**Annual Report for NC1170**
Period Covered: January 1, 2015 to December 31, 2015
Prepared by: Douglas Rhoads

**Institutional Stations (Institutional Abbreviation: Members)**
Beckman Research Institute at the City of Hope (**COH**: M. Miller)
Cornell University (**CU**: P. Johnson)
Iowa State University (**IA**: S. Lamont, J.Dekkers)
Michigan State University (**MI**: J. Dodgson, G. Strasburg)
Mississippi State University (**MS**: C. McDaniel, B. Nanduri)
North Carolina State University (**NC**: C. Ashwell, J. Petitte)
Oregon State University (**OR**: D. Froman)
Pennsylvania State University (**PA**: A. Johnston, R. Ramachandran)
Purdue University (**IN**: W. Muir)
University of Arizona (**AZ**: F. McCarthy, S. Burgess)
University of Arkansas (**AR**: W. Kuenzel, B. Kong, D. Rhoads)
University of California, Davis (**CA**: M. Delany, H. Zhou)
University of Delaware (**DE**: B. Abasht)
University of Georgia (**GA**: S. Aggrey)
University of Maryland (**MD**: T. Porter, J. Song)
University of Minnesota (**MN**: K. Reed)
University of Tennessee-Knoxville (**TN**: A. Saxton, B. Voy)
Virginia Tech (**VA**: E. Wong, E. Smith)
University of Wisconsin (**WI**: G. ROsa)
USDA-ARS-Avian Disease and Oncology Lab (**ADOL**: H. Cheng, H. Zhang).

**Administration**

Executive Director- Jeff Jacobsen, Michigan State University
Lakshmi Matukumalli- NIFA Representative
Christina Hamilton- System Administration.

**NC1170 Business Meeting January 10, 2016**

NC1170 BUSINESS MEETING 2016

Agenda:

1. Call to order
2. Approval of Agenda
3. Approval of 2015 minutes
4. Administrative Advisor: Sue Lamont
5. NIFA/USDA administrator: Lakshmi Matukumalli
6. NRSP8 Poultry co-Coordinators: Delany and Cheng

7. Selection of secretary for NC1170
8. Selection of secretary for NRSP8 poultry
9. Location and time for meeting for 2017
10. Other business
11. Adjourn

Poultry Workshops Plant and Animal Genome, San Diego, CA; January 9 and 10, 2016:

1. Attendance: Saturday AM=100; Sunday AM= 90; peak attendance >120
2. Representatives of 15 stations attended at some point during the weekend
3. One survey showed 1 or more representatives from 22 other institutions including the poultry industry, U.S. government, the United Kingdom, European Union, Thailand, and China
4. Guest Speakers: Kim Pruitt (NCBI), Wes Warren (Washington University), Sandrine Lagarrigue (INRA), Jim Reecy (IA State), Petunia Malatji (Agricultural Research Council, South Africa), Gota Morota (University of Nebraska), Michele Boichard (INRA), Lel Eory (Roslin, UK); Angelica Van Goor (IA State; Jorgenson Travel award winner)
5. Six graduate students gave short presentations about their posters.
6. Updates: J Reecy on NRSP8 bioinformatics; W. Warren on chicken genome reference updates.
7. 19 NC1170 members presented research updates

Business meeting Sunday January 10, 2016.

Attendees:

(DE) Abasht, Behnam, (ADOL) Cheng, Hans, (MI) Strasburg, Gale, (AR) Kong, Byung−Whi, (AR) Kuenzel, Wayne, (IA) Lamont, Susan J., (AZ) McCarthy, Fiona, (MN) Reed, Kent, (AR) Rhoads, Douglas, (MD) Song, Jiuzhou, (TN) Voy, Brynn, (VA) Wong, Eric A., (ADOL) Zhang, Huanmin, (CA) Zhou, Huaijun

Administrators: Lamont, Sue (Admin Advisor), Matukumalli, Lakshmi (NIFA/USDA administrator)

Guests: Fulton, Janet (Hyline)

1. Convened 11:30 AM in San Diego, CA, Sunset Room, Town and Country Convention Center.
2. Minutes from 2015 approved as modified for spelling errors
3. Administrative Advisor: Sue Lamont
    a. Project is going well.
    b. New members have added a lot.
    c. Mid term report was submitted by Sue Lamont. Project was rated highly, but the system changed it to “fair” because of a glitch in the system.
4. Lakshmi Matukumalli :
    a. RFP for 2016 not yet available. Maybe end of this month or in February.
    b. Working on more “inter agency” grants so expect something to come out this year.
    c. There may be a microbiome pilot program in Animal Health.
5. NRSP8 Poultry co-Coordinators: Delany and Cheng
    a. Funding is secure. Small but steady pot of money.
    b. Supported a few small projects, and some travel.
    c. With move to UC Davis we are allowed to have some carry over which is good.

d. Members need to put in requests for pilot studies or small projects that will impact the project. Needs rationale to help the group. Need ideas for leveraging the coordinator funds
e. Members can expect some partial travel money for students/postdocs to attend PAG, but members need to request.

6. Next meeting of NC1170 will be coordinated with PAG at Town and Country in January 2017.
7. NC1170 Leadership for coming year: Behnam Abasht - leader, Gale Strasburg – secretary
8. NRSP8 Poultry for coming year: Huaijun Zhou – leader, Kent Reed – secretary.
9. Other Business: none

**Accomplishments by Objective**

This document summarizes by objective the major accomplishments achieved by the NC1170 Multistate Research Project covering 2015. Achievements included the application of next generation technologies in sequencing to poultry genomes and numerous contributions to advances in bioinformatics, annotation, and transcriptomics as applied to a variety of fundamental disciplines in poultry biology. Our work is conducted under the auspices of many collaborative arrangements with stakeholders involved in the allied poultry industries for the purpose of improving poultry production. The scholarly publications resulting from this project (see appendix) includes over 87 articles in peer-reviewed journals and books/chapters. Special note should be made of the large number of publications involving collaboration between members of this project as well as scholars from around the world. Participants were listed as PI or coPI on $28,418,106 in funding/grant support that was active during 2015. During the past three years this project has added new participants from Cornell University, Mississippi State University, Pennsylvania State University, and University of Tennessee-Knoxville, to a current membership of 32 participants.

**Objective 1: Create and share data and technology to enhance the development and application of genomics and systems biology in poultry.**

**ADOL**

Enhance chicken genetic map. Genetic maps and, in particular those from East Lansing and Wageningen, form the foundation for the chicken genome assembly. Despite being the first agricultural animal to have its genome sequence in 2004, and many additional revisions that have included the latest technologies (e.g., Moleculo, PacBio, optical mapping), there are still gaps with the most notable being the lack of any assemblies for 8 microchromosomes. To improve the genetic map, ADOL unassigned sequence contigs from the latest genome assembly build (galGal 5) were surveyed. This screened identified 9,585 contigs 2 Kb or larger in size of UCD001 (Jungle Fowl and one parent of the East Lansing mapping population); theoretically, this would only miss ~1.4 Mb of the 76.6 Mb unassigned to the chicken genome. Aligning existing UCD003 (White Leghorn and other parent of the East Lansing mapping population) reads, they identified 4,160 scaffolds that cover ~39.6 Mb with at least 1 SNP of reasonable confidence; thus, 5,425 scaffolds that account for ~37 Mb had no SNPs for potential genetic mapping. Further filtering based on Affymetrix design scores gave 5,907 SNPs on 547 contigs that could be potential genotyped. However, in the end, 3,440 SNPs and 510 contigs could be assayed and successfully scored. Following genetic mapping, 3,437 SNPs were assigned to 29 new linkage groups (called E101 to E129), none of which were linked to existing markers. Three SNPs were unlinked and assigned to E00.

This information is being used to build new sequence contigs for the genome assembly. Hopefully many will be for the “missing” microchomosomes, which subsequently could be confirmed by FISH. Additional rounds of genetic mapping and assemblies including longer PacBio reads should facilitate the completion of the chicken genome assembly. And as the chicken genome forms the foundation for many other avian species, this improved assembly will also aid these efforts.

**AZ**

Further development of AgBase. AgBase (http://www.agbase.msstate.edu/) provides resources to facilitate modeling of functional genomics data and structural and functional annotation of agriculturally important animal, plant, microbe and parasite genomes. AgBase currently (15 October 2015) provides 1,539,447 GO annotations for 310,971 gene products from 504 species, including more than 40 agriculturally important species and their pathogens. This information includes 392,101 GO annotations for 57,589 avian gene products, 96% of which are associated with chicken and turkey. During 2015 manual biocuration focused on extending detailed, literature-based functional annotations to well studied genes in additional livestock and aquaculture species, including turkey. With the release of more than 40 bird genomes from the Avian Phylogenomics project, we are also comparing genes and transferring functional information to other avian species.

Improvement of iAnimal. As part of the USDA funded iAnimal Project AgBase personnel also developed functional analysis pipelines within iPlant. 2015 saw the deployment of improved tools to support (a) functional annotation of large scale transcript data and (b) GO and pathways enrichment analyses of differentially expressed data. Future work will focus on documentation and beta-testing with experimental data sets

Development of a Host Pathogen Interaction Database. In 2015 the Host-Pathogen Interaction Database (HPIDb) extended manual biocuration to include host-pathogen interaction data, supporting network modeling of animal health data sets. HPIDB currently provides 2,478 manually curated molecular interactions, including host-pathogen interactions from 28 different livestock pathogen strains. Future work with collaborators at Mississippi State focuses on developing tools to support interaction prediction.

Improvements in gene nomenclature. The Chicken Gene Nomenclature Committee (CGNC) biocurators worked closely with NCBI Entrez Curators to ensure that updated gene annotations had revised nomenclature. During 2015 CGNC reviewed and updated 2, 735 genes (including > 100 genes annotated in conjunction with NCBI Entrez curators). CGNC currently provide standardized nomenclature for 22,172 chicken genes and there is a pending grant application to support continued annotation of bird genes. CGNC especially thanks avian researchers who have sent feedback or lent their expertise to this project.

Chicken expression atlas. The Chickspress resource (http://geneatlas.arl.arizona.edu/) provides a detailed “atlas” of chicken gene expression, collating experimental information from Red Jungle Fowl and chicken gene expression studies. Initial mRNA analysis identified 66,309 transcripts, of which 35% differ from current gene models provided by NCBI or Ensembl; most represent alternate transcripts from predicted gene models. Also identified from these same tissues were 16,496 miRNAs, including15,291 novel miRNAs. Target prediction is complete for these novel miRNAs. Likewise proteomics was used to identify 16,003 NCBI and Ensembl proteins from these same tissues, as well as 77,418 peptides that are not supported by current chicken protein sequences. An ongoing collaboration with INRA researchers (lead by Elisabetta Giuffra) is investigating miRNA an lcnRNA expression.

**CA**

Genome-wide functional annotation of regulatory elements in livestock species. Within a USDA funded FAANG project (H. Zhou, PD) through a partnership of the University of California at Davis (H. Zhou, P. Ross, I. Korf, M. Delany, J. Medrano, Alison Van Eenennaam), Iowa State University (C. Tuggle), USDA ARS ADOL (H. Cheng), and Michigan State University (C. Ernst). The overall objective of this project is to functionally annotate genome-wide regulatory elements in farm animal species. The identification of regulatory elements is a key step in understanding how an organism's genotype determines the phenotype. The technologies and assays developed in human and mouse ENCODE projects provide a solid foundation to functionally annotate chicken, pig and cattle genomes. They are major farm animals in providing the world's food production. Robust functional annotations of their genomes could be leveraged to improve the production efficiency of these industries. We will present the current progress in generating and analyzing data from these three important species, including analysis of forty-eight RNA-seq libraries (sixteen per species) collected from two biological replicates across eight tissues: adipose, cerebellum, cortex, hypothalamus, liver, lung, muscle and spleen. Transcripts detected in these tissues show good coverage of the Ensembl gene sets for the three species, and an initial analysis has identified putative long non- coding RNAs, both tissue-specific and expressed across all tissues. For chicken, an analysis of eight DNase-seq libraries from liver and spleen (two replicates) and cerebellum, lung, muscle and adipose (one replicate) show that identified tissue-specific DNase hypersensitivity (DHS) sites are associated with genes that relate to unique biological functions of the organs or tissues. Integrative analysis of DHS sites, ChIP-seq (H3K4me3 and H3K27me3 histone modification marks), and RNA-seq allows the identification of genome-wide active and inactive promoter regions in chickens, which enables an in-depth comparison of the regulatory landscapes of multiple tissues within these species.

