M44 Impact of six different intestinal bacteria on broiler breeder sperm motility in vitro Melissa Haines1SC, Holly Parker, Chris McDaniel, Aaron Kiess Mississippi State University, Mississippi State, MS

Male fertility is most often evaluated using 3 sperm parameters: concentration, viability, and motility. In some mammalian species, including humans, sperm samples have been shown to have reduced motility when bacteria are present. In male broiler breeders, bacteria has been shown to be associated with spermatozoa, but their effect on motility has not been thoroughly investigated. Additionally, the sperm quality index (SQI) is a modern, rapid method of evaluating avian sperm motility. Therefore, the objective of this study was to use the SQI to determine if broiler breeder sperm motility is reduced when various bacteria are introduced to the ejaculate. In this experiment, semen was collected from 20 Cobb MX broiler breeders by abdominal massage. Individual semen samples were pooled and stored at room temperature on a rotary shaker to provide aeration. Six different intestinal bacteria, Salmonella enterica, Escherichia coli, Campylobacter jejuni, Clostridium perfringens, Lactobacillus acidophilus and Bifidobacterium animals were cultured overnight and used to determine if they inhibited the SQI of broiler breeder sperm. For each bacterium, 50µl of semen was diluted in 450µl of saline, broth alone or broth containing bacteria creating a saline control, broth control, and broth containing bacteria treatment, respectively. In every treatment, 3 replicates were evaluated at each time post inoculation (0, 10, and 20 min) creating a completely randomized design with a split plot in time. All broths containing bacteria immediately reduced sperm motility when compared to the controls (P < 0.0001). Broths containing B. animalis or L. acidophilus immediately and completely inhibited sperm motility. Although, broth containing S. enterica immediately reduced sperm motility, the reduction did not change over time. On the other hand, C. jejuni, C. bifermentans, and E. coli reduced sperm motility immediately, but as time passed the motility continued to decreased (P = 0.0043, 0.0001, and 0.0002, respectively). In conclusion, intestinal bacteria are capable of reducing the motility of broiler breeder sperm. It is also apparent that the degree to which motility is affected is dependent upon the bacteria and in some cases the length of exposure to the bacteria.

Key Words: Yeast, Carbohydrates, Probiotic, Organic minerals, Pullets

M45 Enhancement of sperm-mediated gene transfer and semen characteristics by laser irradiation Mohamed Abdel-Fattah1SC, Essam El-Gendy1, Mona Mohamed1, Yahia Bada1, Mohamed Salama1,2 Cairo University, Giza, Egypt, 3 Ain Shams University, Cairo, Cairo, Egypt

An experiment was conducted to assess the use of laser irradiation to enhance semen characteristics and sperm-mediated gene transfer. Fresh semen samples of native Egyptian cocks were used. The samples were diluted with BPSE and were treated with lipofectin (5%), while a plasmid DNA was used as a genetic material. The semen samples were subjected to 655-nm continuous diode laser at energy dose of 4 J/cm². Spermatozoa reduction capacity was also evaluated by the reduction of tetrazolium dye MTT to the purple formazan pigment. Laser irradiation increased sperm reduction capacity by 19.6% in semen treated with plasmid and the increase was only 6.4% when lipofectin was added to the semen treated with plasmid. The sperm motility was significantly the highest and reached to 75.7% in laser irradiated semen and insignificantly and slightly decreased to 72.3% when plasmid was conjugated. The sperm motility index was significantly the highest in laser irradiated samples with or without plasmid. Sperm viability was 81% for laser irradiated samples and significantly decreased to 77.9% when plasmid was conjugated. Lipofectin significantly reduced the viability and increased abnormality. The results indicated that the plasmid successfully invaded the sperm when semen samples were laser irradiated and no lipofectin was added. Also, plasmid was successfully transferred into the sperm in both samples containing lipofectin and with or without laser irradiation. It is concluded that laser irradiation with low power enhances semen characteristics, however the enhancement of gene transfer through sperm mediation is limited.

