05$) and growth was facilitated. Feed conversion, abdominal fat weight, liver

weight, spleen weight and serum GOT were not changed by BBA. Muscle TBARS (thiobarbituric-acid reactive substances) as an index of lipid peroxidation was significantly decreased by BBA. Protein content of the cultured muscle cell was increased (P<.05) by 1 and 10 ppm BBA, but decreased by 100 ppm BBA.

Key Words: Broiler, Growth Promotor, Distillery By-product

M142 Bone mineralization in nine pedigree lines of meat-type chickens. P. Talaty*[1], M. N. Katanbaf[2], and P. Y. Hester[1], [1]*Purdue University*, *West Lafayette, IN*, [2]*Cobb-Vantress, Inc.*, *Monticello, KY*.

The variability of bone mineral density (BMD) and bone mineral content (BMC) of the tibia, ulna, and radius of 9 Cobb pure lines (L) of chickens were accessed at 6 wk of age. Bone mineralization of 9 birds/line/sex was determined using dual energy X-ray absorptiometry. Using the mixed model of SAS, data for BMC, bone length, and bone area were analyzed using an analysis of covariance with BW as the covariant. An ANOVA was used for BMD and bone width as BW was nonsignificant as a covariant. Bone within bird (used a subplot), line of chicken, and sex of the bird were considered fixed effects. The BMD did not differ among pedigree lines. Interactions with sex and bone were nonsignificant indicating that the BMD response was the same for all 9 pedigree lines with respect to the sex of the bird and the type of bone scanned. However, all other traits measured in this study including BW, BMC, bone length, bone width, and bone area were different among lines ($P < 0.001$). The two pedigree lines (L 7 and L8) with the lightest 6-wk-old BW (2033 and 2055 g, respectively) had diverse skeletal traits. Birds of L7 had the lowest BMC (1.05 g), shortest bone length (69.2 mm) and smallest bone area (8.0 sq cm); however, the other pedigree line low in BW (L8) showed the opposite trend in that bones from these birds were the highest in BMC (1.38 g), bone length (74.6 mm), and area (9.2 sq cm) when compared to all of the other lines. It was the BMC of the tibia and not the radius and ulna that caused the differences in BMC among pedigree lines of chickens (line × bone interaction, $P < 0.0001$). The BMD of L8 was also the highest of all pedigree lines that were compared, but the difference was non-significant ($P = 0.12$). The overall CVs calculated for skeletal traits across all genetic lines were high with BMD at 51% and BMC at 80%. It is concluded that large differences in skeletal traits exist among pedigree lines of meat-type chickens.

Key Words: Bone Mineralization, Bone Mineral Density, Pedigree Chickens

M143 The expression of neutral amino acid transporter B^0 and mTOR proteins along the gut mucosal crypt-villus axis in the formula-fed neonatal pig. C. Yang[1], X. Yang[1], D. Lackeyram[1], Y. L. Yin[2], K. Swanson[1], F. Verrey[3], and M. Z. Fan*[1], [1]*University of Guelph*, *Guelph, ON, Canada*, [2]*Institute of Subtropical Agriculture, the Chinese Academy of Sciences*, *Changsha, Hunan, China*, [3]*Institute of Physiology, University of Zurich*, *CH-8057 Zurich, Switzerland*.

The intestinal apical Na+-neutral amino acid (AA) transporter B^0 is believed to be the primary transporter for the uptake of luminal neutral AA across the apical membrane. Our previous work demonstrated a high level of apical B^0 transporter activity along the jejunal crypt-villus axis in fed neonatal pigs. However, the B^0 gene (mRNA) expression is low in proliferating crypt cells and increases with cell differentiation in the villus cells. This study was conducted to examine how B^0 protein is expressed along the jejunal crypt-villus axis in fed neonatal pigs. Six Yorkshire gilts were removed from sows at d 5 of age and fed a milk protein-based liquid formula till 14-16 d of age before being sacrificed for tissue collection. Three major intestinal epithelial cell fractions from the jejunum, representing cells from the upper villus, the middle villus and the crypt regions, were sequentially isolated, with cell viability of 92-95%, as assessed by trypan blue exclusion, by the distended sac method. Western blot analyses were used to examine the protein abundance of Na+-neutral amino acid (AA) transporter B^0 and the mammalian target of rapamycin (mTOR) protein phosphorylated (Pi). No differences ($P>0.05$) were observed in B^0 protein abundance among the cell homogenate, soluble, and the apical membrane along the three cell fractions. A higher ($P<0.05$) mTOR protein-Pi was observed in the middle villus cells. The crypt cell also expressed relatively high level of the activated mTOR protein. The results suggest that the B^0 protein expression along the gut crypt-villus axis may be regulated at posttranscriptional level and the mTOR-signaling pathway may be involved in the regulation in the fed neonate.