Inducible overexpression and targeted genome editing of chicken IRF7 in DF-1 cell line**.** In order to develop new antiviral strategy against more virulent pathogens emerged from the birds, a better understanding of antiviral host response mechanism is essential. Type I interferon (IFN) is the first line of antiviral response and interferon regulatory factors (IRFs) are the master modulators of IFN signaling and it has been suggested that IRF7 plays an important role in the regulation of host immune response to Avian influenza viruses (AIV) infection in chickens. Our previous study revealed a link between chicken IRF7 and the regulation of RLR and TLR signaling pathways in response to dsRNA. In order to better understand underlying mechanism of how chicken IRF7 regulates the antiviral response against avian influenza virus (AIV), IRF7 conditional over-expression and knock-out DF-1 cell models were further developed. Cumate inducible over-expression system was chosen to avoid the antibiotics (eg. Tetracycline for Tet-on & off system) exposure to the cells during the in vitro pathogen infection. The expression level of IRF7 was linearly induced as increasing amounts of cumate were added and IFNB expression had significant positive correlation with the IRF7 induction. Multiplex genome editing approach was applied using CRISPR/Cas system to mediate targeted genomic deletion. Two spacers that are targeting near the start and stop codon of IRF7 were designed to induce concurrent double-stranded breaks (DSB) followed by nonhomologous end joining (NHEJ) to delete the complete IRF7 CDS on the genome (~3kb). The results revealed that 4.5% (4/88) of alleles had large targeted genomic deletion that does not have the IRF7 CDS in the clonal DF-1 colonies. Further In vitro AIV infection studies are followed by functional genomics approaches using these modified cell lines to elucidate the molecular mechanism of avian host immune response against AIV.

Marek's disease virus, vaccine and host genome interactions by cytogenetic profiling using Marek's resistant and susceptible genetic lines. Tumors from line 6x7 crosses were scored for heterogeneity and provided as cell suspensions suitable for cytogenetic profiling. The goal was to study in tandem the cytogenetics and molecular sequencing of the same samples. At UCD nearly 50 tumors were assessed for mitotic indices, profiles of null, associated MDVvirus, associated and integrated MDV

virus and integrated-only percentages. In addition although mapping studies have not been performed, the frequency of macrochromosomes exhibiting integrations has been determined at UCD.

Developmental Mutant Candidate Region Analysis: Wingless-2 Further fine mapping of the wg-2 mutation in congenic line birds was undertaken. Affected homozygous progeny display multisystem developmental abnormalities, most notably absent wings. Phenotypically normal heterozygotes (+/-) were bred after genotype determination by sequencing a tightly-linked variant (SNP 390) with transmission status confirmed by test crossing. Initial SNP studies in 2004 indicated a candidate region of 2 Mb on GGA 12 in the breeding population which was updated to a size of 260 kb five years ago using a study of mutant (-/-) progeny. However, since no genotype-specific selection criteria were imposed on the breeding scheme beyond SNP 390 genotype, the size of the *wg-2* candidate region segregating in the carrier population was unknown. Herein we set out to investigate whether recombination has further reduced the candidate region size in the breeding population. Two carrier populations were screened (2015 and 2014 parents) using twelve SNPs across the 260 kb region. Results indicate that recombination events have occurred and as such have further reduced the region linked to the *wg-2* phenotype to a minimum of 220 kb in the 2014 carriers. Test matings and sequencing at SNPs across that region in the 2015 progeny are under way to determine if additional events have occurred. As the candidate region shrinks in overall size, additional recombination becomes unlikely and so additional genomic technologies are also being employed to inform candidate gene analyses.

**COH**

Finishing sequence determinations for the MHC-*Y* gene region on GGA 16 (Miller, Goto, Schmidt, Delgado, Warden, Wu, Dalton, Balendiran, Torres, McPherson and Delany). Chicken major histocompatibility complex (MHC) genes are arranged in such a way that there are two specialized gene groups in separate regions with haplotypes that assort independently of each other even though the regions are relatively close to one another on the same arm of the GGA 16 microchromosome. These are the MHC-*B* region, which contains a small number of typical MHC genes and the MHC-*Y* regions, an unusual MHC region with no closely similar counterpart in human and mice. The MHC-*Y* region contains numerous specialized MHC class I (*YF*) genes that may present specialized forms of antigen, multiple c-type lectin-like loci, several class IIβ genes, and a few other genes[1-4]. MHC-*Y* is linked to additional interesting genes nearby including olfactory and scavenger receptor genes [5]. The *YF* genes are polymorphic, but encode molecules structurally similar to the monomorphic human molecule MR1. Human MR1 is now known to participate in recognition of microbes and to present microbial vitamin B metabolites to specialized T cells that respond to microbial infections [6]. In an effort to create sequence data for the *MHC-Y* region that might be used to enhance the application of genomics to poultry selection we isolated and sequenced a number of Red Jungle Fowl BAC clones for *MHC-Y*. Although extraordinarily difficult to sequence because of the large number of highly similar sequences, assembly of *MHC-Y* sequences for the BAC clones into two contigs was completed with Sanger sequencing by T. Shiina and K. Hosomichi at Tokai U. Because these Sanger sequences were very difficult to assemble additional sequencing was sought to verify these initial determinations and to complete sequencing of additional BAC clones. PacBio and Illumina sequence data proved very difficult to assemble *de novo*, but these data were valuable for verifying the high accuracy of the original assemblies. We are now confident that sequences of two contigs are correct. In addition to the gene sequences identified within MHC-*Y*, the region is filled with many repeat sequences (LTR and LINE sequences) that are interesting in themselves and, which have, without doubt, contributed to the challenge of sequence assembly. We initially assumed that Red Jungle Fowl MHC-*Y* would be contained within a single contig. FISH mapping suggests the Red Jungle Fowl *MHC-Y* haplotype (McPherson, Goto, & Miller) is actually an assemblage of at least three

discrete segments. A similar arrangement was previously noted in an unidentified MHC-Y haplotype [7]. We are now moving forward with the sequence data we have to complete a full analysis and publish the findings.

Structural studies of YF1*7.1 (Miller, Goto, Delgado, M. Soekamto, Gugui, Stadtmueller and Bjorkman). We hypothesize that YF class I-like molecules present non-peptide antigens. Given their similarity to mammalian MR1 molecules the YF class I molecules may be part of an immunosurveillance system for microbial pathogens. Because MHC Y class I genes are polymorphic different alleles may be associated with presentation of different ligands. Our goal is to understand what ligands are bound are bound by different chicken MHC-Y isoforms and to learn if structurally different YF isoforms bind different ligands. The first X-ray crystallography of the YF1*7.1 showed that it had the architecture typical of a classical MHC class I molecule, but that it possesses a somewhat hydrophobic and narrow (apparently too narrow to accommodate peptide) binding groove [8, 9]. An unidentified ligand (thought to be a laboratory-derived contaminating cationic detergent) was found within the binding groove. We are now working to define the natural ligands of YF1*7.1. During the past year we have developed the methods required for preparing large quantities of purified fusion-tag-free YF1*7.1 and β2-microglobulin proteins. These have been used to refold the YF1*7.1 with natural ligands. Mass spectrometry was used to identify candidate ligands. Candidate ligands in pure form were then tested for capacity to induce YF refolding. Structural studies are now underway to learn if the candidate ligand selected is present within the binding groove.

**IA**

Transcriptome data. Data from RNAseq experiments were deposited in public databases upon submission of the manuscripts describing those studies.

**MI**

Chicken genome sequence analysis and annotation. MSU has been examining the latest chicken genome sequence assembly (galGal4) and exploring approaches to fill gaps and obtain missing segments of the assembly (particularly on microchromosomes). Optical map and Moleculo sequence data have been obtained for the UCD001 reference bird (#256). These efforts parallel work at the Wash. U. Genome Center that is doing targeted gap filling and PacBio sequencing. In addition, a full manual annotation of the transcripts in the latest galGal4 reference sequence assembly is nearly complete.

**MS**

Manual biocuration of Agbase. AgBase is a curated, open-source, web-accessible resource for functional analysis of agricultural plant and animal gene products. AgBase facilitates post-genome biology for agriculture by providing data, tools, training and support to translate functional genomics data into gains for agriculture and society. AgBase uses vocabularies developed by the Gene Ontology (GO) Consortium to describe molecular function, biological process, and cellular component for genes and gene products in agricultural species. We provide 1,539,447 GO annotations for 310,971 gene products from 504 species, including more than 40 agriculturally important species and their pathogens (15 October 2015). This information includes 392,101 GO annotations for 57,589 avian gene products, 96% of which are associated with chicken and turkey. During 2015 we also continued manual biocuration of host-pathogen interaction data to support network modeling of animal health data sets. Via the Host-Pathogen Interaction Database (HPIDb) we provide 2,478 manually curated molecular interactions, including host-pathogen interactions from 28 different livestock pathogen strains. With collaborators at University of Arizona we are developing tools to support interaction prediction.

**NC**

Protein identification in the developing follicle. The avian ovary contains hundreds of follicles but has about 5 large yolky follicles arranged in a hierarchy based upon size from about 9 to 40 mm in diameter. The largest follicle is designated as the F1 follicle, which is the most mature and is destined for the next ovulation. A reproductively active domestic hen ovulates roughly every day, and an active ovary generally contains a succession of preovulatory follicles (F2–F5) that mature to the next size to take the place of the newly released F1 follicle. A new F5 follicle is recruited from a pool of 7–9 small yellow follicles (SYF) that range 5–9 mm in diameter. Below SYF, lies a pool of large white follicles (LWF) 2–5 mm in size that contain no apparent yellow yolk. The smallest and most immature of the avian ovarian follicles are the numerous small white follicles (SWF) that are less than 2 mm in diameter. Most research to understand the biology of the smallest follicles of the avian ovary has consisted of removing the follicles from the stroma of the ovary for the culture of whole follicles. To gain access to the smallest follicles and, if possible, apply global proteomics to the various ovarian compartments, laser microdissection (LMD) was utilized for the separation of the yolk, follicular wall (granulosa and theca), and surrounding stromal cells of small white follicles obtained from reproductively active domestic fowl. This study resulted in a total of 2889 proteins identified from the three specific isolated compartments. White yolk from the smallest avian follicles resulted in the identification of 1984 proteins, while isolated follicular wall and ovarian stroma yielded 2470 and 2456 proteins, respectively. GO annotations highlighted the functional differences between the compartments. Among the three compartments examined, the relative abundance of vitellogenins, steroidogenic enzymes, anti-Mullerian hormone, transcription factors, and proteins involved in retinoic acid receptors/retinoic acid synthesis, transcription factors, and cell surface receptors such as EGFR and their associated signaling pathways reflected known cellular function of the ovary.

**WI**

Assessment of bagging GBLUP for whole-genome prediction of broiler chicken traits. Bootstrap aggregation (bagging) is a resampling method known to produce more accurate predictions when predictors are unstable or when the number of markers is much larger than sample size, because of variance reduction capabilities. The purpose of this study was to compare genomic best linear unbiased prediction (GBLUP) with bootstrap aggregated sampling GBLUP (Bagged GBLUP, or BGBLUP) in terms of prediction accuracy. A 600K Affymetrix platform was used to genotype 1351 birds with phenotypic information on three traits in broiler chickens: body weight, ultrasound measurement of breast muscle and hen house egg production. The predictive performance of GBLUP versus BGBLUP was evaluated in different scenarios consisting of including or excluding the TOP 20 markers from a standard genome-wide association study (GWAS) as fixed effects in the GBLUP model, and varying training sample sizes and allelic frequency bins. Predictive performance was assessed via five replications of a threefold cross-validation using the correlation between observed and predicted values, and prediction mean-squared error. GBLUP over fitted the training set data, and BGBLUP delivered a better predictive ability in testing sets. Treating the TOP 20 markers from the GWAS into the model as fixed effects improved prediction accuracy and added advantages to BGBLUP over GBLUP. The performance of GBLUP and BGBLUP at different allele frequency bins and training sample sizes was similar. In general, results of this study confirm that BGBLUP can be valuable for enhancing genome-enabled prediction of complex traits.

Using multiple regression, Bayesian networks and artificial neural networks for prediction of total egg production in European quails based on earlier expressed phenotypes. The prediction of total egg production (TEP) potential in poultry is an important task to aid optimized management decisions in commercial enterprises. The objective of the present study was to compare different modeling approaches for prediction of TEP in meat type quails (*Coturnix coturnix coturnix*) using phenotypes such

as weight, weight gain, egg production and egg quality measurements. Phenotypic data on 30 traits from two lines (L1, n = 180; and L2, n = 205) of quail were modeled to predict TEP. Prediction models included multiple linear regression and artificial neural network (ANN). Moreover, Bayesian network (BN) and a stepwise approach were used as variable selection methods. BN results showed that TEP is independent from other earlier expressed traits when conditioned on egg production from 35 to 80 days of age (EP1). In addition, the prediction accuracy was much lower when EP1 was not included in the model. The best predictive model was ANN, after feature selection, showing prediction correlations of r = 0.792 and r = 0.714 for L1 and L2, respectively. In conclusion, machine learning methods may be useful, but reasonable prediction accuracies are obtained only when partial egg production measurements are included in the model.