Key Words: fowl spermatozoa, laser irradiation, semen quality, sperm-mediated, gene transfer

M46 Novel method of producing chimeric chicks using piggyBac and JetPEI Brian Jordan1SC, Mike Stark1, Robert Beckstead1 University of Georgia, Athens, GA, 2Brigham Young University, Provo, UT

The chicken is a well-established model system for studying vertebrate embryogenesis, but creating transgenics has proven difficult. Viral infections have been predominantly used to insert transgenes and have been moderately successful, however, the rate of germline infection is low and the virus is not easily manipulated in the lab. To increase efficiency and ease of production we are using piggyBac, a transposable element (TE) system, paired with an in-vivo transfection reagent to generate transgenic chicks. The TE system utilizes a transposase enzyme, which recognizes a specific DNA sequence called a transposon. The enzyme excises the transposon from its original location and inserts it into another genomic location. The transposon contains a constitutively expressed GFP gene for tracking of insertion by fluorescent microscopy. The TE system is delivered to cells using JetPEI, an in-vivo transfection reagent. JetPEI forms a bundle around DNA, which is endocytosed by cells. Once inside, the bundles rupture and TE DNA is released.
The current study was conducted to compare and contrast the uses of various devices (temperature transponder or infrared thermometer) and their locations (inner air cell membrane or outer egg shell surface) in estimating embryo body temperature in Ross × Ross 708 broiler hatching eggs. The air cells of embryonated and non-embryonated eggs were implanted with temperature transponders on d 13.5 of incubation. Likewise, for these same eggs, egg shell surface temperature was detected with the use of transponders and an infrared thermometer. Temperatures were recorded every 12 h between 14.5 and 18 d of incubation. Mean air cell temperature readings of embryonated eggs using transponders were higher than those of the egg shell as determined with the use of transponders or an infrared thermometer. All readings in the embryonated eggs were higher than those in the non-embryonated eggs. In non-embryonated eggs, transponder temperatures in the air cell were higher than those on the egg shell, and on the egg shell, transponder readings were higher than those from the infrared thermometer. Graphs and corresponding regression values were used to track temperatures every 12 h between 14.5 and 18 d of incubation. These readings confirmed increased embryonic heat production during the incubational period examined, and correlations further confirmed positive relationships among the various device-location combinations within embryonated and non-embryonated eggs. It was concluded that the use of transponders in the air cells of broiler hatching eggs allowed for a closer estimation of embryo body temperature than egg shell surface temperature by circumventing the confounding effects of the thermal barrier properties of the egg shell and the flow of air across its surface.

**Key Words:** air cell, egg shell, embryo, temperature, transponder

M49 Effects of nicotine on blood glucose and liver glucose and glycogen concentrations in male broilers

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One-d-old Ross × Ross 708 male broilers from a breeder flock at peak egg production were tagged and placed in 12 individual pens. In 6 of the 12 pens, nicarbazin (NCZ) was added to basal broiler diets at a rate of 125 ppm (113.4 g/ton), and the other 6 pens were control pens in which birds received salinomycin instead of NCZ, at a rate of 66 ppm (60 g/ton). Feed and water were provided ad libitum, feed was closely monitored in all pens to avoid shortages, and no coccidial challenge was imposed. At the end of the trial, a photoperiod increase was imposed in an effort to induce post-traumatic stress due to feed enjoyment. On d 0, 4, 12, and 13, blood and feed weights on a pen basis were recorded for calculation of BW gain, feed intake, and feed conversion in age intervals. Mortality was also recorded on a daily basis. Blood glucose, liver weight, and liver glucose and glycogen content were determined on d 12 and 13. In response to NCZ, 0 to 4, 0 to 12, and 0 to 13 d feed conversions were higher; whereas, 4 to 12 d BW gain, and 0 to 12 and 0 to 13 d cumulative BW gains were lower. The added NCZ increased blood glucose on d 13, but did not affect at any time the liver constituents analyzed. In conclusion, the un-interrupted feeding of NCZ at 125 ppm in the starter and grower diets of male Ross × Ross 708 broilers increased feed conversion and reduced BW gain between d 0 and 13 d of growth-out, whereas liver glucose and glycogen concentrations were not affected. Nevertheless, a 16 h photoperiod increase may elicit an increase in the blood glucose levels of broilers fed NCZ.

