

in % for N, P2O5, and K2O (with standard deviations in parenthesis) for the various categories of poultry litters were:

Fresh Broiler Litter (2,903 samples): 3.15(0.60), 2.77(0.81), 2.33(0.62)  
 Stockpiled Broiler Litter (262 samples): 2.78(0.86), 2.84(0.94), 2.29(0.69)  
 Composted Broiler Litter (62 samples): 2.80(0.98), 3.00(1.00), 2.30(0.83)  
 Fresh Layer Litter (209 samples): 2.26(0.83), 3.16(1.34), 2.05(0.81)  
 Broiler Breeder Litter (325 samples): 2.12(0.79), 3.14(1.17), 1.93(0.63)

As indicated by the standard deviations, litter composition can be quite variable. One possible reason for the variability might be that the samples taken by farmers do not represent the true average nutrient composition of litter within houses because of spatial variability of nutrient

concentrations. In order to characterize this spatial variability, a broiler house was sampled at 30 uniformly spaced locations, and each sample analyzed for N and P concentrations. Both N and P concentrations varied nearly twofold. Results indicate that care must be taken in sampling poultry houses, by sub-sampling from areas such as feeders and waterers in proportion to their corresponding areas within a house. With the increased awareness of potential environmental impacts of land application of manures, poultry producers can more effectively utilize poultry litter within a NMP through improved sampling procedures.

*Key Words:* Poultry, Nutrient, Litter

## Extension and Instruction

**254 A poultry industry manager survey used to characterize employable skills for undergraduate students.** K. M. Downs\* and J. E. Mehlhorn, *Middle Tennessee State University*.

A 27-question survey instrument was developed to assess skills important to poultry managers in achieving success in the poultry industry. Twenty questions focused on evaluating subject matter competencies and employable skills. Seven questions evaluated the importance of specific recruitment efforts. A 1-5 Likert scale (1=unimportant, 5=critically important) was used for response quantification. The survey instrument was tested by 15 outside observers. Managers in all phases of the broiler and table egg industry (i.e., live production, processing, hatchery, feed mill, human resources, marketing, and distribution) were targeted. Surveys (one front page, blue paper) were mailed with an explicative cover letter, self-addressed stamped envelope, and appreciation gift (MTSU decal). At present, response rate has been low (56.2%). A majority (57%) of managers classified their current position as either live production or processing. Managers completing the survey (n=9) indicated teamwork skills (4.89) and integrity (4.89) as the most important employable skills, while originality (3.78) was considered the least important. Oral communication abilities were considered more important than written skills (4.33 vs. 4.00), and undergraduate major was moderately important in career success (3.00). Knowledge of business (3.89) and foreign language (2.67) were evaluated, respectfully, as the most and least important subject matter competencies. Likewise, departmental career fairs were considered the most important means of recruitment (3.89), while web-based job sites were considered only minimally important (2.75). In an effort to substantiate database validity, survey administration is ongoing. However, according to the current results, efforts to foster team-building skills, problem-solving abilities, and business acumen in

undergraduate students may enhance poultry career success. Moreover, industry-academia interface remains vitally important for student job placement.

*Key Words:* Poultry managers, Job placement, Undergraduate education

**255 An undergraduate laboratory course on animal cell culture techniques.** P. E. Mozdziak\*, *North Carolina State University, Raleigh NC*.

A new laboratory course emphasizing practical training in animal cell culture techniques has recently been developed at North Carolina State University. The new course is an advanced elective for the newly developed undergraduate minor in Biotechnology, which was first instituted in the fall of 2001. The course is also an elective for students in the Department of Poultry Science. The goal of the Animal Cell Culture Techniques course is for students to acquire the necessary practical skills for the isolation of animals cells for in vitro studies, maintenance of animal cells in vitro, manipulation of animal cells in vitro, and application of molecular techniques to in vitro situations. Avian cells are used as the model organism for most laboratory exercises. The course was first taught to a total of 6 graduate students in the summer session of 2001. It was successfully taught to a total of 9 undergraduate and graduate students in the spring of 2002, and there are currently 19 students registered for the spring of 2003 semester. Overall, student demand is greater than the facilities and faculty resources available to teach the course. Training in animal cell culture techniques is an essential opportunity for students studying Poultry Science and related disciplines.