**Objective 2: Facilitate the creation and sharing of poultry research populations and the collection and analysis of relevant new phenotypes including those produced by gene transfer.**

**ADOL**

Maintenance and distribution of experimental chicken lines. A major strength of ADOL is the large number of chicken lines that are characterized for a number of traits, especially those associated with viral diseases, and maintained under specific pathogen free (SPF) conditions. Besides providing unique genetic resources to ADOL, ~1500 embryos or chicks are supplied yearly to academic institutions or companies in the United States. The lines and maintenance are briefly summarized below. ADOL maintains 39 chicken lines with special genetic characteristics for tumor or viral susceptibility that also differ remarkably for immunological and physiological traits. All but 3 (C, N and P) were developed at the ADOL over the last 67 years. These include 4 of the world's most highly inbred lines ($6_3$, $7_1$, $7_2$, and $15I_5$,), all of which are well defined for avian leukosis virus (ALV) receptor genes, endogenous virus loci (EV), and resistance to MD. Two of the lines are outbred, 2 of which are highly utilized worldwide for ALV analyses (0 and 15B1). Four congenic lines exist for analysis of EV genes; 3 (0.44-TVBS1-EV21, 0.44-TVBS3-EV21, and RFS) were developed from line 0 and 1 (100B) from line 72. Eight congenic lines exist for analysis of the influence of the MHC (B haplotype) on resistance to tumor diseases, immune responses or vaccinal immunity; 7 (15.6-2, 15.7-2, 15.15I-5, 15.C-12, 15.P-13, 15.P-19, and 15.N-21) were developed from line $15I_5$, and 1 (15.N-21) from line 0. Lines $6_3$ and $7_2$ differ markedly for MD resistance and immune function traits, as well as ALV and EV genes, but have the same B haplotype. Nineteen recombinant congenic strains (RCS) are under development to identify non-MHC genes that influence traits differing between lines $6_3$ and $7_2$. ADOL also developed one transgenic chicken line (0.ALV6) that is very beneficial for analysis of ALV. ADOL lines are routinely tested by blood-typing using 40 antisera either to ensure purity or to maintain heterozygosity (EV21, 100B, and O.P-13) during annual line reproduction. The breeders are unique in that they are maintained in a quarantined state and, on the basis of frequent serologic tests for 11 pathogens, are considered free of infection from common poultry pathogens. With the planned moved to of ADOL staff to new facilities in Athens, GA, they are coordinating efforts to transfer all the lines in the next few years.

**AZ**

Chicken anatomy ontology. Continuing work on the chicken anatomy ontology is based upon UA biocurator funds, with work focusing on adding adult chicken anatomy terms to the avian ontology provided by Uberon. The avian subset of the Uberon ontology contains 15,300 anatomy terms, with 1,774 terms added by the UA biocuration group. Since this ontology will be required for the Functional Annotation of Animal Genomes (FAANG) Funding is pending to support a full-time biocurator to complete this ontology.

VERVE Net is a virus ecology research and virtual exchange network (http://vervenet.us) collaboration with Dr Bonnie Hurwitz (U Azriona). VERVE Net is funded by the Gordon Betty Moore Foundation to develop a forum for virus ecology research. As part of this effort and in partnership with Protocols.io, a forum for groups share, discuss, and adapt protocols was developed. Initially the platform only supported molecular biology protocols, but was recently updated to include support for bioinformatics protocols. Drs. Hurwitz and McCarthy have partnered to begin to develop a similar forum specific to poultry research and breeding that uses the same software platform.

**IA**

Maintenance of chicken resource populations. Thirteen unique chicken research lines [including highly inbred, MHC-congenic, closed populations; and advanced intercross lines (AIL)] were maintained by Iowa State University to serve as resources for identifying genes and QTL of economic importance. All adult breeders were housed in individual cages and matings done by artificial insemination to ensure pedigree accuracy. All MHC-defined lines were blood-typed to verify MHC serologic haplotype. Two AILs (now at generation F24) were maintained to facilitate fine-mapping of QTL with the goal of identifying genomic regions and candidate genes controlling important phenotypes.

Utilization and sharing of research populations. The ISU genetic lines formed a discovery platform for research on the genomics of heat resistance in a USDA-AFRI-NIFA project (PD: C Schmidt, U Del) and a USAID project on genomics of resistance to Newcastle disease virus and heat (PD: H Zhou, UC-Davis). Genetic material (chicks, fertile eggs, blood, tissues, DNA or RNA) was shared with many cooperating investigators to expand studies on the chicken genome. Active collaborations utilizing ISU chicken genetic lines include the international Avian RNA-seq consortium, H Zhou, UC-Davis (NDV and heat-stress response), J Womack, Texas A&M (defensins), A Clark, Cornell University (imprinting), R Coulombe, Utah State (aflatoxin sensitivity), B Abasht, U Delaware (imprinting), C Keeler, U Delaware (microarray analysis of host response to Salmonella), and H Lillehoj, USDA-ARS (response to Eimeria and other pathogens). The outbreak of high path avian influenza in the state of Iowa prevented the sharing of live birds in 2015.

**MS**

Turkey and quail parthenogenic lines. We continue to select Beltsville Small White turkeys and Chinese Painted quail for parthenogenetic development. We are currently in our 10th generation of selection with approximately 40% of the unfertilized quail eggs from virgin hens exhibiting macroscopic parthenogenetic development.

**NC**

Primordial germ cells**.** Using a transgenic line of chicken expressing GFP, clonal cultures of male and female PGCs were injected into male or female recipient embryos. The resulting chicks were hatched and the genetically male chicks were raise to sexual maturity and mated to wild- type hens. Semen from males injected with female PGCs resulted in no fertility, while male PGCs injected into male embryos were capable of normal germ line transmission. GFP FACS analysis of the semen from roosters injected with female PGCs indicated that the infertility was not the result of incomplete spermatogenesis, and in several cases GFP+ sperm made up over 50% of the semen. In additional to the absence of GFP+ offspring, overall fertility was negatively correlated with the proportion of GFP+ germ cells in the semen. These observations suggests Z-bearing female germ cells undergo normal spermatogenesis, but are incapable of fertilization. The mechanism for this observation currently is unknown.

**Objective 3: Elucidate genetic mechanisms that underlie economic traits and develop new methods to apply that knowledge to poultry breeding practices.**

**ADOL**

Association of CD8 and cecal microbiome with Marek's disease genetic resistance**.** Marek's disease (MD) is an important neoplastic disease of chickens caused by Marek's disease virus (MDV), an oncogenic alphaherpesvirus. In a recent study using two chicken lines, one resistant (line 6) and another susceptible (line 7) to MD, ADOL scientists profiled splenic T cells and the cecal microbiome in both uninfected and MDV- infected birds to gain a better understanding of the primary differences associated with MD phenotype in these lines. They find that the percent of splenic CD4+ T cells were similar regardless of MDV challenge status in both resistant and susceptible birds. In contrast, CD8αα profiles were different ($P < 0.005$) between the chicken lines under mock control and MDV challenge, suggesting that CD8αα T cells play a key role in mediating MDV infection. Genera level analysis of the microbiome composition showed differences between lines in both control and MDV challenged treatments ($P < 0.05$) in both chicken lines, suggesting that MDV affects cecal microbial community structure during the course of infection. Furthermore, community metabolic profiles profile due to MDV infection relates to changes in functional metabolic profile in the birds. These results provide insights into the immune response and the potential interplay with the microbiome during infection with an oncogenic virus.

Identifying driver mutations for Marek's disease**.** Marek's disease virus (MDV) is a highly oncogenic virus in susceptible chickens as lymphomas, characteristic of Marek's disease (MD), are induced as early as 2-4 weeks after infection. Unlike other herpesviruses, MDV integrates into the chicken genome and encodes an oncoprotein, known as Meq, a bZIP transcription factor that homodimerizes or heterodimerizes preferably with c-Jun. However, as all MDV-infected chicken do not develop gross tumors and most tumors are clonal, it is likely that somatic driver mutations are required for transformation. To identify potential driver mutations, ~200 line 6 x 7 F1 progeny were challenged with MDV by ADOL scientists, which produced 72 tumor samples. All samples were genotyped with a 15K SNP array to screen mainly for copy number variants (CNV) and loss of heterozygosity (LOH). Twenty-two samples that were the most homogeneous had both their DNA and RNA sequenced. To aid in the screens, even though lines 6 and 7 are highly inbred, germline and CD4 T cells also had their DNA and RNAs sequenced. After establishing computational pipelines, preliminary results indicate there are ~0.6 SNPs per Mb, which is similar to juvenile cancers in human, though this varies greatly depending on the algorithm used. Commonly called SNPs give a relatively small list of candidate genes. Similar results have been obtained based on screens to find structural variations.

Global Profiling of microRNA expressions in response to MD vaccines and MD virus challenge in MD resistance and susceptible lines of chickens**.** Mounting evidence shows microRNAs (miRNAs) directly regulate gene expression post-transcriptionally through base-pairing with regions in the 3'-untranslated sequences of target gene mRNAs, which results in dysregulation of gene expression/translation and subsequently modulates cellular processes. The latest ADOL study was designed to identify miRNAs differentially expressed in spleen in response to vaccination followed by MDV challenge at cytolytic stage by small RNA deep sequencing. Twenty-four and 36 differentially expressed miRNAs were identified in response to HVT followed by MDV challenge, while CVI988/Rispens and MDV induced 6 and 31 differentially expressed miRNAs in lines $6_3$ and $7_2$, respectively, in contrast to the control counterparts (Log2 fold change ≥ 2.0). Hundreds to thousands of target genes were predicted for the differentially expressed miRNAs. Over 30 important pathways were likely involved with the lists of target genes of the differentially expressed miRNAs, which included TGF-beta signaling, MARK signaling, and Wnt signaling pathways, etc. suggesting that the differentially expressed miRNAs play important roles in immunity, vaccinal protection, and suppression of tumorigenesis against MD through complicated networks. It is

anticipated that further studies in extension of this project should lead to better understanding on how microRNAs mediate vaccine protective efficacy in chickens.

**AR**

Identification of Genes Affecting Sperm Mobility**.** AR- in collaboration with DE and OR, performed RNAseq on white leghorn and broiler primordial germ cells for both males and females. Initial analysis identified specific pathways distinguishing genders as well as broiler from leghorn. RNAseq data were also analyzed with respect to relevant data from chick embryo fibroblasts and DF1 cells to identify possible germ cell specific transcripts.

Identification of Genes Affecting Ascites Susceptibility**.** AR- in collaboration with other investigators at AR continued to investigate the genetics of ascites. GWAS identified a region around 60 Mbp on GgaZ that is associated with male susceptibility. Selected matings suggest that the heterozygotes are more susceptible. The heterozygote also shows differences in production traits. GWAS also identified a region on Gga2 as female biased, but further analysis has found no association with ascites phenotype but there is an association with cardiac hypertrophy which is associated with ascites. Prior GWAS identified two regions on Gga9 that was confirmed with microsatellite data (Schmid et al., 2015). Further SNP analysis of the region around 14 Mbp does not find any association with any of 5 SNPs from the region. Current work is examining Allele Specific Expression (ASE) for candidate genes in this region. For analysis of the 16Mbp region, the research has focused on the 5HTRB serotonin receptor. SNP and microsatellite genotyping support a minor association and ASE is in progress.

Bacterial chondronecrosis with osteomyelitis and lameness in broilers. AR in collaboration with other investigators at AR have worked out the sites in the skeleton where opportunistic bacteria colonize to cause infections leading to lameness in broilers. An experimental model has been developed, blood flow issues identified, prophylactic and therapeutic treatments identified and the condition was named bacterial chondronecrosis with osteomyelitis (BCO) (Wideman, 2015). The major bacterial species causing BCO and lameness was identified as *Staphylococcus agnetis* along with its genome assembly (Al-Rubaye et al., 2015). Most of the recent work is subject to a non-disclosure agreement. The work has focused on an early bacteremia leading to BCO and a manuscript has been submitted on characterization of the early blood microbiome. Recent work has indicated that the bacterium can be communicated from broilers exposed to the bacterium.

Global genome sequencing for genetically selected chicken lines. Rous sarcoma virus. AR continued genome-wide SNP analysis for genetically selected chicken lines. By the genome re-sequencing data and a pathway analysis tool, various potential genetic markers showing amino acid changes and potential roles in phenotype development were identified. Particularly, we focused more on analyzing genetic variations found in Arkansas Progressor (AP) and Regressor (AR) chicken lines, that are important animal models for the study of susceptibility for and resistance to tumor development induced by Rous sarcoma virus (RSV) (Kong et al., 2015a). The AP line is susceptible and develops viral src (v-src) oncogene induced tumors while the AR line regresses tumors. In this study, a total number of 7.1 and 7.3 million SNPs were identified in AR and AP genomes, respectively. Through a series of filtration processes, 12,242 SNPs were identified in AR chicken line to be linked with inducible mutations such as non-synonymous, frameshift, nonsense, no-start and no-stop. After additional filtering of SNPs on the basis of ≥ 10 read depth, 63 reliable marker SNPs were found which were chosen for further study. Among the 63 causal SNPs, 10 potential markers were randomly chosen for the further validation using the genotyping PCR method. Gene network evaluation using Ingenuity Pathway Analysis revealed that genetic marker SNPs for tumor regression have roles in networks centered to UBC, PI3K complex and NF-kB suggesting that the tumor regression property of the AR chicken line is likely associated with

protein degradation by ubiquitination and protein kinase pathways. Such findings aid to provide insight into genetic components responsible for tumor regression.