**Key Words:** broiler, glycogen, liver, nicarbazin, photoperiod

M50 The impact of divergent selection for ascites on blood parameters of altitude challenged broilers

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Previous ascites research has indicated that increased hematocrit levels are significant to the diagnosis of ascites as the bird responds to oxygen challenge. Therefore, it is expected that divergent selection for ascites suscepti-
bility should result in associated changes in hematocrit response to altitude challenge. Using broiler lines divergently selected 16 generations for ascites susceptibility broilers were raised at local altitude or exposed to hypobaric challenge (simulated 11,500 ft). In both environments birds were reared in identical cage systems and provided feed and water ad libitum. Beginning at hatch birds were bled in two week increments, to six weeks of age. The experiment was then duplicated (simulated 5000 ft) to slow the mortality rate and provide additional results for the research lines. Contrary to results reported in literature the ascites resistant line exhibited higher hematocrit levels, than the random (control) or ascites susceptible line at both increases altitudes, while maintaining similar hematocrit levels at local altitude. These results may be indicative of hematocrit levels, being less influential in ascites development.

Key Words: Domestic fowl, Broilers, Ascites, Hematocrit, Altitude

M51 Preliminary studies in light turkey syndrome: histopathology of select gut and immune tissue. Alamanda Calvert1,2, Andre Ziegler, Sally Noll1 University of Minnesota, St. Paul, MN Light weight Turkey Syndrome (LTS) as described by Minnesota turkey producers is characterized by flocks that are below weight at market. LTS is not well understood, although it is thought to originate during brooding and is seen primarily in toms. The purpose of this experiment was to determine if different characteristics were seen between heavy and light weight poults in flocks. Six commercial (CF) and two research flocks (RF) were sampled at 1, 2, and 3 wks of age. Four of the CF were raised in MN, two in ND and the RF were raised in broader battery cages in MN. Heavy and light weight groups were characterized as 10-15% above or below the flock average sample weight. Four duodenal, ileal, cecal, bursal, splenic and eight jejunal tissues were scored per group. Tissues were fixed in 10% NBF and stained with H&E. Logistic regression (SAS 9.2) was used to determine flock, age and weight group effects. Tissues were scored for distribution and severity of lymphocytes (L) and heterophils (H). Distribution of L in the small intestine increased from 18% to 81% with sample age (P <0.001). At 2 wks of age, generalized L were present in the jejunum in 68% of heavy and 46% of light poults. In the ceca, 29% of heavy and 9% of light poults had severe L present (P<0.005). Two CF had decreased H over time and more light poults with generalized H than heavy poults (P<.1). Generalized L necrosis of the bursa was present in 42% of light and 27% of heavy poults (P<.03). Bursal atrophy was higher in light (46%) versus heavy (19%) poults at 3 weeks of age (P<.02). All small intestine and immune tissue showed some differences among flocks (P<.05). Heavy weight poults appear to have a faster maturing and healthier immune system because of the presence of gut L. In contrast, light poults have increased gut H and bursal atrophy probably due to infection. The many differences seen among flocks in the gut and immune tissue demonstrate that this is a complex syndrome that most likely has different combinations of causes for each flock.