*Key Words:* Tissue culture, Undergraduate education, Biotechnology

## Genetics

**256 Functional analysis of Salmonella genome for virulence genes using transposon sequence tag profiles.** M. M. Cox<sup>1</sup>, R. L. Ziprin<sup>2</sup>, L. F. Kubena<sup>2</sup>, D. J. Nisbet<sup>2</sup>, S. C. Ricke<sup>3</sup>, and Y. M. Kwon\*<sup>1</sup>, <sup>1</sup>*University of Arkansas, Fayetteville, AR*, <sup>2</sup>*USDA-ARS, College Station, TX*, <sup>3</sup>*Texas A&M University, College Station, TX*.

An increasing number of bacterial genomes have been completely sequenced. However, the biological functions of the sequences are largely unknown for the bacteria mainly due to the lack of a comprehensive method for functional screening of bacterial genome. We devised a quantitative method for functional analysis of bacterial genomes in genome-wide scale using transposon mutagenesis and sequence tags that are defined by their positions in the transposon-flanking sequences. We demonstrated that the identity and frequency of the sequence tags provide information on the level of each mutant in the pool. The results suggest that comparing the sequence tag profiles of the mutant pools before and after selection could allow identification of transposon mutants with competitive disadvantages. We applied this method to identify the Tn5 mutants of *Salmonella enteritidis* that are attenuated during infection in 5-day-old chicks. This method is a powerful approach for categorizing gene function that should be applicable to a variety of microorganisms.

*Key Words:* Bacterial genomes, Transposon, *Salmonella enteritidis*, Virulence genes, Chicks

**257 Relationship between PGC concentration and morphological parameters in early chick embryos.** C. Tomita, K. Nomura, and A. Tajima\*, *Institute of Agriculture and Forestry, University of Tsukuba*.

Stage 14 (Hamburger & Hamilton, 1951) chick embryos have been used to collect circulating primordial germ cells (PGCs) for producing germline chimeras. However, the recovery rate of circulating PGCs from the embryo varies considerably among replicates. The present study was conducted to elucidate the relationship between the concentration of circulating PGCs and morphological parameters in the stage 14 chick embryos. Three roosters and 12 hens were used in the present study. Each hen was artificially inseminated weekly with semen collected from same rooster. Collected eggs were incubated for 57.5-69.5 hours to obtain stage 14 embryos. Blood was collected from embryos using a fine glass pipette and blood cell (BC) as well as primordial germ cell (PGC) concentration was determined. After blood collection, embryo proper and vascular system surrounding embryo was removed altogether and the digital image was recorded. The morphological analysis of the embryo was conducted using the digital image. The total number of embryo samples used in the present study was 50. Average PGC and BC concentrations were  $15 \pm 1.8/\mu\text{l}$  and  $112,742 \pm 7,458/\mu\text{l}$ , respectively. Average circumference of marginal sinus, area pellucida and embryo proper were  $55.2 \pm 1.5\text{mm}$ ,  $31.6 \pm 0.8\text{ mm}$  and  $21.2 \pm 0.6\text{ mm}$ , respectively. A high positive correlation was observed among three morphological parameters. However, no significant correlation was observed between PGC or BC concentration and any of the 3 morphological parameters. On the other hand, a large variation was observed among embryos for all parameters observed in the

present study. The result from the present study showed that the size of the stage 14 embryo differed considerably among embryos and the concentration of circulating PGCs is not correlated with the morphological parameters used in the present study.