Feed efficiency of broilers. AR continued analysis on the feed efficiency of broiler lines. We analyzed global gene expression on breast muscles depending on feed efficiency phenotypes resulting in new potential pathways characterized by cellular mechanisms and processes associated with the regulation of feed efficiency (Kong BW et al., 2015b,c,d).

Genes and their products involved in the stress response of birds. AR – in collaboration with other investigators at AR and MO have continued the investigation of genes involved in the stress response of broilers. The focus has been on receptors, particularly the vasotocin 4 receptor (VT4R) and the vasotocin 2 receptor (VT2R). Due to gene sequence and functional similarity to mammals, it has been proposed that a new terminology be used. Specifically the VT4R be termed the avian V1aR and the VT2R be called the V1bR to follow the homologous receptor gene abbreviations used in mammals. The avianV1aR has been shown to occur in corticotropes (cells of the anterior pituitary gland that produce a hormone ultimately stimulating the release of the stress hormone, corticosterone, by the adrenal gland). The avian V1aR was mapped throughout the entire brain and found in neurons of several brain regions and also found in glial cells located in all ten circumventricular organs of the broiler brain (Selvam et al., 2015). Circumventricular organs are specialized brain areas lacking a blood-brain barrier and highly vascular. Significant differences in gene expression of four receptors within the pituitary gland (V1aR, V1bR, corticotropin releasing hormone receptor 1 and 2, CRH-R1, -R2) following an acute stressor suggest the receptors are involved in avian psychological stress. Within the brain two structures have been shown significantly activated using Fos protein as a marker of activated neurons. The paraventricular nucleus containing arginine vasotocin neurons and the nucleus of the hippocampal commissure containing CRH neurons showed significantly increased Fos immunoreactivity (Nagarajan et al., 2015). Identification of high utility genetic marker genes for chronic stress in chickens have been identified using high-throughput genome sequencing of genetically selected stress lines of Japanese quail (Kang et al., 2014).

Genes involved in reproductive development of broilers. AR in collaboration with CT have investigated genes and neurons involved in sensing photoperiod. Broilers transferred to long photoperiods develop their reproductive system. The receptors are not in the eyes nor the pineal as determined in mammals. The receptors, called deep-brain photoreceptors (DBPs) are located in neurons located at the base of the brain in three proposed brain regions. Three primitive photopigments, different from pigments in rods and cones in the retina, have been proposed responsible for sensing photoperiod. We have examined the three brain regions containing 4 loci proposed to house DBPs. Significantly increased gene expression for all three photopigments in all three brain regions were found following the transfer of broilers to a long photoperiod (Kang and Kuenzel, 2015). Using the patch clamp technique in electrophysiology to examine single neurons, it was found that in the septal brain region including the lateral septal organ, are neurons showing clear depolarization following direct photostimulation. This major characteristic along with others recorded provided evidence that the lateral septum houses DBPs involved in reproductive function (Kuenzel et al., 2015).

**CA**

Improving food security in Africa by enhancing resistance to Newcastle disease virus and heat stress in chickens. Within a USAID funded Feed the Future Innovation Lab for Genomics to Improve Poultry project (H. Zhou, PI) through a partnership of the University of California at Davis (H. Zhou, D. Bunn, R. Gallardo), Iowa State University (S.J. Lamont, J. Dekkers), Sokoine University of Agriculture - Tanzania, the University of Ghana, and the University of Delaware (C. Schmidt). The five-year research program will apply advanced genetics and genomics approaches to sustainably enhance

innate resistance to Newcastle disease virus (NDV) and heat stress in chickens to improve production. This project directly addresses the President's FY2012 "Feed the Future" initiative. We are investigating two stressors (biotic: NDV and abiotic: heat stress). Birds of two genetically distinct and highly inbred lines (Fayoumi and Leghorn), and Hy-Line Brown were either exposed to NDV only (Iowa State) or NDV and heat stress (UCD). Measures of body temperature, blood gas parameters, NDV titers from tears, and antibody response in serum were taken on the live birds, and tissues were collected for transcriptome analysis. Three ecotypes each in Ghana and Tanzania will be exposed to NDV. DNA isolated from Hy-Line Brown were genotyped using chicken 600K SNP for GWAS. At UCD, 100 birds from two inbred lines (50 birds per Fayoumi and Leghorn) and 540 birds from Hy-Line International were used. At 14 days of age (one week before NDV challenge) the heat-stress treatment will begin. To mimic parts of the world that are subject to relatively continuous high heat and humidity, the heat-stress rooms were continuously at 35 C with 60- 70% relative humidity until day 31. Phenotypic measurements using iSTAT were collected at four stages: (1) at day 13 before the temperature increase ("pre-heat"), (2) at day 14 ("acute heat"), (3) at day 20 before the NDV challenge ("chronic heat before pathogen challenge") and (4) at day 23 ("chronic heat after pathogen challenge"). The cloacal temperatures were measured, too. Virus titers from tears were measured at 2 and 6 days post-infection (dpi). Antibody response to NDV was measured at 10dpi. There were significant differences between Fayoumi and Leghorn birds in virus titers, antibody response to NDV infection and heat stress (Fayoumi is resistant and Leghorn is susceptible to both stressors). For inbred line study, tissues at 2, 6, and 10 dpi were collected to focus on NDV study. RNA isolated from trachea, lung, and Harderian glands were used to generate 192 libraries for the sequencing. Tissues at 4 hours, 10 day post-treated with heat stress were collected to focus on heat stress study. A total of 128 libraries from hypothalamus, liver and muscle were constructed and sequencing are under analysis. Initial bioinformatics analysis to identify genes and signal pathways associated with NDV and heat stress have been done in some tissues. Further bioinformatics analysis will be done next year. For Hy-Line Brown, 600K SNP chip has been used to genotype 540 birds with phenotype of virus titers, antibody response, and 13 physiological parameters in serum in responding to heat stress. Initial bioinformatics analysis to identify QTL affecting immune response and heat stress were done and further analysis are undergoing. In addition, additional haplotypes on several immune related genes such as MHC, TLRs, Mx1 etc in collaborating with J. Fulton from Hy-Line International have been genotyped and associations between haplotypes and phenotypes of immune response will be further analyzed.

*Salmonella enterica* serovars Enteritidis infection alter the indigenous microbiota diversity in young layer chicks. Microbiota plays an important role in maintaining gastrointestinal homeostasis by performing beneficial metabolic functions, development of the immune system, and most importantly, enhancing the colonization resistance to incoming pathogenic microorganisms. Avian gastrointestinal tracts are highly populated with a diverse array of microorganisms that share a symbiotic relationship with their hosts and contribute to the overall health and disease state of the intestinal tract. The microbiome of the young chick is easily prone to alteration in its composition by both exogenous and endogenous factors especially during the early post-hatch period. The genetic background of the host and exposure to pathogens can impact the diversity of the microbial profile that consequently contributes to the disease progression in the host. The objective of this study was to profile the composition and structure of the gut microbiota in young chickens from two genetically distinct highly inbred lines. Furthermore, the effect of the Salmonella Enteritidis infection on altering the composition makeup of the chicken microbiome was evaluated through the 16S rRNA gene sequencing analysis. One-day-old chickens were challenged with S. Enteritidis and the host cecal microbiota profile was examined at 2 and 7 days post-infection. Alpha diversity, beta diversity, and

overall microbiota composition was analyzed for four factors: host genotype, age, treatment, and post-infection time-points. The results revealed that S. Enteritidis infection in young chickens significantly reduced the overall diversity of the microbiota population with an enrichment of bacteria from the Enterobacteriaceae family in both genetic lines compared to the non-infected group. These changes indicated that Salmonella colonization in the gastrointestinal tract of the chickens has a direct effect on altering the natural development of the gastrointestinal microbiota. Significant inverse correlation between Enterobacteriaceae and Lachnospiraceae family in both non-infected and infected groups, suggest possible antagonistic interaction between members of these two taxa, which could potentially influences the overall microbial population in the gut. Our results also revealed that genetic difference between two genetic lines had minimal effect on the establishment of microbiota population. Overall, this study provided preliminary insights into the contributing role of Salmonella Enteritidis in influencing the overall makeup of chicken's gut microbiota.

**CU**

RNAseq analysis of hepatocytes and follicular development. Intense selection pressure for postnatal growth has led to great improvements in the broiler industry. In parent flocks, however, broiler breeder hens develop excessive follicles resulting in decreased egg production. Intensive feed restriction decreases these reproductive inefficiencies, but the hormonal mechanisms underlying the differential effect of feeding level on follicle development and whole animal metabolism are unknown. Follicle development in the laying hen is a highly efficient and regulated process. Maintenance of a well ordered follicular hierarchy is essential for optimum follicle selection and subsequent egg production in hens. We examined the relationship between the metabolic and reproductive axis in broiler breeder hens by investigating the reproductive parameters and the liver transcriptome of broiler breeders maintained on a feed- restricted diet (RF) or an *ad libitum* (FF) diet. Cobb 700 broiler breeder hens in their first year of lay were reared using a RF feeding program according to primary breeder guidelines. During peak production (determined by the highest percentage of laying hens), birds were randomly divided into two groups. One pen of hens was given FF (n=11), and one pen remained on RF (146 g/bird/day, as suggested by the primary breeder; n=11) for six weeks prior to euthanasia. FF hens had decreased egg production ($p<0.01$), an increased ovarian weight ($p<0.05$), increased number of large follicles ($p<0.01$), and a disordered follicular hierarchy compared to RF hens. Interestingly, liver weights were also significantly lower in the RF group ($p<0.01$) and liver morphology (examined by hematoxylin and eosin staining) showed lipid accumulation in hepatocytes of FF hens. In the second experiment, high-throughput RNA sequencing technology (Illumina HiSeq) was used to investigate the gene expression pattern in FF and RF broiler breeder hens. Liver ssRNA-seq libraries were prepared and run on Illumina HiSeq, generating 22-33 million reads per sample (n = 3; each group). Reads were aligned to UCSC galGal4 using TopHat (v2.0.13) and transcripts were counted using Cufflinks/Cuffdiff (v.2.2.1). A MDS plot was generated to evaluate the variance among biological replicates, and identify sample outliers. Differentially expressed (DE) genes were determined using EdgeR's generalized linear models (glm), which identified 120 DE genes in FF and RF hens ($p<0.01$; FDR<0.05). Of these, 51 genes were up-regulated and 69 genes were down-regulated. Further functional annotation and pathway analysis indicated that differentially expressed genes in the data set were involved in lipid metabolism. These studies suggest that increased feed intake in FF hens may over-stimulate GH and IGF1 signaling and increase de novo lipogenesis which could contribute to the reproductive dysfunction in FF hens. Several of the differentially expressed genes were validated by qPCR. One of the primary IGF1 binding proteins (IGFBP2) was significantly increased in the RF hens, indicating that there was likely decreased bioavailability of IGF1 in this condition. Enzymes involved in thyroid hormone metabolism were also affected by the dietary treatment resulting in increased production of the active form of thyroid hormone (T3) in FF hens.

