

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® 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*¹, H. Nakasu¹, T. Tozaki², T. Hasegawa³, M. K. Akbar⁴, and K. Maruyama¹, ¹Meiji University, Kawasaki, Japan, ²Laboratory of Racing Chemistry, Utsunomiya, Japan, ³Japan Racing Association, Utsunomiya, Japan, ⁴Maple 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)₁₀ 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

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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

as measured by immunogenicity criteria of HI test for ND, Elisa test for IBD and challenge with either ND or IBD viruses. HI titers and Elisa titers for IBD reached its peak at 2 weeks p.v. and lasted till 6 weeks of age. Chicks were protected from challenge with either NDV or IBDV.

Key Words: ND, IBD, Virus-antibody vaccine

269 Avian intestinal antimicrobial peptides. C.J. Nile*¹, C. Townes¹, G. Michailidis¹, B.H. Hirst¹, and J. Hall¹, ¹The University of Newcastle Upon Tyne, Newcastle Upon Tyne, UK.

Antimicrobial peptides are important mediators of innate disease resistance in tissues exposed to microbial pathogens such as the gastrointestinal (GI) tract. They provide a natural defence mechanism and act in protecting the host against infection. To identify novel cationic antimicrobials in the avian GI tract, intestinal material from five day old chicks was extracted in 60% acetonitrile (ACN): 1% trifluoroacetic acid (TFA) and subjected to reverse phase HPLC. Elution was performed on a Vydac C18 column using 0.18% TFA in water and a gradient of ACN. Fractions were collected and assayed for antimicrobial activity using a phoP *Salmonella* mutant sensitive to antimicrobial peptides. Fractions showing antimicrobial activity were further analysed by C8 chromatography and putative peptides identified by MALDI-TOF-MS. One such peptide, designated Avian AMP-1, is 76 amino acids in length, contains a putative signal peptide and regions of basic and hydrophobic amino acids common to cationic antimicrobial peptides. In addition the C terminus of the peptide contains four cysteine amino acids. The pattern of the cysteines is unique and suggests Avian AMP-1 belongs to a family of as yet uncharacterised antimicrobials. RT-PCR analyses of RNA extracted from chick tissues showed that this peptide was expressed in the intestines of five-day old birds and 18-day old chick embryos. This may be related to its putative function of preventing microbial infection or consistent with additional, as yet unknown, functions of the encoded peptide. These data suggest that the chick intestine is a source of novel peptides with antimicrobial activity.

Key Words: Intestine, Antimicrobials, Peptides

270 Appearance of carotenoids in avian macrophage cell lines is time- and cell-line dependent. E. Koutsos*, California Polytechnic State University, San Luis Obispo, CA.

Three avian macrophage cell lines (HD11, HTC, and MQNCSU) were examined for their ability to accumulate carotenoids from media. Each cell line was proliferated in media (RPMI 1640) containing fetal bovine serum (no detectable carotenoids). After 4 d, cells (1×10^6 cells/well) were plated in a 6-well plate (2 wells/cell line/plate) in 5 cc media containing 10% chick serum. Serum was collected from 3 wk old broiler chicks fed 38 mg lutein/kg diet, and contained $57.2 \mu\text{mol}$ lutein/mL + $5.9 \mu\text{mol}$ zeaxanthin/mL. Cells were scraped, collected, washed and counted from 6 wells/cell type (3 plates/time) at 0, 12, 24 and 48 h post-plating. MQNCSU cells were also collected at 72 h, and HD11 cells were collected at 72 and 96 h post-plating; for these cells, media was refreshed by adding 3 cc media at 48 h. All cell lines contained lutein, but a cell line x time interaction ($P < 0.01$) demonstrated that at 24 h, HD11 and MQNCSU contained more lutein than did HTC cells ($P < 0.01$). At 48 h, HTC cells had higher lutein concentration than did HD11 ($P < 0.01$), which had higher lutein concentration than did MQNCSU ($P < 0.01$). Compared to the same cell line at 0 h, MQNCSU cells had significantly increased lutein at 24 and 72 h ($P < 0.05$ for each), HD11 cells had increased lutein at 24, 72, and 96 h ($P < 0.05$ for each), and HTC cells had increased lutein at 48 h ($P < 0.05$). HD11 and MQNCSU, but not HTC cells, contained zeaxanthin, which was increased for both cell line at 24 h ($P < 0.05$) but not at other time points. MQNCSU cells (24 h) contained $7.0 \times 1.8 \times 10^{-10}$ nmol lutein/cell + $1.2 \times 0.3 \times 10^{-9}$ nmol zeaxanthin/cell, HD11 cells (24 h) contained $2.2 \times 1.3 \times 10^{-9}$ nmol lutein/cell + $2.1 \times 0.6 \times 10^{-9}$ nmol zeaxanthin/cell, and HTC cells (48 h) contained $3.6 \times 2.5 \times 10^{-9}$ nmol lutein/cell and no detectable zeaxanthin. These data provide a framework for choosing appropriate cell types for *in vitro* carotenoid assays, in addition to the appropriate time course of carotenoid addition prior to sampling.