**Key Words:** PGC, Embryo morphology, Chick

**258 Estimation of growth parameters using a nonlinear mixed Gompertz growth model.** Z. Wang\* and M. J. Zuidhof, *Livestock Development Division, Alberta Agriculture, Food and Rural Development.*

In order to maximize the utility of simulation models for decision-making, accurate estimation of growth parameters and associated variance is crucial. Mature weight ( $W_m$ ) and rate of maturing ( $b$ ) were estimated for the Gompertz growth model, using a nonlinear mixed model approach. A total of 3266 body weight observations from 96 males and 96 females of six commercial broiler strains were used in the analysis. The  $W_m$  of each individual is considered a random effect in the model, and it accounts for heterogeneous BW variances and correlations across age. A comparison is made between the mixed and fixed effects models using the same data set. The average fit statistics of BIC (955.7 vs. 487.6 for female and 103.9 vs. 331.5 for male) indicate that the mixed model provides a much better fit to the data. The mixed model resulted in a significant reduction of the standard errors. In addition, the mixed effects model reduces the impact of selective sampling and provides an estimate of between-bird  $W_m$  variation.

**Key Words:** Broiler, Nonlinear mixed model, Gompertz growth model, Growth parameters

**259 Ghrelin gene sequence and expression in lines of chickens selected for high and low body weight.** A. Y. Kuo\*<sup>1</sup>, C. M. Ashwell<sup>2</sup>, M. P. Richards<sup>2</sup>, S. M. Poch<sup>2</sup>, P. B. Siegel<sup>1</sup>, and D. M. Denbow<sup>1</sup>, <sup>1</sup>*Virginia Polytechnic Institute and State University*, <sup>2</sup>*Growth Biology Laboratory, USDA-ARS.*

Ghrelin, a recently discovered neuropeptide, is a natural ligand for the growth hormone secretagogue receptor. Ghrelin, which is secreted mainly from the gastrointestinal tract stimulates food intake in mammalian species. The study report here was conducted to investigate possible differences in chicken ghrelin gene sequence, and gene expression in lines of chickens that had undergone 46 generations of selection for high (HWS) or low juvenile body weight (LWS). Utilizing reported chicken ghrelin mRNA sequence information from broiler proventriculus (GenebankAB075215), primers were designed to amplify pancreas cDNA from Leghorns by PCR. Using these PCR products, it was found that Leghorn ghrelin contained 8 extra base pairs in the 5' untranslated region (UTR). Then, based on broiler and Leghorn ghrelin sequences, primers were designed to amplify proventriculus cDNA from HWS and LWS chicken using PCR. Both HWS and LWS chickens have 6 extra base pairs at the 5' UTR, similar to that of Leghorns. Ghrelin gene expression levels in HWS and LWS chickens were detected using Real Time quantitative PCR. There was a line by sex interaction for ghrelin expression levels. In the HWS line ghrelin gene expression level was significantly higher in males than females, and for males ghrelin expression was significantly higher in line HWS than in LWS. In contrast to mammals, intracerebroventricular injection of ghrelin decreases feed intake in chickens. Therefore, it appears, that ghrelin has a different effect on feed intake in birds than mammals. In conclusion, there is a difference in the ghrelin gene sequence between various types of chickens, and selection for high or low body weight has influenced expression of the ghrelin gene.

**Key Words:** Gene expression, Ghrelin, mRNA, PCR, Proventriculus

**260 Rfp-Y haplotypes characterizing NIU White Leghorn parent lines 1 and 4.** R. Kopulos\*<sup>1</sup>, W. E. Briles<sup>1</sup>, and M. Miller<sup>2</sup>, <sup>1</sup>*Department of Biological Sciences, Northern Illinois University, DeKalb, IL 60115*, <sup>2</sup>*Beckman Medical Research Institute, City of Hope, Duarte, CA 91010.*

The chicken major histocompatibility complex (MHC) is comprised of the *B* system and the *Rfp-Y* system. Both are located on chromosome 16 even though they are genetically independent. The second system, *Rfp-Y*, was found by DNA hybridization to contain a cluster of additional MHC genes. Since its discovery, the *Rfp-Y* has been demonstrated to influence immune response in several disease challenge trials. NIU White Leghorn lines 1 and 4 serve as parental lines to produce cross-line progeny