**DE**

Gene expression Analysis Using 3'-RNA Sequencing: Application in a Chicken Study**.** The high cost of RNAseq has limited this technology mainly to experiments with limited numbers of samples. To examine a cost-effective alternative, we used a method, which confines sequencing to the 3'-end of mRNA and produces just one fragment per transcript, resulting in a dramatic decrease in sequencing cost. Total RNA isolated from chicken adipose tissue samples was used for cDNA library preparation using QuantSeq 3'mRNA-seq library Prep Kit. Sixty-one uniquely indexed DNA libraries were pooled and sequenced on one lane on the Illumina Hiseq 2500. On average, 2.24 million reads per sample were generated, 90.1% of which were mapped to the chicken reference genome (Ensembl Galgal4). For more than 70% of the genes with detectable expression, we redefined the 3'-end and identified alternative polyadenylation sites within the 3'-untranslated regions. To compare gene expression measures between 3'-RNA-seq and RNA-seq technologies, we used data from a subset of 20 samples that were previously used in a RNA-seq study of feed efficiency. The correlation of the log10(fold-change) for gene expression (high- *vs.* low-feed efficiency birds) between these two methods was 0.90. In conclusion, 3'-RNA-seq is a cost effective method amenable to global gene expression studies at population-level, e.g., expression QTL (eQTL) mapping. Also, it allows for accurate detection of the 3'-end of transcripts, enabling verification of the current gene model annotations and global characterization of alternative polyadenylation

Detection of Recent Selection Signatures in Commercial Broiler Chickens. Strong human-driven selection in broilers has led to major changes in traits of economic importance, most likely through changes in the frequency of alleles conferring desired phenotypes. Therefore, identification of genomic regions of such alleles may help uncover genes responsible for economic traits. In our study, we applied two methods, cross-population extended haplotype homozygosity (XP-EHH) and cross-population composite likelihood ratio (XP-CLR), to perform a genome-wide scan of recent selection signatures in five commercial elite broiler chicken lines, including three broiler sire (male) lines and two broiler dam (female) lines. A total of 322 candidate selection regions were detected by both methods, representing approximately 1.5% of the genome in each line. Of all candidate selection regions, 42 regions were detected in multiple liens. Through analyzing haplotypes in candidate selection regions, we found evidence for selection acting on the same or opposite alleles in multiple lines. Two examples of genes in candidate regions detected in multiple lines are *Sex determining region Y-box 6 (SOX6)* and *Thyroid hormone receptor beta* (*cTR*). These genes may have been under recent selection because of their essential roles in chickens' growth, development and reproduction. The candidate genes identified in the present study are of interests for future research into the genetic architecture of traits relevant to modern broiler breeding.

Transcriptomic Investigation of Genomic Imprinting in Chicken Embryonic Brain and Liver**.** Epigenetic and genetic *cis*-regulatory elements may cause unequal expression of the two autosomal gene copies in diploid organisms. This imbalance is referred to as allele specific expression. An intriguing type of allele specific expression is that some genes are expressed monoallelically from either paternal allele or maternal allele, a phenomenon commonly termed genomic imprinting. Previous studies reported conflicting evidence regarding the existence of genomic imprinting in chickens. Albeit no genomic imprinting has been reported in the chicken embryo as a whole, we investigated whether certain embryonic tissues exhibit genomic imprinting. The present study aims to identify genomic imprinting in chicken embryonic brain and liver by examining the mRNA expression of parental alleles in an F1 generation. Eggs from two inbred chicken lines (Fayoumi and Leghorn) and their reciprocal crosses were collected and incubated for 12 days; then brain and liver were harvested from embryos for cDNA library preparation. Of 65 million reads per sample generated using the Illumina HiSeq 2000 sequencer, 88%

were mapped to the chicken reference genome (Ensembl Galgal4). To establish the genotypes of the inbred lines and their F1 hybrids and to minimize reference bias of RNA-Seq sequence alignment, genomic DNA from inbred Fayoumi and Leghorn chickens was pooled separately and each pool was sequenced at 20X coverage. Our results indicate that in the F1 crosses, 9.2% of the heterozygous loci show allele specific expression (binominal test, p value ≤ 0.05), but genomic imprinting is not present at 12-day chicken embryonic brain or liver.

Gene Expression Analysis of Wooden Breast Disease in three Distinct Modern Broiler Lines. Wooden Breast Disease (WBD) is a relatively new muscle disorder known to affect the pectoral muscles in modern broiler chickens raised under modern commercial conditions. This disorder significantly decreases meat quality resulting in substantial economic losses in the poultry industry. To establish the molecular profile associated with this disorder, we compared gene expression profiles between affected and unaffected birds from 2 purebred lines and one crossbred commercial broiler (CB) population. Breast muscle samples were taken from affected and unaffected birds at day 47 or 48 post-hatch and subsequently processed for RNA-seq analysis using the Ilumina Hiseq platform. The numbers of differentially expressed (DE) genes at >1.3 fold change (FC) with a false discovery rate (FDR) of <0.05 between affected and unaffected birds in lines 1, 2 and CB were 3854, 3119 and 3682, respectively. Of all DE genes, 1328 overlapped and exhibited the same expression direction in all three populations. A comparative analysis of all DE genes using Ingenuity Pathways Analysis (IPA) revealed 47 canonical pathways with relatively consistent Z-scores across three populations. The same presentation was also reflected on upstream regulators. The top canonical pathways such as RhoA signaling and upstream regulators such as TGFβ1 were activated in affected chickens. Based on the relative similarities of molecular and biological processes in the three distinct broiler populations, the present study demonstrates the existence of a unique gene expression signature for this disorder, which could aid in diagnosis of WBD and in understanding its cause and pathogenesis.

**GA**

Molecular mechanisms associated with dietary methionine deficiency**.** We studied the molecular mechanisms that underlie SAA restrictions in broiler chickens from 3-5 weeks of age in the *Pectoralis major* muscle using next generation sequencing. Fold change ≥1.5 and false discovery rate ≤0.05 were used as criteria for declaring differentially expressed genes (DEGs). In the *P. major, t*here were 454 downregulated DEGs and 465 unregulated DEGs in the deficient group compared to the controls. Signaling pathway impact analysis showed that the Fc gamma R-mediated phagocytosis, NK cell mediated cytotoxicity and NF-kappa B signaling pathways were activated whereas the B cell receptor signaling pathway was inhibited in the *P. major* of the SAA restricted birds. The current study suggests that restriction in dietary methionine in growing chickens leads to a potential increase in oxidative stress, cytoskeleton modification that reduces cell to cell communication and abnormal methylation which induces inflammation response. Birds showing such inflammatory response may compromise their immune system and their ability to fight infections.

Genetics of leg problems and bone quality in meat-type chickens**.** Although skeletal issues have been directly associated to the improvement of growth rate, the genetic association among leg problems, bone quality traits and growth rate has not been conclusively evidenced or characterized yet. In order to devise practical breeding strategies it is necessary to ascertain the nature of that genetic relationship. We report the genetic relationship between growth and bone quality traits in a random mating broiler control population. Traits studied were growth rates from week 0 to 4 [body weight gain (BWG) 0 to 4], from week 0 to 6 (BWG 0 to 6), and residual feed intake (RFI) from week 5 to 6 (RFI 5 to 6). Bone quality traits were obtained at 6 weeks of age. These traits were shank weight (SW), shank length (SL), shank diameter (SDIAM), tibia weight (TW), tibia length (TL), and tibia diameter (TDIAM). Likewise, tibia was

used to obtain the tibia density (TDEN), tibia breaking strength (TBS), tibia mineral density (TMD), tibia mineral content (TMC), and tibia ash content (TAC). At the phenotypic level, growth traits were positively correlated with most of the bone quality traits except with TDEN and TAC which tended to show unfavorable associations (-0.04 to -0.31). Heritability of bone quality traits ranged from 0.08 to 0.54. The additive genetic associations of growth traits with weight, length, and diameter of shank and tibia were positive (0.37 to 0.80). A similar pattern was observed with TMD and TMC (0.06 to 0.65). In contrast, growth traits showed unfavorable genetic associations with TDEN, TBS, and TAC (-0.03 to -0.18). It was concluded that bone quality traits have an additive genetic background and they can be improved by means of genetic tools. It appears that selection for growth is negatively correlated with some traits involved in the integrity, health, and maturity of leg bones. We also report on the genetic association between leg problems and bone quality traits in a random mating broiler control population. The leg problem traits were valgus (VL), varus (VR), and tibial dyschondroplasia (TD), and that of bone quality were shank weight (SW), shank length (SL), shank diameter (SDIAM), tibia weight (TW), tibia length (TL), tibia diameter (TDIAM), tibia density (TDEN), tibia breaking strength (TBS), tibia mineral density (TMD), tibia mineral content (TMC), and tibia ash content (TAC). A threshold-linear mixed model, implemented via a Bayesian approach, was employed for the joint analysis of the traits. Genetic correlations of leg problems with bone quality traits ranged from -0.06 to 0.11 suggesting that genetic relationship between leg problems and quality is weak, and management strategies could better alleviate leg problems than genetic improvement.

**IA**

Segmental duplications initially identified as heterozygous loci in highly inbred lines. Segmental duplications (SD) are genomic regions with nearly identical sequence. We identified segmental duplications on chromosome 1 in two highly inbred pooled lines by SNV validation analysis. SDs in both lines were discovered during the validation process for novel SNV in these lines. This resulted in variants that appeared to segregate, as some genotyped individuals incorrectly appeared as heterozygous. Duplication identification was carried out using the KBiosciences Kompetitive Allele Specific PCR genotyping system (KASP). This allowed for both a numerical and visual examination of the existence of variants. Follow up analysis of the possible duplications included comparison of the mean depth of coverage (DP) across the genome versus the DP for the variants showing duplication (DP ≥ ~2 x mean). The flanking sequences for the duplicated variants were also queried using NCBI Blast to search for multiple hits to different genomic regions. Independent commercial lines were also used to confirm the existence of the duplications. Results from the KASP assay, Blast, and DP analysis confirmed that selected variants on chromosome 1 had flanking sequences that mapped to multiple genomic locations with SNV loci that showed ≥ ~2 the mean genomic DP. Results suggest SDs caused primers to amplify both the alternate and reference alleles making them appear as segregating. This was due to fixation of different alleles in the genome that are incorrectly mapped to the same genomic location within regions of a SD.

Selection signatures in African ecotypes assessed using a 600K SNP chip. Indigenous populations of animals have developed unique adaptations to their local environments, which may include factors such as response to thermal stress, drought, pathogens and suboptimal nutrition. The survival and subsequent evolution within these local environments can be the result of both natural and artificial selection driving the acquisition of favorable traits, which over time leave genomic signatures in a population. This study's goals are to characterize genomic diversity and identify selection signatures in chickens from equatorial Africa to identify genomic regions that may confer adaptive advantages of these ecotypes to their environments. Indigenous chickens from Uganda (n = 72) and Rwanda (n = 100), plus Kuroilers (n = 24, an Indian breed imported to Africa), were genotyped using the Axiom® 600k

Chicken Genotyping Array. Indigenous ecotypes were defined based upon location of sampling within Africa. The results revealed the presence of admixture among the Ugandan, Rwandan, and Kuroiler populations. Genes within ROH consensus regions are linked to GO terms for lipid metabolism, immune functions and stress stress-mediated responses (FDR < 0.15). The genes within regions of signatures of selection are enriched for gene ontology terms for health and oxidative stress processes. Key genes in these regions had anti-oxidant, apoptosis, and inflammation functions. The study suggests that these populations have alleles under selective pressure from their environment that are associated with stress tolerance, which may aid in adaptation to harsh environments. The similar responses across the populations could be related to the similarity of environments or an artifact of the detected admixture.

Gut microbiome of laying hens altered by exposure to high ambient temperature. The gut microbiome has garnered attention in the recent years for its contribution toward host response to stress. For production chickens, both broilers and layers, heat stress has a significant negative impact on production traits and overall well-being of the birds. The gut microbiome of chickens under heat stress is poorly understood. In this study, we characterized the changes in chicken cecal microbiome during a 4 week heat stress experiment with mature layer hens during active egg production. Eighty mature egg- layers were randomly split into 2 groups: heated and not heated. Eight birds from each group were euthanized to harvest samples at 5 time points during heat treatment: day 1 (acute), and weekly in weeks 1 through 4 (chronic). Bacterial DNA were isolated from cecal digesta, and the V1-V3 region of the 16S rRNA gene was amplified. The barcoded amplicons were pooled and submitted for sequencing on the Illumina MiSeq.

Liver transcriptome response to hyperthermia stress in three distinct chicken lines. High ambient temperatures cause stress in poultry, especially broiler lines, which are genetically selected for rapid muscle growth. RNA-seq technology provides powerful insight into environmental response of a highly metabolic tissue, liver. We investigated the effects of acute (3h, 35°) and chronic (7d of 35° for 7h/d) heat stress on the transcriptome response of liver of 3 wk-old chicks of heat-susceptible broiler, heat-resistant Fayoumi and advanced intercross line (AIL) chickens. Transcriptome sequencing of 48 male chickens using Illumina HiSeq 2500 technology yielded an average 3.4 gigabase sequence per sample. There were 8 times more (N=627) differentially expressed genes (DEGs) in the broiler acute-heat group compared to control (25°) samples, than in Fayoumi. Contrasting genetic lines under similar heat treatments, the highest number of DEGs appeared between Fa and Br. Of 12 expressed heat shock protein genes, 8 were DEGs in at least one heat-control contrast. Ingenuity Pathway Analysis analysis of the 627 broiler DEGs revealed 83 overrepresented canonical pathways (*P<0.01*). Six comparison groups shared 7 common overrepresented functions, related to liver metabolism, including Hepatic Fibrosis Cell Activation, Fatty Acid β-oxidation I, and Fatty Acid Activation. “Lipid Metabolism, Vitamin and Mineral Metabolism, Small Molecule Biochemistry” was the highest ranking network in all genetic lines. Our results establish that acute heat stress has greater impact on liver metabolism than chronic heat in broiler and AIL, but not Fayoumi. This study extends our understanding of the liver transcriptome response to different heat treatments in novel genetic chicken lines.