Key Words: Macrophage, Carotenoid, Lutein, Zeaxanthin

271 The effects of cloacal inoculation with *Salmonella typhimurium* on gene expression in the chicken bursa of fabricius. C. M. Oubre^{1,2}, N. Puebla-Osorio¹, J. Delrow³, B. D. Shamblyn², R. B. Moyes², and L. R. Berghman*¹, ¹Poultry Science Department, Texas A&M University, College Station, Texas, ²Biology Department, Texas A&M University, ³Fred Hutchinson Cancer Research Center, Seattle, WA.

The Bursa of Fabricius is unique to birds and is the anatomical site of differentiation and development of B-lymphocytes. It is essentially a dorsal diverticulum of the cloaca and is used by the bird to sample the environment through a natural reflex known as "cloacal drinking". The present study was aimed at describing the effect of cloacal inoculation with *Salmonella typhimurium* on the gene expression profile of the bursa of Fabricius using a chicken immune cDNA microarray available at the Fred Hutchinson Cancer Research Center (FHCRC). Four-day old chicks were challenged cloacally with *S. typhimurium* at 10^5 cfu/ml in a 0.5 ml inoculum and tissue was harvested at 30 minutes, three hours, and 24 hours post inoculation. Once collected, RNA extractions were performed on the samples and they were sent to the FHCRC for hybridization onto the immune cDNA microarray. The microarray data were analyzed with GenePix for the identification of genes that were significantly up- or down regulated by bursal challenge with *Salmonella*. Numerous genes showed significant change as compared to the unchallenged control, ranging from two-fold decreases to ten-fold increases in gene expression. Results showed marked increases in expression of genes such as: the chicken Ig rearranged light chain, Ig a heavy chain, Interferon regulatory factor, lysosomal transmembrane protein, and Il-6-, CD9-, and CD63-related genes. Increased expression of chicken Ig rearranged light chain is in line with a recent report suggesting that intra-bursal antigenic stimulation plays a role in the selective amplification of B-cells with a productive V-J rearrangement. This amplification may be facilitated by an increase in Il-6 production which can play the role of B-cell growth factor. The expression profile of a number of pivotal genes will be verified by real-time PCR and a more refined working hypothesis will be developed.

Key Words: Cloacal inoculation, Bursa of Fabricius, *Salmonella typhimurium*

272 Changes in delayed type hypersensitivity, egg antibody content and immune cell fatty acid composition of layer birds fed conjugated linoleic acid, n-6 or n-3 fatty acids. R. K Selvaraj* and G. Cherian, Oregon State University, Corvallis, OR, USA..

The effects of dietary conjugated linoleic, n-6 or n-3 polyunsaturated fatty acids on delayed type hypersensitivity response (DTH), egg yolk antibody content, immune tissue fatty acid profile and lipid oxidation products of layer birds were investigated. One hundred and twenty thirty-week-old single comb white leghorn laying hens were distributed randomly to four treatments (3 replications of 10 birds per replication) and were fed diets containing conjugated linoleic acid (CLA) (Diet I), sunflower oil (Diet II), canola+flax oil (Diet III) or fish oil (Diet IV). The total lipid content of the diet was 3%. Birds fed Diets III and IV had higher content of n-3 fatty acids in lymphocyte and splenocytes than Diet I and II. Thiobarbituric reactive substances were higher ($P < 0.05$) in the breast and thigh muscle of Diet IV-fed birds than other diets. Serum and yolk anti-BSA antibody contents were higher ($P < 0.05$) in birds fed Diets III and IV when compared with Diets I and II. DTH response, measured as the foot pad thickness increased in Diets III and IV when compared with birds fed Diet I and II ($P < 0.05$). The number of peripheral blood lymphocyte CD4^+ and CD8^+ cells and the splenocyte mononuclear cell CD4^+ , CD8^+ and IgM^+ cells did not differ ($P > 0.05$) between treatment groups. Feeding n-3 fatty acids increased antibody mediated immune response while n-6 fatty acids and CLA increased cell mediated immune response.

Key Words: Polyunsaturated fatty acids, Delayed type hypersensitivity, Immunoglobulins