for evaluating immune response to selected immunological challenges. The lines transmit segregating alloantigen haplotypes of eight non-MHC gene systems. To define the *Rfp-Y* haplotypes in lines 1 and 4, Southern blot analysis was done with a *YF*-specific probe, *163/164f*, using DNA digested with *BglI* enzyme. Each of the parent lines possesses at least four *YF* haplotypes, with 3 haplotypes in common between the two lines. Having the *Rfp-Y* haplotypes identified in addition to the regular *B* types will allow segregation among cross-line progeny of two independently segregating portions of the total MHC, plus haplotypes of the *A*, *C*, *D*, *E*, *H*, *I*, *L*, and *P* alloantigen systems. Immunological challenge of the crossline progeny will allow simultaneous determination of genotypic effects on specific immune response associated with *Rfp-Y* along with each of the alloantigen systems, including possible interactions between genotypes at the several loci.

**Key Words:** Major histocompatibility complex (MHC), *Rfp-Y*, Immune response, Alloantigen systems

**261 The identification of MHC haplotypes in bobwhites using hemagglutination and single-stranded conformational polymorphism (SSCP).** L. Yates\*<sup>1</sup>, M. Miller<sup>2</sup>, and W. E. Briles<sup>1</sup>, <sup>1</sup>*Department of Biological Sciences, Northern Illinois University, DeKalb, IL 60115*, <sup>2</sup>*Beckman Medical Research Institute, City of Hope, Duarte, CA 91010.*

Maintenance of diversity at the major histocompatibility complex (MHC) may be important to the survival and fecundity of small breeding populations of endangered species. The MHC of the chicken was initially discovered as an erythrocyte alloantigen system by using antisera prepared by alloimmunizations. Northern bobwhites and the endangered masked bobwhites are maintained at NIU for study of MHC genes, along with other non-MHC erythrocyte alloantigen systems. Hemagglutination testing using specially prepared alloantisera proves to be an extremely effective and inexpensive method for typing large numbers of birds. Antisera specific for B-F and B-G regions of the chicken MHC were tested on bobwhites. Differential reactivity patterns showed genetic segregation of MHC alleles within bobwhite families. Matings and immunizations within families were designed based on these hemagglutination data. The harvested alloantisera were subsequently tested on all bobwhite families. The production of alloantisera within bobwhites is hindered due to the small quantity that is obtained and that many non-MHC erythrocyte alloantigen systems have yet to be characterized. Therefore SSCP analysis was used in conjunction with hemagglutination testing to determine MHC haplotypes in bobwhites. PCR with primers designed for specific chicken B-F and B-L gene regions were applied to bobwhite DNA. The PCR products were sequenced and a blast search was done to confirm that MHC genes were amplified. The B-L specific primers generated the expected single PCR product of 277 bp as seen in the chicken. SSCP analysis revealed distinct B-L gene differences in the pedigreed bobwhites. The combination of hemagglutination and SSCP analysis will be useful in evaluating the role of segregating MHC haplotypes in wild populations of bobwhites.

**Key Words:** Major histocompatibility complex (MHC), Alloantigen, Single-stranded conformational polymorphism (SSCP), Bobwhite

**262 Disclosure of immune effects associated with non-MHC alloantigen systems.** W. E. Briles\*, <sup>1</sup>*Department of Biological Sciences, Northern Illinois University, DeKalb, IL 60115.*

Alloantigens are cell surface molecules that are immunogenic among certain individuals of a species. The *A* system of alloantigens was discovered in 1950 along with the *B* system, now designated as the major histocompatibility complex (MHC). Subsequently, systems *C*, *D*, *E*, *H*, *I*, *J*, *K*, *L*, *P* and *R* have been reported. All segregate independently of the others except that *E* is linked to the *A* system by 0.5% crossing over. Data indicating the existence of immune effects of one or more of these alloantigen systems have been reported from two distinctly different types of experimentation. Initially the procedure was to observe the alloantigen haplotype frequency in lines differing in a trait of interest or to observe the degree of gene frequency change following selection for a particular trait or a response to an immunological challenge. A shift in gene frequency following continuous selection is considered to be indicative of possible alloantigen haplotype function. Such data have been reported for the *A*, *C*, *D*, *E*, *H*, *I*, and *L* systems. A second, more genetically definitive experimental determination of immunological effects, consists of comparative data for alloantigen genotypes within families, including

possible interaction between systems. A limited number of recent within-family comparisons have disclosed immunological effects of genotypes for the *C*, *D*, *I*, *L* and *P* systems. The data comparing genotypes within families stems primarily from comparisons utilizing progeny produced by crossing NIU line 4 males with NIU line 1 females. Each line possesses two or more alleles for each of the *A*, *B*, *C*, *D*, *E*, *H*, *I*, *L*, and *P* systems, allowing challenge performance among genotypes within families for each of the nine different alloantigen systems. Additional alloantigen systems will be incorporated into the parent lines as circumstances permit.