Key Words: turkey, tissue score, histopathology, growth depression, poult

M52 Effects of spray dosage of a live attenuated Mycoplasma gallisepticum vaccine on the vaccination efficiency and associated in vivo M. gallisepticum populations in layers Roy Jacob1,2,3, E. David Peebles1, Spencer Leigh5, Scott Branton2, Jeff Evans2 Mississippi State University, Mississippi State, MS, 2USDA/ARS Poultry Research Unit, Mississippi State, MS Live attenuated vaccines (LAVs) are commonly utilized within the table egg industry to limit economic losses associated with virulent Mycoplasma gallisepticum (MG) outbreaks. To determine the effect of dosage of a recently released LAV, (Avipro® MG F) when applied via spray on vaccination efficiencies and in vivo MG populations, 240 mycoplasma-free Hy-Line W-36 pullets were caged individually in a commercial layer facility with four rooms, 60 birds per room, through 19 wk of age (woa). A randomized control study design was used. At 11 woa, birds of each room were spray vaccinated at one of 4 levels: 0× (negative control), 1×, 2×, or 4× of the manufacturer’s recommended dosage. The reconstituted LAV source tier was 2.8 × 10^6 cfu/1 × dose. At 5 wk post-vaccination (p.v.) in vivo MG LAV populations were estimated via palatine fissure swabs and subsequent quantitative Taqman®-based Real Time PCR assays. At 7 wk p.v., all groups were challenged with the virulent MG strain R66. Vaccination efficiency was assessed pre-challenge (6 wk p.v.) by measuring seroconversion rates via Serum Plate Agglutination assays (SPA) and post-challenge by measuring the degree of airsacculitis in virulent MG-challenged birds. SPA results demonstrated a dose dependent response as 0%, 5%, 37% and 42% birds showing seroconversion in the 0×, 1×, 2× and 4× dosage groups, respectively. The incidence of detectable in vivo MG increased with higher dosages, but MG population estimates did not correlate directly with dosage. Viable in vivo MG populations were detected in all SPA positive birds. Following challenge, airsacculitis was observed in 36%, 32%, 25% and 21% birds in the 0×, 1×, 2×, and 4× groups respectively, which also showed dose dependent protection. The results demonstrate that the dosage may have direct implications vaccination efficiency of AviPro® MG F.

Key Words: Layer, Vaccine, Mycoplasma gallisepticum, Quantitative PCR

M53 A comparative study of the virulence of different strains of Histomonas meleagridis in turkeys Mathew Abraham1,2, Larry McDougald, Robert Beckstead The University of Georgia, Athens, GA Histomonas meleagridis is a flagellated protozoan causing histomoniasis or blackhead disease, a great threat to the turkey industry with its heavy mortality and morbidity. Aim of our experiment was to study the virulence of multiple strains of H. meleagridis by inoculating turkeys with strains isolated from Michigan and Buford (Georgia) and monocultures generated from the Buford strain. Two week old birds were weighed, banded and divided into 15 groups of 10 birds with 12 groups infected with histomonas (20,000/1ml) by means of direct cloacal inoculation and 3 groups remaining uninfected. Each of the 4 strains of Histomonas Buford Pass 1(BP-1), Buford Pass 100(BP-100), Buford monoculture 16(BM-16) and Buford monoculture 57(BM-57) were used to infect 3 replicate cages of 10 birds each. Unmedicated feed and water were provided ad-libitum. At 10 days post infection (dpi) birds were weighed, euthanized, necropsied and cecal and liver lesions were scored in 0-4 scale based on the severity. Similarly, turkey poult grown for 4 weeks were weighed, banded and divided into 9 groups of 7 birds with 6 groups infected with Michigan strain of H. meleagridis (10,000/1ml) by means of direct cloacal inoculation. Among which, 3 infected groups were fed with feed mixed with Histostat 50. At 7 dpi birds were weighed, euthanized and necropsied and lesions were scored in 0-4 scale. Average lesion score of cecum obtained for Control, BF 1, BF 100 and BM 57 was 0, 1, 0.5, 0.67, 2.5 and 1 respectively. Average lesion score of liver was 0, 0.57, 0, 0.9 and 0.07 and a weight gain of 615, 541, 597, 513 and 617 grams (g) for each group. For birds infected with Michigan strain, there were no lesions found for the control and Histostat fed group. Infected group of Michigan strain with unmedicated feed showed an average cecal and liver lesion score of 1.62 and 0.33 respectively. Average weight gain of Control, MI infected and MI infected (Histostat) were found to be 375, 298 and 197 g respectively. From the above observations we found that BF-1 and BM-16 are more virulent on the experimental infection with Buford strain. Even though Histostat provides a complete protection against MI strain, it accounted for a decreased weight gain in our study.