Distinct cardiac transcriptome response to heat stress between two broiler lines. Prolonged exposure to elevated temperature can significantly increase metabolism, depress appetite, reduce meat production and raise mortality in broilers. Since the cardiovascular system plays an important role to dissipate heat and transport oxygen, strong cardiac function is indispensable for birds to adapt and survive under heat stress. However, due to small hearts relative to body weight, modern broilers have high risk of heart failure when exposed to high temperature. In this study, a Ross 708 line (modern broilers) and a Heritage broiler line (selectively bred until the 1950s) were subjected to heat stress at 35℃ for 7 hours/day from 21 to 42 day posthatch and compared to the control groups kept at ambient

temperature. Twenty-three libraries averaging 10 million 50-base pair single end reads were generated after transcriptome sequencing, trimming and filtering. After differential expression analysis with EdgeR, more than 300 genes were differentially expressed genes were identified in Ross 708 broilers, while only three genes were differentially expressed in Heritage broilers at a significance level of False Discovery Rate (FDR)<0.1 and $\log_2$ fold-change (LFC)>1. By Ingenuity Pathway Analysis (IPA) the significantly differentially expressed genes in Ross 708 broilers are mainly involved in apoptosis, cell proliferation, cell migration and inflammatory response. In heart disease, those genes are highly related to ventricular hypotrophy, cardiac arrhythmia and tachycardia. This information provides an insight into the susceptibility of modern broiler's heart to heat stress and a foundation for future breeding in broilers to improve their adaptation to global climate change.

Related studies. Several studies complementary to those reported in the NC1170 project are reported as part of the Iowa contribution to the NE1334 project (chickens) or elsewhere. These include (1) investigating the interaction of two stressors [heat stress and exposure to an inflammation-inducing PAMP (LPS)] on the transcriptome of birds of two distinct, highly inbred lines (broiler, Fayoumi) using RNA-seq of individual samples of thymus, bursa and spleen, (2) using two highly inbred lines (Fayoumi and Leghorn) and RNA-seq, identifying genes and pathways associated with NDV challenge, (3) using a commercial egg-laying line (Hy-Line Brown), conducting GWAS of response to NDV challenge, with NDV titer and anti-NDV antibodies as phenotypes, (4) genomic, molecular and cellular characterization of the host-pathogen interactions between chickens and avian pathogenic *E. coli*, (5) impact of fitting dominance and additive effects on accuracy of genomic prediction of breeding values in egg-laying chickens, and (6) fitting lactation curves to egg production curves.

**IN**

Detecting Loci Under Selection in Domesticated and Wild Populations Using Re-sequencing Analysis**.** Rapid fixation of new, favorable alleles through directional selection (a 'selective sweep') generates a sudden drop of genetic variability at linked loci by hitchhiking. The beneficial substitution of an allele shapes patterns of genetic variation at linked sites, and may provide important insights into (1) the mechanisms of evolutionary change; (2) guide selection of loci for inclusion in population genetic studies; (3) facilitate significant genomic regions; and (4) help elucidate genotype-phenotype correlations in complex traits. Thus, in principle, adaptation can be mapped by locating the signature of directional selection in polymorphism data. When a new beneficial mutation increases its frequency in a population, the standing genetic variation in neighboring regions will be affected. The level of variability is reduced, the level of LD increased, and the pattern of allele frequencies are skewed. However, detection of loci under selection is much more complicated than simple examination for changes in allele frequency and LD. These changes can also be brought about by random genetic drift. The goal of my research is to develop methods to detect selection independent of genetic drift and other complicating factors, such as relationships and population structures.

**MD**

Transcriptome analysis reveals an activation of major histocompatibility complex 1 and 2 pathways in chicken trachea immunized with infectious laryngotracheitis virus vaccine. Infectious laryngotracheitis is an acute, contagious, upper respiratory disease of chickens caused by gallid herpes virus 1. Due to mortality rates that can reach up to 70% depending on the virulence of the virus, the disease is of great economic importance to the poultry industry. In this study, 15-d-old specific pathogen-free White Leghorn chickens were used to perform transcriptome analysis of chicken trachea immunized with infectious laryngotracheitis virus vaccine. Myosin and several collagen-related genes were downregulated in the immunized group, suggesting that normal function and structure may be

compromised. In addition, some cytokine receptors and several immune genes, such as Granzyme A (GZMA), CD4 molecule (CD4), CD8a molecule (CD8A), and CD8b molecule (CD8B), were upregulated upon vaccination. The gene ontology analysis shows that genes included in the biological process cluster were related to antigen processing and presentation, positive regulation of immune system processes, T cell selection, and positive regulation of T cell activation. In conclusion, chicken embryo origin vaccine activation of the major histocompatibility complex 1 and 2 pathways provides insight for evaluation and design of infectious laryngotracheitis vaccines.

Methylome Analysis in Chickens Immunized with Infectious Laryngotracheitis Vaccine**.** In this study Song and colleagues investigated the methylome of chickens immunized with Infectious laryngotracheitis (ILT) vaccine derived from chicken embryos. Methyl- CpG binding domain protein-enriched genome sequencing (MBD-Seq) method was employed in the detection of the 1,155 differentially methylated regions (DMRs) across the entire genome. After validation, they ascertained the genomic DMRs distribution and annotated them regarding genes, transcription start sites (TSS) and CpG islands. Global DNA methylation decreased in vaccinated birds, presenting 704 hypomethylated and 451 hypermethylated DMRs, respectively. Additionally, an enrichment analysis detecting gene networks was performed, in which cancer and RNA post-transcriptional modification appeared in the first place, followed by humoral immune response, immunological disease and inflammatory disease. The top four identified canonical pathways were EIF2 signaling, regulation of EIF4 and p70S6K signaling, axonal guidance signaling and mTOR signaling, providing new insight regarding the mechanisms of ILT etiology. Lastly, the association between DNA methylation and differentially expressed genes was examined, and detected negative correlation in seventeen of the eighteen genes.

Develop and evaluate methodologies and reagents to assess immune function and disease resistance to enhance production efficiency through genetic selection in poultry**.** Marek's disease (MD) is a highly contagious, lymphomatous disease of chickens induced by a herpesvirus, Marek's disease virus (MDV) that is the cause of major annual losses to the poultry industry. MD pathogenesis involves multiple stages including an early cytolytic phase and latency, and transitions between these stages are governed by several host and environmental factors. The success of vaccination strategies has led to the increased virulence of MDV and selective breeding of naturally resistant chickens is seen as a viable alternative. While multiple gene expression studies have been performed in resistant and susceptible populations, little is known about the epigenetic effects of infection. In this study, Song and colleagues investigated temporal chromatin signatures induced by MDV by analyzing early cytolytic and latent phases of infection in the bursa of Fabricius of MD-resistant and –susceptible birds. Major global variations in chromatin marks were observed at different stages of MD in the two lines. Differential H3K27me3 marks were associated with immune-related pathways, such as MAP kinase signaling, focal adhesion and neuroactive ligand receptor interaction, and suggested varying degrees of silencing in response to infection. Immune-related microRNAs, e.g. *gga-miR-155* and *gga-miR-10b*, bore chromatin signatures, which suggested their contribution to MD- susceptibility. Finally, several members of the focal adhesion pathway, e.g. *THBS4* and *ITGA1*, showed marked concordance between gene expression and chromatin marks indicating putative epigenetic regulation in response to MDV infection. This comprehensive analysis of chromatin signatures, therefore, revealed further clues about the epigenetic effects of MDV infection although further studies are necessary to elucidate the functional implications of the observed variations in histone modifications.

Transcriptional analysis of Rathke's pouch formation. The anterior pituitary gland plays an essential role in the regulation of many physiological processes such as growth, reproduction, lactation, stress, and metabolism in vertebrates. Formation of Rathke's pouch, the precursor of the anterior pituitary gland,

is a multi-step process regulated by cell interactions, signaling pathways, and transcription factors, which starts with evagination of the oral ectoderm. Previously, laser capture microdissection, RNA amplification, and reverse transcription quantitative real-time PCR (RT-qPCR) were used to characterize mRNA levels for selected genes in the oral ectoderm and adjacent neural ectoderm during Rathke's pouch formation in chicken embryos (embryonic day 2.5 to 7). Changes in levels of mRNA for *Bmp4*, *Fgf8*, *Hesx1*, *Pitx1*, and *Pitx2* were associated with formation of Rathke's pouch. However, each of the selected genes analyzed had been implicated previously in pituitary development in mammals. In recent research, Porter and colleagues performed RNAseq on these samples to analyze changes in mRNA levels on a genome-wide scale and identify novel genes associated with Rathke's pouch formation and pituitary development. Pathway analysis was performed to identify gene networks involving the differentially expressed transcripts identified. Among the top biological functions identified were tissue development, organismal development, endocrine system development, and embryonic development. Results implicated NOTCH1 and NEUROG1 signaling within the oral ectoderm and PACAP and other neuroendocrine peptides within the neural ectoderm. Efforts are underway to define the functional roles of the novel genes and gene networks identified in Rathke's pouch formation and pituitary development.

**MN**

Genomics to increase aflatoxin resistance in turkeys. To investigate the response to aflatoxin exposure we are using RNA-Seq approaches to characterize the transcriptome level changes in the liver, intestine and spleen of birds exposed to $AFB_1$. First, we have completed a laboratory experiment using and *in ovo* assay for $AFB_1$ exposure in the turkey. This experiment examined the effects of early exposure on liver and spleen tissues of domestic and wild turkeys to investigate gene expression differences related to aflatoxin exposure have been sequenced and RNA-seq data are in analysis. Results obtained from the liver are summarized in a manuscript currently under review (Monson et al., in review). A comprehensive review of aflatoxicosis in poultry was also compiled and published (Monson et al., 2015). Second, we are currently completing analysis of an RNAseq analysis from an $AFB_1$ challenge of 16wk wild and domestic turkeys conducted at our collaborating institution (Utah State University, RA Coulombe). Included in this analysis are intestine (primary site of absorption), liver (primary site of metabolism), and spleen (site of secondary immune response). Finally, we continue comparative sequencing of GSTA genes in domestic, heritage and wild turkeys in an effort to identify sequences responsible for gene silencing. The information obtained from these experiments can be used in the development of future in-depth studies of AFB1 responses.

Influence of thermal challenge on turkey muscle development and meat quality. This project in collaboration with Michigan State University and Ohio State University seeks to quantify climate change impacts on poultry breast muscle growth and development, morphological structure, intramuscular fat deposition, and protein functionality to develop appropriate strategies to mitigate the undesirable changes in meat quality. To this effect we are currently working to determine changes in transcriptional profiles of thermally challenged and non-challenged satellite cells, and turkey poults by deep transcriptome RNA sequence analysis. Analysis of satellite cell RNA (24 RNAseq libraries) has provided insight into the transcriptional activity of these stem cells under thermal challenge and the differential responses by Temperature, Developmental time and Genetic line are being summarized for publication (Reed et al. in prep). Analysis of data from a second RNAseq experiment examining skeletal muscle gene expression in your turkey poults (30 libraries) will evaluate Temperature and Line effects.

**PA**

Genetic control of granulosa cell differentiation. Differentiation of the granulosa cell (GC) layer is characterized by the enhanced capacity for FSH---induced cell signaling via the protein kinase A/cyclic

adenosine monophosphate (cAMP) pathway. Critical consequences of such signaling within the GC layer include the initial capacity for steroidogenesis and the enhancement of follicle vascularization observed at selection. Bone Morphogenetic Protein 6 (BMP6) enhances both CYP11A expression and FSH responsiveness in GC collected from 1---2mm, 3---5mm and 6---8mm follicles, with the greatest response again occurring in GCs from 6---8mm follicles. Factors that activate mitogen activated protein kinase (MAPK) or protein kinase C (PKC) signaling prevent BMP6 from initiating FSH---responsiveness and the initiation of GC differentiation at each stage of development. The process by which the GC layer from the single follicle selected each ovulatory cycle ultimately escapes inhibitory signaling to initiate FSH---responsiveness is currently under investigation. These studies are intended to help direct the genetic selection of broiler breeder hens, which inherently are predisposed to multiple selection/double ovulations. Moreover, the results will assist in the selection of turkeys for the purpose of enhancing overall egg production.