**Key Words:** Alloantigen, Genotypes, Haplotype, Immune effect

**263 Genetic polymorphisms in Guinea Fowl and chickens revealed by random amplification of polymorphic DNA (RAPD) and simple sequence repeat based primers.** S. Nahashon\*, N. Adefope, A. Amenyenu, and D. Wright, *Cooperative Agricultural Research Program, Tennessee State University.*

Information on genetic relationships in livestock both within and between species has several important applications when designing nutritional, genetic improvement and breeding programs. In the present study, randomly amplified polymorphic DNA (RAPD) and simple sequence repeat (SSR)-based amplification of genomic DNA were used to evaluate genetic polymorphisms and diversity within and between populations of chicken and guinea fowl. Blood samples (0.5 mL) were collected in 0.5M EDTA by brachial venipuncture and DNA was isolated using Dr. Gentle<sup>®</sup> DNA Isolation kit (Takara Bio Inc., Japan). DNA from ten birds of either sex was pooled within chicken and guinea fowl populations and eight pools from each population were analyzed for polymorphism. Fifteen and ten arbitrary and SSR-based primers, respectively, were used to amplify DNA fragments using the Polymerase Chain Reaction (PCR). PCR products were analyzed on 1.2% agarose gel. The amplified fragments in both species ranged in molecular weight from 200 to 2650 base pairs. A total of 56 fragments in chicken and 59 in guinea fowl were amplified, of which 19 and 8% in chickens and guinea fowl, respectively, were polymorphic. Average band sharing frequency was higher within chicken and guinea fowl populations (0.90 to 0.98 and 0.89 to 0.98, respectively) than between the two populations (0.00 to 0.46). Based on this study, the RAPD and SSR based amplification of genomic DNA seem to reveal more polymorphisms within chickens than guinea fowl populations. These findings also reveal high genetic diversity between chicken and guinea fowl populations.

**Key Words:** Guinea fowl, Chickens, Genetic polymorphisms, Genetic diversity

**264 Mapping the chicken glycoprotein pituitary hormone alpha subunit gene.** J. Yang\*, R. Okimoto, K. Scarbrough, and J. Kirby, *Center of Excellence for Poultry Science, University of Arkansas, Fayetteville, AR 72701.*

The pituitary glycoprotein hormones (luteinizing hormone, follicle-stimulating hormone, and thyroid-stimulating hormone) are composed

of two subunits called the alpha subunit and beta subunit. The chicken glycoprotein pituitary hormone alpha subunit (GPHA) is identical in all three hormones, but the beta subunit is hormone specific. Primers were developed from the cDNA sequence of the chicken alpha subunit, which amplified a 1.7kb fragment that contained two partial exons and one intron. This fragment was amplified from Red Jungle Fowl and White Leghorn DNA, parental types of the East Lansing Reference Population. The fragments were then gel purified, sequenced, and analyzed. A Nla III restriction site single nucleotide polymorphism (SNP) was present in the RJF that was not present in the WL. PCR amplification and restriction enzyme digestion were performed on the East Lansing reference population, and the results were used in mapping the GPHA gene. It is located between the known genes GSTA2 and ME1 on Chromosome 3.