Key Words: Histomonas meleagridis, Blackhead, Virulence, Turkey, Histostat

M54 Oral vaccination with modified chitosan and a synthetic Salmonella peptide induces mucosal immune response H.M. Fleming1,2, O.B. Faulkner, S. Shivaramiah, M.J. Morgan, N.R. Pumford, C. Lester, A.D. Wolfenden, L.R. Biekle, G. Tellez, B.M. Hargis University of Arkansas, Fayetteville, AR A proprietary modification of chitosan was used as an adjuvant with a 19 amino acid synthetic peptide sequence (NNP) conjugated to KLH to vac-
The primary objective of any poultry production system is to optimize the economic efficiency of converting poultry feed into human food. Highly successful breeding and selection programs have provided the platform for annual improvements in biological efficiency as measured by the output of the resident microbiota (flora) (2). The physical nature of both the intestinal lining and its content display detectable changes in the early stages of disease. Villus height to crypt depth ratios have for example been used to indicate intestinal integrity (3; 5). This is possible because the length of an intestinal villus is kept constant by continuous enterocyte replacement. The delicate cells lining the intestinal tract are continuously exposed to potentially damaging luminal content and not surprisingly they require frequent replacement. It has been shown that the life span of a typical enterocyte is 3 to 4 days and consequently complete replacement of the intestinal epithelial lining occurs in this period of time by a process of cell division in the crypt area, sequential migration of the enterocyte to the tip of the villus and finally extrusion from the tip into the lumen (1). The body’s first homeostatic response to accelerated enterocyte attrition is enhanced cell division in the crypt area and to achieve this, the crypt increases in size. It stands to reason that a slight decrease in villus height to crypt depth ratio in the absence of any change in villus height is the first indicator that the conditions within the intestinal tract have

**Key Words:** Broilers, cassava waste meal, growth, nutrient digestibility

**M56 Effects of feeding processed cassava waste meal on growth and apparent nutrient digestibility of broiler chickens.** Stanley Omoh Omoikojo1, Daniel Maya, Clement Ebanehita Isidahomen, Ermosele Theophilus Ehebha. *Ambrose Alli University, P.M.B 14, Ekpoma, Edo State, Nigeria*

In an eight-week feeding trial, four broiler starter and finisher diets formulated to contain 0, 15, 30 and 45% processed cassava waste meal (PCWM) were randomly assigned to a total of 144 Anak 2000 broiler chickens in a completely randomized design to assess their growth and apparent nutrient digestibility. Each treatment group contained three replicates of eight chicks each, thus making a total of twenty four chicks per treatment group. Results on the growth performance of broilers showed that only the average live weight was not significantly (P>0.05) increased with a corresponding increase in the inclusion levels of PCWM at the starter phase, but at the finisher phase all the growth performance indices except feed:gain ratio followed a similar pattern with that of the live weight at the starter phase. The nutrient digestibility results showed that only the apparent digestible ash was significantly (P<0.05) increased by the inclusion level of PCWM at the starter phase. At the finisher phase, the values of the apparent digestible dry matter, crude protein, crude fibre, ash and nitrogen free extract were significantly (P<0.05) increased with corresponding increase in the inclusion levels of PCWM from 0 &ndash; 45%, whereas apparent digestible ether extract values were not affected by the test diet. The over all results showed that PCWM can successfully replace maize up to 45% level in the diets of broiler chickens without any adverse effect. Key: Broilers, cassava waste meal, growth, nutrient digestibility.

**Key Words:** Broilers, cassava waste meal, growth, nutrient digestibility