Key Words: Na+-Neutral Amino Acid Transporter B^0, Neonates, The Mammalian Target of Rapamycin (mTOR)

M144 Modulation of protein synthesis by somatotropin and insulin in skeletal muscle of growing pigs. F. A. Wilson*, H. V. Nguyen, A. Suryawan, R. A. Orellana, J. G. Fleming, A. S. Jeyapalan, and T. A. Davis, *Childrens Nutrition Research Center, Baylor College of Medicine*, *Houston, TX*.

Chronic treatment of pigs with porcine somatotropin (pST) for 7 days increases feed efficiency by both promoting whole body protein synthesis in the fed state and reducing whole body protein degradation during fasting. pST-treated pigs have higher plasma insulin levels than vehicle-treated controls. This study aims to determine whether the increase in protein synthesis of pST-treated pigs is mediated through an insulin-induced stimulation of translation initiation. Growing pair-fed, weight-matched pigs were treated with pST (150 μg/kg/day, n=18) or vehicle (n=18) for 7 to 10 days. Pancreatic glucose-amino acid clamps were performed in overnight fasted pigs to attain plasma insulin levels of 5, 25 and 50 μU/ml, equivalent to reported insulin levels in 1) fasted control and fasted ST-treated, 2) fed control, and 3) fed-pST treated pigs, respectively. Amino acid and glucose levels were maintained at fasting levels. Skeletal muscle protein synthesis was measured with the flooding dose method and western blotting was used to identify changes in the abundance and activation of translation initiation factors in muscle. Statistical analysis was performed using ANOVA. Plasma levels of urea nitrogen were lowered ($P<0.001$) and insulin-like growth factor-1 levels increased ($P<0.005$) in pST-treated pigs indicating effectiveness of pST treatment. Insulin increased protein synthesis in muscle of control ($P=0.01$) and pST-treated ($P=0.07$) pigs. Treatment with pST also increased muscle protein synthesis ($P<0.05$). The abundance of the active translation initiation complex eIF4G-eIF4E and the phosphorylation of eIF4G in muscle mirrored the changes in muscle protein synthesis in response to insulin, however there was no clear effect of pST. We conclude that both insulin and pST stimulate protein synthesis in skeletal muscle of growing pigs.

(Supported by USDA NRI 2005-35206-15273)

Key Words: Somatotrohin, Pig, Insulin

M145 Impact of different doses of ractopamine in swine carcass and meat characteristics from Large White and Duroc breeds. E. F. Leonardo[1], I. L. Stella[1], A. C. M. S. Pedreira[2], G. B. Mourão[1], and E. F. Delgado*[1], [1]*Escola Superior de Agricultura "Luiz de Queiroz"*, *Piracicaba, SP, Brazil*, [2]*Agência Paulista de Tecnologia do Agronegócio*, *Piracicaba, SP, Brazil*.

Lean meat production has been an important issue for health and economical reasons in swine meat industry. Sixty animals (30 castrated males and 30 females) being 30 Large White (LW) and 30 Duroc (DU) were randomly assigned to different doses of ractopamine (RAC): 0 (control), 10 ppm and 20 ppm in the diet. The experiment started when animals reached 85 kg of live weight and ended after 4 weeks when animals were 110 kg. The *L. dorsi* muscle samples were collected 24 h postmortem (PM) from the left carcass side in a commercial abattoir. The WB shear force (WBS) was measured 24 h PM (d 1) and 5 d after slaughter (d 5). Rib eye area (REA) and fat thickness (FT) were measured 24 h PM. WBS on d 1 were higher (P≤0.01) for LW (7.58±0.32 kgf) than DU breed (6.09±0.38 kgf). Differences in WBS remained on day 5 (P≤0.01) for LW and DU breeds (4.83±0.21 and 3.86±0.25 kgf, respectively). The WBS for d 1 were not different between RAC doses but higher than control diet (0: 6.12±0.44; 10 ppm: 6.86±0.43; 20 ppm: 7.51±0.44 kgf; P≤0.05). On d 5, WBS was different between RAC doses and also compared to the control diet (0: 3.74±0.29; 10 ppm: 4.23±0.28; 20 ppm: 5.07±0.29 kgf; P≤0.05). LW animals had greater REA (P≤0.05) than DU breed (37.7±1.0 and 34.3±1.2 cm^2, respectively). Rib-eye area for females and males were different (37.7±1.1 and 34.3±1.2 cm^2, respectively). There was a FT interaction between breeds and sex condition (P=0.083). FT were different between RAC doses (P≤0.05) and also compared to the control diet (P=0.054) (0: 2.62±0.13 cm; 10 ppm: 2.53±0.12 cm; 20 ppm: 2.10±0.13 cm). The results confirmed the leaner carcass production when RAC is added to diet. However, in those pure breeds doses of RAC did not modified REA. The negative effect of increased RAC dose on swine meat tenderness was also observed.