**Key Words:** GPHA, SNP, Mapping, Chicken

**265 Isolation and sequence determination of microsatellites from ducks (*Anas platyrhynchos*).** M. Nagai\*<sup>1</sup>, H. Nakasu<sup>1</sup>, T. Tozaki<sup>2</sup>, T. Hasegawa<sup>3</sup>, M. K. Akbar<sup>4</sup>, and K. Maruyama<sup>1</sup>, <sup>1</sup>Meiji University, Kawasaki, Japan, <sup>2</sup>Laboratory of Racing Chemistry, Utsunomiya, Japan, <sup>3</sup>Japan Racing Association, Utsunomiya, Japan, <sup>4</sup>Maple Leaf Farms, Indiana, USA.

Microsatellites are polymorphic, short tandem repeats and needed for the construction of linkage map and QTL analysis, and for the individual and family identification. In chickens, swine, and cattle, a large number of microsatellites have been isolated and utilized for the above purposes. However, in ducks, only seven microsatellites have been reported to date, not sufficient even for the use in individual identification. In this study, we constructed an enrichment library for CA-repeats from duck genome, picked microsatellite clones and determined their DNA sequence to complete the isolation and sequence determination of CA-microsatellites. Genomic DNA was prepared from dry blood cells of one female commercial hybrid Pekin duck. After genomic DNA was digested with *Sau*3AI, DNA fragments were ligated to a pair of adapters compatible to *Sau*3AI ends. DNA fragments were amplified by PCR with the same adapters as the primer. PCR products were probed for CA-repeats within their sequences with a (CA)<sub>10</sub> biotin-labeled oligonucleotide. PCR products with CA-repeats were retrieved using streptavidin-coated magnet beads. CA-microsatellites were released from streptavidin by washing with alkaline buffer at 80°C to complete the enrichment. Microsatellites were amplified by PCR with the adapter-primers and were subcloned into the T-vector by topoisomerase. Microsatellite plasmids were introduced in *E. coli*, JM109 for DNA propagation and sequence determination. In total, 186 microsatellite clones have been picked and DNA sequence determination is in progress. The sequence determination has been completed in more than 60 clones. Thus far, every single clone from the enrichment library was a microsatellite with CA-repeats. For each microsatellite, a primer pair was being designed and evaluated for the use in individual identification.

**Key Words:** Microsatellite, CA-repeat, Ducks, Genome, Individual identification

## Immunology

**266 WITHDRAWN.** , .

**267 Field evaluation of Mycoplasma gallisepticum vaccines in broiler breeder chickens.** Salah Mousa\*, Mostafa Saif-edin, and Mohamed Aly, *Fac. Vet. Med. Assiut Univ.*

Different Mycoplasma gallisepticum (*M. gallisepticum*) living vaccines were compared in meat type breeder chicken flocks under field conditions. The living vaccines (F, ts71 1, and 6/85 strains) together with inactivated vaccine were applied before egg production in Mycoplasma free flocks. Seroconversion as measured by ELISA, haemagglutination inhibition and serum plate agglutination tests; transmission of *M. gallisepticum* in eggs; egg production, and hatchability rate indicated that living as well as inactivated vaccines were protective. On the other hand, the F strain vaccine produced superior seroconversion and good protection than other types of *M. gallisepticum* vaccines. The statistical analysis of results of egg production, hatchability rates, and cumulative mortalities indicated a significant difference ( $p < 0.05$ ) between vaccinated

groups and the nonvaccinated control group. However, no significant difference was observed between vaccinated groups. The body weights and protection against challenge were higher in chicks derived from vaccinated parents as compared with chicks derived from nonvaccinated control parents.

**Key Words:** Mycoplasma gallisepticum, Mycoplasma vaccines

**268 Field evaluation of a novel bivalent vaccine against Infectious Bursal Disease (IBD) and Newcastle Disease (ND) by mixing viruses and antibodies contained in hyperimmune egg yolk.** Heba Mousa, *Assiut Univ., Egypt.*

A bivalent vaccine against Infectious bursal disease (IBD) and Newcastle disease (ND) was prepared by mixing viruses with antibodies contained in hyper immune-egg yolk. Experiments were conducted to test the efficacy of the vaccine in presence of maternally derived antibodies. The vaccine was evaluated after injection of one-week old commercial chicks by subcutaneous route. Serum samples were collected weekly and subjected to HI and Elisa tests. The vaccine initiated high immune response