Analysis of a supplement to regulate the adiponectin axis. Adiponectin, a hormone secreted from adipose tissue, plays a major role in adipose tissue deposition and energy metabolism. Plasma adiponectin levels decrease as broiler breeder chickens accumulate visceral adipose tissue. Augmenting circulating levels of adiponectin with recombinant adiponectin is impractical as adiponectin circulates at a very high level (4---18 ug/ml) and has short biological half---life (45---60 minutes). Recent studies tested an orally active compound, adipoRon, that is likely to stimulate adiponectin receptors (AdipoR1 and AdipoR2). AdipoRon has been found to ameliorate type 2 diabetes, improve myocyte functions and prevent obesity in murine models. AdipoRon treatment at 0---50 micromolar concentrations resulted in an increase in phospho mitogen activated protein kinase 1/2 and phospho---protein kinase B (Akt) abundance levels in a dose---dependent manner in granulosa cells of preovulatory and prehierarchical follicles. Both adipoRon and recombinant chicken adiponectin dramatically reduced the expression of steroidogenic acute regulatory protein (StAR) in granulosa cells isolated from preovulatory and prehierarchical follicles suggesting a possible downregulation of steroidogenesis in ovarian follicular cells.

**TN**

Adipocyte metabolism regulation. The overarching focus of this project is that adipose tissue is a crossroads for energy utilization, and that a better understanding of this tissue in avians will provide insight into pathways that support optimal growth, including under the effects of environmental stressors. A large set of genes associated with the induction of fatty acid oxidation have been identified, a number of which are incompletely annotated. Putative transcripts of interest are being sequenced and characterized to determine their regulation in adipose tissue under a variety of nutritional manipulations. Significant effort has been focused this year on defining the sequential metabolic events in response to fasting, and to identify the molecular “switches” that confer metabolic plasticity in adipose tissue. This effort utilizes LC-MS to comprehensively identify metabolite pathways that are altered by feed restriction. Expanded instrumentation in the Campagna lab allows profiling of phospholipids, acetyl-carnitines and cholesterol metabolites, as well as small polar metabolites. A number of metabolic changes of interest have been identified. One specific focus is to screen for signaling lipids (e.g., oxysterols) that activate PPARalpha, LXRalpha and other transcription factors that we have linked to control of fatty acid oxidation and metabolic plasticity in broiler adipose tissue. We also established two in vitro adipocyte models that will be used to knockdown or overexpress specific candidate genes for their roles in adipogenesis and lipid metabolism in chickens.

**VA**

Is variation in oxidative stress, a physiological underpinning for many disorders in vertebrates, genetic? Smith continues to investigate oxidative stress in birds and whether it underlies many phenotypes

including longevity as well as those of interest to the poultry industry like dilated cardiomyopathy. Smith's use of genomic tools to dissect the genetic basis of immune response also continues. In the past year, Smith reported work that showed the biphasic effects of ethanol consumption on both oxidative status and adaptive immunity of the chicken, *Gallus gallus*. The effect of ethanol consumption on immune response continues to be of broad interest. Although there is a consensus about the immunosuppressive effect of excessive ethanol use, our understanding of the physiological basis of the influence of alcohol toxicity on the immune system is still unclear. In addition, the potential benefit of moderate alcohol use remains ambiguous. Therefore, this study used an avian model to assess the dose-dependent effect of ethanol on adaptive immunity, and to investigate the role of oxidant status in ethanol-induced changes in the immune system. A total of 96 White Leghorn chickens were randomly divided into 4 groups and provided ad libitum drinking water containing 0, 2, 6, and 10% ethanol for 2 weeks. Total plasma IgG and IgM were determined by ELISA before and after sheep red blood cell (SRBC) challenge. Immune capacity was further evaluated based on the weights of different lymphoid organs. Oxidant status was estimated using biomarkers. Birds exposed to water containing 2% ethanol appeared to have a higher plasma antioxidant status and increased total IgG ($P < 0.05$). Those exposed to 6 and 10% alcohol had decreased levels of plasma antioxidants but increased oxidant status. Additionally, birds exposed to water containing 10% ethanol produced significantly lower IgG following SRBC challenge and had lower average lymphoid organ weights compared with those given water containing 2% ethanol ($P < 0.01$). Significant positive correlations were apparent between lymphoid organ weights and oxidant status ($P < 0.01$). These results appear to suggest positive effect of exposure of chickens to moderate ethanol use on adaptive immunity and a deleterious effect of acute ethanol consumption. Further, oxidant status may influence ethanol-induced polarized changes in the immune system. The work supports earlier suggestions that the chicken could be used as a model to investigate the biological effects of alcohol in vertebrates.

Ontogeny of nutrient transporter gene expression in turkeys during late embryonic and post hatch periods. Growth of poultry post-hatch is dependent upon the uptake of nutrients from ingested feed. Nutrients such as amino acids, peptides and sugars are taken up by transporter proteins located in the brush border membrane of intestinal epithelial cells, which face the intestinal lumen. The objective of this project was to profile the mRNA expression patterns of a number of nutrient transporters during the post-hatch period in chickens and turkeys. The mRNA abundance of a number of amino acid, peptide, and sugar transporters was found to increase from day of hatch until 2-4 weeks post-hatch in both chickens and turkeys, which reflects the maturation of the intestine and the increasing need for nutrients to support rapid growth. One interesting difference between chickens and turkeys was the level of mRNA abundance of the anionic amino acid transporter EAAT3, which transports glutamate, the major energy source for intestinal epithelial cells. EAAT3 mRNA expression was found to be 6-fold greater in the ileum of turkeys than chickens. In addition, male and female turkeys were found to express different levels of nutrient transporters. Of the 11 digestive enzymes and amino acid, peptide and sugar transporters examined, there was greater mRNA abundance of aminopeptidase N (APN) and eight transporters (bo,+AT, EAAT3, ASCT1, PepT1, CAT1, LAT1, y+LAT2, and GLUT5) in female turkeys than male turkeys. The mRNA for the sodium glucose transporter (SGLT1) was expressed greater in male than female turkeys.

Cellular response to Eimeria infection. Avian coccidiosis, which is caused by the intestinal protozoa Eimeria, is characterized by reduced feed efficiency and body weight gain. The sites of lesions in the intestine vary for different Eimeria species, for example E. acervulina, E. maxima and E. tenella primarily affect the duodenum, jejunum, and ceca, respectively. E. praecox, which produces only micro lesions also affects the duodenum. The observed growth depression may be due to the intestinal lesions and the resultant changes in expression of digestive enzymes and nutrient transporters. The

objective of this project was to examine differential expression of digestive enzymes, nutrient transporters and a host defense protein in the small intestine and ceca of broilers challenged with different Eimeria species. Following infection with the four different Eimeria species, there was downregulation of a common set of nutrient transporters (i.e., the anionic amino acid transporter EAAT3 and the zinc transporter ZnT1) and an antimicrobial peptide (LEAP2). Although the four Eimeria species differ in their clinical symptoms, the cellular response to infection by these four Eimeria is similar. The downregulation of nutrient transporters would also explain the observed decrease in growth rate following Eimeria infection.

**Brief Impact Statements**

- Genetic mapping of SNPs on unassigned sequence contigs identified 29 new linkage groups that should aid in the completion of the chicken genome assembly.
- CD8αα T cells and cecal microbes are associated with response to MDV infection and genetic resistance.
- Candidate driver mutations for MD tumors have been identified, which may provide targets for selection or MD vaccines.
- Profiling of miRNAs and miRNA expression in response to MD vaccination and virus challenge is a critical step to explore epigenetics influence over tumorigenesis and vaccine protective efficacy. Advancement in knowledge of this area would lay foundation necessary to understand interactions between vaccination, virus challenge, and host response, which should play important roles determining MD incidence.
- Identification of genome regions affecting ascites phenotype will lead to improved selection to reduce ascites costs.
- Understanding the pathogenesis of BCO will lead to new management practices to reduce lameness in broilers.
- Genome-wide analysis of genetic variations in Arkansas Progressor (AP) and Arkansas Regressor (AR) chicken lines may provide valuable genetic marker information and fundamental cellular mechanisms responsible for regression of virus induced- tumor.
- Characterizing global gene expression of breast muscles, conducting genome-wide SNP analysis and cellular mechanisms involved in the regulation of feed efficiency will provide a means of identifying genetic biomarkers applicable to meat producing animals.
- Identifying an effective antagonist of the avian V1bR will provide an opportunity to attenuate the stress response of broilers and enable us to determine whether an augmentation in productivity results.
- Data obtained with genes responsible for enabling neurons within the brain of birds to sense photoperiods and seasonal information will aid in locating neurons within the broiler brain responsible for activating the reproductive system when birds are transferred to long-day photostimulation.
- Fundamental annotation information (both structural and functional) underpins functional modeling of genomic data sets. This enables poultry researchers to more accurately identify genes involved in the systems they are studying and translate long lists generated by functional genomics into a biological model that they can use to improve poultry production.
- Identification of genes that are associated with resistance to heat stress and Newcastle disease virus and can be used to genetic enhancement of disease resistance of chicken in adaption to hot climate.
- Knowledge of genes associated with enhanced immune response may inform further

information on vaccine efficacy in poultry production.

- Understanding impact of gut associated pathogen on microbiota composition at different development stages will provide great insights in improve gut health and subsequently increase production efficiency and animal well-being.
- The MHC-Y region contributes to differences in immune responses to infections. Defining the role of YF class I-like genes in immunity will define genetic differences at MHC-Y likely to differentially affect immune responses.
- Understanding hormonal mechanisms of the integrated metabolic and reproductive axis in broiler breeder hens is necessary to improve ovarian function and egg production.
- 3'-RNA-seq is a cost effective method amenable to global gene expression studies at population-level, e.g., expression QTL (eQTL) mapping. Also, it allows for accurate detection of the 3'-end of transcripts, enabling verification of the current gene model annotations and global characterization of alternative polyadenylation.
- Several candidate genes for recent selection have been identified in multiple lines
- In F1 crosses generated from inbred Fayoumi and Leghorn lines, 9.2% of the heterozygous loci show allele specific expression
- Genomic imprinting is not present at 12-day chicken embryonic brain or liver.
- A unique gene expression has been identified for wooden breast disease in commercial broiler chickens, which could aid in diagnosis of this disease and in understanding its cause and pathogenesis.
- Dietary methionine deficiency seems to be associated with: muscular inflammation, compromised, immunity, and reduced bone quality
- Selection for growth mildly affects integrity, health and maturity of leg bones.
- Genetic association between leg problems and growth is weak at best, so management strategies would ameliorate leg problems better than genetic solutions.
- The feasibility of applying molecular genetics and genomics to analysis of variation in structure, function and gene expression within the chicken genome was demonstrated.
- Genes, pathways and genomic regions associated with important biological traits in chickens were identified.
- Genetic variation in commercial research lines, research lines and indigenous lines of chickens was characterized.
- Heat exposure was found to alter the layer microbiome.
- Single-step Bayesian methods for large-scale genomic prediction using genotyped and non-genotyped animals were further developed and optimized for computing speed.
- Fitting dominance effects did not improve the accuracy of genomic prediction of breeding values in a commercial layer population.
- A modified version of the Wilmink lactation curve function was found to provide an improved fit to egg production data while maintaining linearity in parameters
- Detection of loci under selection using whole genome selection is potentially much more powerful than classic QTL detection methods based on association of phenotype with genotypes, such as GWAS and F2 design. Combined with ASE analysis, differentiated populations, and re-sequencing analysis, many of the genes associated with economic traits (e.g., behavior, wellbeing, disease traits) can be identified.
- Efforts to map causal alleles for single or complex traits are compromised by the incomplete nature of the reference genome sequence and the annotation that links it with genes and transcripts. New methodologies have substantially improved the reference sequence and better annotation will make that reference more useful to all users.

- Genetic markers to improve AFB1-resistance have a potentially high commercial value and positive economic impact to industry, owing to improvements in health and well-being, productivity, and a safer product for consumers.
- Identification of molecular mechanisms associated with altered muscle development will result in development of mitigation strategies based on improved genetic selection, nutritional intervention, and other strategies to improve poultry muscle food quality and quantity.
- Functional information for genes leverages functional genomics data to understand diseases in poultry through applyication of “omics” approaches.
- A global proteome for small white follicles, white yolk, and ovarian stroma of the avian ovary will provide a baseline for future studies of follicular development.
- Use of cultured PGCs in germ plasm preservation, development of transgenic birds, and assisted reproduction of endangered species, requires that the sex of the donor PGCs match the sex of the recipient embryo.
- Working models have been developed for early ovarian follicle growth and selection plus the role for adiponectin in adipose tissue deposition and energy metabolism. Mechanisms regulating follicle growth, selection and differentiation will help predict the cellular mechanisms and role of energy balance that regulate sequence/clutch size, together with causes of ovarian dysfunction in broiler breeder hens.
- A number of lipids that potentially control adipose development and metabolism in broiler adipose tissue have been identified.
- Two in vitro models were developed to test the effects of manipulating specific candidate genes on adipose deposition and metabolism.
- Comprehensive methods for lipidomics and metabolomics were developed to expand ability to link gene function to metabolic pathways
- Previously identified genetic markers have been validated for a non-specific immune response. These could be a resource for testing the potential for replacing the use of antibiotics to improve immune capacity in chickens.
- Correlation analysis suggested that oxidative stress may play a role in biphasic immunoregulatory effects of ethanol, possibly through their regulatory function on cell proliferation and oxidative damage of cellular components.
- The responses in the chicken as well as the bird’s unique features including the bursa of Fabricius and PUA suggested that avians could be promising models for understanding the biological basis of alcohol toxicity on the immune system and host defense.
- A common set of nutrient transporters and an antimicrobial peptide LEAP2 were downregulated following challenge with E. acervulina, E. maxima, E. tenella and E. praecox. The downregulation of nutrient transporters would partly explain the weight loss and may represent a common cellular response to counter an Eimeria infection. The suppression of the host defense peptide LEAP2 may be a general Eimeria-mediated mechanism for propagating the infection.
- For most transporter genes examined mRNA abundance was greater in female turkeys than male turkeys. Turkeys also expressed 6-fold greater amounts of mRNA for the anionic amino acid transporter EAAT3 compared to chickens. This new knowledge can be used to not only better formulate turkey diets to accommodate increased glutamate absorption, but also to optimize nutrition for both sexes.
- The genome-wide analysis of transcriptional changes in the oral and neural ectoderm layers during chick embryonic development unveiled numerous novel candidate genes that potentially play pivotal roles in pituitary development and function.