Key Words: Warner-Bratzler Shear Force, Meat Tenderness, Swine Meat

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M146 Pro-inflammatory response of chicken thrombocytes to lipopolysaccharide. T. R. Scott* and M. D. Owens, *Clemson University*, *Clemson, SC*.

Thrombocytes (10^7) from blood of SCWL chickens were cultured 1 hr in the presence of lipopolysaccharide (LPS). LPS concentrations of 0, 0.1, 1 and 10 µg/ml were used. Following culture, thrombocytes and culture media were separated by high speed centrifugation. Cell pellets were resuspended in RNA*later*™. RNA was extracted from cells for real-time PCR of GAPDH, Toll-like receptor 4 (TLR4), IL-1β, IL-6 and IL-12. Culture media supernatant histamine (H) and prostaglandin E2 (PGE2) concentrations were determined with homologous time-resolved fluorescence assays. GAPDH was used as the housekeeping gene, and the expression of this was unaffected by any concentration of LPS used in culture with the thrombocytes. TLR4 was found to be constitutively expressed by thrombocytes and its expression was not altered by any concentration of LPS. Expression of IL-1β, IL-6 and IL-12 were all increased by LPS stimulation of thrombocytes in culture. H release by thrombocytes in culture was not affected by LPS. PGE2 concentrations in culture supernatants were found to be increased following LPS treatment. Although different from the 0 µg/ml control, the expressions of cytokines and PGE2 concentrations were not different among 0.1, 1 and 10 µg/ml LPS used in culture. Chicken thrombocytes express TLR4 and respond to LPS stimulation with increased pro-inflammatory cytokine expression and PGE2 release.

Key Words: Thrombocyte TLR4, Pro-inflammatory Cytokines, Prostaglandin E2

M147 Pro-inflammatory response of broiler chick thrombocytes. F. Ferdous*, D. V. Maurice, and T. R. Scott, *Clemson University*, *Clemson, SC*.

Broiler chicks at 4 weeks of age were bled to obtain thrombocytes for in vitro stimulation with lipopolysacchardie (LPS). The chicks had been fed a chick starter diet or the same diet supplemented with either corticosterone (CS) or vitamin C plus corticosterone (VitC). The diets were fed to the chicks from 2 to 4 weeks of age in order to induce body conditions indicative of stress. After 2 weeks of feeding, the chicks exhibited differences in feathering and body weight with the control chicks being largest and well-feathered while the VitC chicks were intermediate in both features and the CS chicks were small and poorly feathered. Isolated thrombocytes (10^7) were cultured for 1 hour with 0 or 10 µg/mL LPS. Following culture the cells were separated from the supernatants by high speed centrifugation. The thrombocyte pellets were resuspended in RNA*later*™, and RNA was extracted with the RNeasy Kit. RNA samples were processed for real-time PCR of GAPDH, IL-1β, IL-6 and IL-12. GAPDH was used as the housekeeping gene, and its expression was not affected by any dietary treatment nor concentration of LPS. The thrombocyte pro-inflammatory cytokines were unaffected by the diets, but 10 µg/mL LPS significantly induced greater expression of these above 0 µg/mL LPS. Although dietary induced stress can affect other physiological parameters in broiler chicks, the LPS induced expression of thrombocyte IL-1β, IL-6 and IL-12 are not altered.

Key Words: Thrombocytes, Pro-inflammatory Cytokines, Stress

M148 Identification of antimicrobial peptides in avian heterophils using whole cell MALDI-TOF. L. Kannan*[1,2], N. C. Rath[1], R. Liyanage[2], and J. O. Lay[2], [1]*USDA/Agricultural Research Service*, *Fayetteville, AR*, [2]*University of Arkansas*, *Fayetteville*.

Mass spectrometry (MS) is a rapidly emerging tool not only to characterize specific biomolecules but also to characterize and identify prokaryotic cells using whole cell MALDI-TOF-MS (matrix assisted laser desorption ionization time of flight). In order to study the potential of this technique to explore the eukaryotic cell associated peptides we isolated heterophils from the peripheral blood of chickens and turkeys and subjected to whole cell MALDI-TOF in the mass range of 1-20kDa. The mass-spectrum obtained showed a prominent peak at m/z 3915 in chicken and at m/z 4132 in turkey heterophils. Since heterophils occur abundantly in bone marrow, we isolated these peptides from the bone marrow extracts of both the species using