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**Presentations Published in Symposium Proceedings:**

1. Abasht B., W. Fu, W.R. Lee, E.M. Brannick, M.F. Mutryn. 2015. Identification of gene expression biomarkers associated with severity of Wooden Breast disease. Proceeding of XXII European Symposium on the Quality of Poultry Meat, May 10-13, 2015, Nantes, France.
2. Candace Croney, Nicole Olynk Widmar, Bill Muir and Ji-Qin Ni 2015. Animal Welfare and Food Production in the 21st Century: Scientific and Social Responsibility Challenges. Int. Symp. on Animal Environ. & Welfare. Oct. 23–26, 2015, Chongqing, China
3. Hans Cheng, Ph.D.; Sudeep Perumbakkam, Alexis Black Pyrkosz, Ph.D.; John R. Dunn, DVM, Ph.D.; Andres Leggara, William M. Muir, Ph.D. Fine mapping of QTL and genomic prediction using allele-specific expression SNPs demonstrates that the complex trait of genetic resistance to Marek's disease is predominantly determined by transcriptional regulation BMC Genomics 16:816
4. Mutryn M.F., W. Fu, W.R. Lee, B. Abasht. 2015. Incidence of Wooden Breast disease and its correlation with broiler performance and ultimate pH of the breast muscle. Proceeding of XXII European Symposium on the Quality of Poultry Meat, May 10-13, 2015, Nantes, France.
5. X Alencar, K Martin Rainey, W. Muir, S. Xu 2015 NAM: Association Studies in Multiple Populations Bioinformatics 2015- 0712.R1

**Books and Chapters in Books:**

1. Johnson, AL. Reproduction in the female. In: Sturkie's Avian Physiology, 6th Ed., C.G. Scanes, Ed., New York: Elsevier, Chapter 28, pp. 635---665, 2015.
2. Kuenzel WJ. The avian subpallium and autonomic nervous system. In: Sturkie's Avian Physiology. (Ed. C.G. Scanes), Elsevier, 6th Ed., San Diego, CA. 2015. Chapter 9:135163.
3. Petitte JN, Mozdziak, PE. Transgenic Animal Technology and Meat Quality. In: Meat Quality: Genetic and Environmental Factors. 2015, W. Przybylski, D. Hopkins, eds., New York: CRC Press, Chapter 14, 417-430.
4. Voy BH, Dearth S, Campagna SR. Transcriptomic and metabolomic profiling of chicken adipose tissue. In: Genomics, Proteomics and Metabolomics in Nutraceuticals and Functional Foods. Eds., Bagchi D, Swaroop A and Bagchi M. Wiley Blackwell, Hoboken, NJ, pp. 537-43, 2015.

**Dissertations:**

1. Alaamri, O. In Vivo Periodical Monitoring of Immune Cell Infiltration in Response to Feathers and Intramuscular Injection of IONPs Using the Pulp (Dermis) of Growing Feathers as Test Site Tissue in Chickens. PhD dissertation; University of Arkansas; Dec 2015.
2. Hu, Y. *Quantitative Epigenetic Analysis on a Whole Genome Scale.* PhD Thesis, UW-Madison, 2015.
3. Monson, M.S. PhD. Hepatotoxic and Immunomodulatory Transcriptome Responses to Aflatoxin B1 in the Turkey (*Meleagris gallopavo*). PhD dissertation; University of Minnesota; 2015.
4. Peñagaricano, F. *Quantitative Genomic Approaches for the Genetic Analysis of Complex Traits in Livestock Species.* PhD Thesis, UW-Madison, 2015.
5. Stephens, CS. Follicle Selection and Growth in the Domestic Hen Ovary. PhD Thesis, Cornell University, 2015.

**Funding and Leveraging:**

| Grant | Funding |
|---|---|
| Acquisition of a Shared Use Real Time PCR System. Arkansas Biosciences Institute; 7/2014-6/2015;; PI- Rhoads | $40,936 |
| Adapting chicken production to climate change through breeding. USDA-NIFA-AFRI;; PI: Schmidt; CoPIs: Lamont, Rothschild, Persia, Ashwell | $4,700,000 |
| ARS CRIS Project, Employing Genomics, Epigenetics, and Immunogenetics to Control Diseases Induced by Avian Tumor Viruses. PI Cheng | NA |
| ARS CRIS Project, Genetic and Biological Determinants of Avian Tumor Virus Pathogenicity, Transmission, and Evolution. PI Cheng | NA |
| Borlaug Fellow Program, USAID, S.E. Aggrey | $34,852 |
| Developing a dual purpose model: Can dietary fatty acids developmentally program reduced adipose tissue in broiler chicks? University of Tennessee Center of Excellent; 6/1/2015 – 5/31/2016; PI: Voy | NA |
| Development and Application of Novel Glycan-Specific Reagents to Facilitate Early detection of epithelial ovarian cancer; NIH, 09/2011-8/2015,; PI:Muddiman; coPI: Petitte | $1,124,374 |
| Development of colonization resistance in chicks. NIH-NIFA Dual purpose with Dual benefits. 2015-67015-22930. A. Baumler, H. Zhou | $1,600,000 |
| Enabling network analysis of host-pathogen interactions. USDA AFRI; 11/2014-10/32017;; PIs: McCarthy & Nanduri | $487,987 |
| Enhancing genetic resistance to Marek's disease in chicken via allele-specific expression screens and genome-wide selection. USDA, AFRI, award no. 2012-67015-19419,; PI, Cheng; co-PIs, John Dunn (ADOL), Bill Muir (Purdue U.), Sudeep Perumbakkam (ADOL), and Frans van Sambeek (Hendrix Genetics, The Netherlands | $499,960 |
| Experimental annotation of the Chicken Genome. NIH NIGMS; 2009-2014;; PI: Burgess; coPI: McCarthy | $1,000,000 |
| Exploring Causal Relationships Underlying Economically Important Traits in Dairy Sheep. USDA-HATCH; 10/2013-9/2017;; PI: Rosa; coPI: Rhoads | $166,312 |

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| Follicle Selection and Differentiation in the Avian Ovary. National Science Foundation; 3/2014---2/2017. PI: A.L. Johnson | $261,000 |
| Follow-on: Reducing Disease In Livestock; Gates Foundation, 2/2012-3/2015;; PI: Petitte; coPI: Mozdziak | $100,000 |
| Functional genomics to enhance aflatoxin resistance in poultry. USDA-NIFA-AFRI. 2013-2016, (co-PD w/ Coulombe). | $499,822 |
| Genome biology of Marek's disease: Viral integration and genome alterations in genetically resistant and susceptible stocks. USDA-NIFA, 2013-67015-21330; Hans Cheng (USDA- ARS, MI), Mary Delany (UC Davis) and Bill Muir (Purdue University) (CG no. 2013-01125) | $499,997 |
| Genome wide identification and annotation of functional regulatory regions in livestock species. USDA NIFA 2015-67015-22940 H. Zhou, P. Ross, I. Korf | $499,842 |
| Genomic Selection for Improved Fertility of Dairy Cows With Emphasis on Cyclicity and Pregnancy. USDA-AFRI; 9/2012-8/2017;; PI: Pinedo; coPI: Rosa | $3,000,000 |
| Genomics for improving animal production. USDA NIFA National Need Training Grant 2014-38420-21796 H. Zhou, J. Murray, P. Ross | $238,500 |
| Genomics for Resistance to Disease in Animals: An Integrated Educational Approach. USDA-National Needs training grant;; PI: Lamont; coPIs: Carpenter, Rothschild, Tuggle | $254,000 |
| Genotype-based selection for ascites susceptible and resistant broilers. USDA Animal Health; 7/2014-8/2016;; PI: Anthony; coPI-Rhoads | $22,000 |
| Glucocorticoid induction of endogenous growth hormone (GH) in chicken embryos. USDA-AFRI; 1/14-12/16;; PI: Porter | $500,000 |
| Hatch funds to UC Davis ; US Poultry, Cattle and Swine Genome Coordinators Funds for farm animal ENCODE Aviagen Limited ; National Pork Board | NA |
| Hepatic gene expression profiling to identify changes associated with altered ovarian follicle selection. Cornell University Center for Vertebrate Genomics 1/2014- 12/2015;; PI: Johnson | $7000 |
| iAnimal: Cyberinfrastructure Enabling Animal Breeding, Genetics, And Genomics. USDA AFRI; 09/13-08/16;; PI: Lyons; coPI McCarthy | $500,000 |
| Identifying the onset of a novel muscle disorder in chickens through differential gene expression and histologic analyses. U.S. Poultry & Egg Association; 06/01/14-05/30/16;; PI: Abasht; coPIs: Erin Brannick, Carl Schmidt | $57,919 |
| Improving Food Security of Africa by Enhancing Resistance to Disease and Heat in Chickens. USAID;; PI: Zhou; coPIs: Bunn, Lamont, Dekkers, et al. | $6,000,000 |
| Improving the chicken genome assembly and annotation. USDA, AFRI, award no. 2013-67015-21357,; PI, Wes Warren (Wash. U. St. Louis); co-PIs, C. Titus Brown (Michigan State U.) and Cheng. | $485,690 |
| Inferring Causal Phenotype Networks Using Genomic Information. USDA-AFRI; 3/2011-2/2016;; PI: Rosa | $467,290 |
| Influence of thermal challenge on turkey muscle development and meat quality. USDA-NIFA-AFRI. 2013-2016, co-PD Reed, Strasburg and Velleman. | $975,000 |
| Integrated training of graduate students in quantitative methods (Co-PDs: Aggrey and Rekaya)-USDA-NIFA | $238,500 |
| Knowledge representation resources for agricultural researchers. USDA AFRI; 01/11-09/15;; PI: McCarthy; coPI: Burgess | $733,845 |
| Marker Assisted Selection for Ascites Resistance in Broilers. NIFA-AFRI; 11/2014-10/2017;; PI-Rhoads; coPI-Anthony, Kong, Schmidt | $467,000 |

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| MHC-Y Class I determinants in Innate and Adaptive Immune Responses to Marek's Disease. NIFA-AFRI; 09/2009-08/2014;; PI-Miller | $370,000 |
| Molecular and metabolomics mechanisms underlying Met isomers and their analogues in broilers. EVONIK Industries AG. S. E. Aggrey | $170,000 |
| Nutrient transporter research intramural funding and extramural funding. industry sources (Evonik, Kemin);; PI: E. Wong | $80,000 |
| PathBubbles for Dynamic Visualization and Integration of Biological Information. NSF ABI; 03/12-06/16;; PI: Schmidt; coPI: McCarthy | $218,690 |
| Single-step Bayesian Method for Genomic Prediction that Combines Information from Genotyped and Non-genotyped Animals. USDA-NIFA-AFRI;; PI: Fernando; CoPIs: Dekkers, Garrick | $350,000 |
| Strategies to enhance de novo biosynthesis of methionine in organic chicken. USDA-NIFA. S. E. Aggrey, et al. | $500,000 |
| System Biology Analysis & Modeling of Complex "OMIC" Data: A Service Center Approach. Agriculture and Food Research Initiative Competitive Grant no. 2011-67015-30196 H. Zhou, K. Drake | $749,891 |
| Transcriptome Analysis and Transformation of Chicken Primordial Germ Cells. Arkansas Biosciences Institute; 7/2014-6/2015;; PI-Rhoads | $17,700 |
| US-UK Collaborative Research: Host Resistance to Avian Pathogenic E. coli. USDA-NIFA-AFRI/BBSRC;; PI: Lamont; coPIs: Wolc, Kaiser, Stevens, Vervelde | $499,999 |
| Total | $28,418,106 |

NA- Not Available