Annual Report for NC1170
Period Covered: January 1, 2016 to December 31, 2016
Prepared by: Behnam Abasht

Institutional Stations (Institutional Abbreviation: Members)
Beckman Research Institute at the City of Hope (**COH**[1]: M. Miller)
Cornell University (**CU**[1]: P. Johnson)
Iowa State University (**IA**[1]: S. Lamont[2,3], J. Dekkers[2])
Michigan State University (**MI**[1]: G. Strasburg[2,3])
Mississippi State University (**MS**[1]: C. McDaniel, B. Nanduri)
North Carolina State University (**NC**[1]: C. Ashwell, J. Petitte)
Pennsylvania State University (**PA**[1]: A. Johnson, R. Ramachandran)
Purdue University (**IN**[1]: W. Muir[2])
Texas AgriLife Research (**TX**: G. Athrey[3]) new member
University of Arizona (**AZ**[1]: F. McCarthy[2,3], S. Burgess)
University of Arkansas (**AR**[1]: W. Kuenzel[2,3], B. Kong, D. Rhoads[2,3])
University of California, Davis (**CA**[1]: M. Delany[2], H. Zhou[2,3])
University of Delaware (**DE**[1]: B. Abasht[2,3])
University of Florida (**FL**[1]: M. Edelmann)
University of Georgia (**GA**[1]: S. Aggrey[2])
University of Maryland (**MD**[1]: T. Porter, J. Song[2])
University of Minnesota (**MN**[1]: K. Reed[2,3])
University of Tennessee (**TN**[1]: B. Voy[2,3])
University of Wisconsin (**WI**[1]: G. Rosa)
USDA-ARS-Avian Disease and Oncology Lab **(ADOL**[1]: H. Cheng[2,3], H. Zhang[2])
Virginia Tech (**VA**[1]: E. Wong[2,3], E. Smith)

1. Written annual report submitted.
2. Oral annual report given at the meeting.
3. Attended the annual business meeting.

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

NC1170 Business Meeting January 15, 2017

NC1170 BUSINESS MEETING 2017
1. Call to order
2. Approval of Agenda
3. USDA Administrative Advisor: Susan Lamont
4. Renewal of the NC1170 project: Susan Lamont
5. Renewal of the NRSP8
6. NIFA/USDA administrator: Lakshmi Matukumalli
7. NRSP8 Poultry co-Coordinators: Delany and Cheng
8. Location and time for meeting for 2018
9. Creating transgenic chickens (inviting Rob Etches): Wayne Kuenzel
10. Other business
11. Adjourn

1. Poultry Workshop PAG:
    a. Attendance: Saturday AM=80; Sunday AM= 70; peak attendance >120

b. Representatives of 16 stations attended at some point during the weekend
c. One survey showed 1 or more representatives from 15 other institutions including the poultry industry, U.S. government, the United Kingdom, Germany, Canada, Sweden, Netherlands, Thailand, and China
d. Guest Speakers: Walter Bottje (University of Arkansas), Carl Schmidt (University of Delaware) Jim Reecy (IA State), Wes Warren (Washington University), Thibaut Hourlier (European Molecular Biology Laboratory - EBI, Cambridge UK), Michèle Tixier-Boichard, (INRA, France), Richard Kuo (Roslin, UK), Richard Crooijmans (Wageningen, Netherlands), Mirte Bosse (Wageningen, Netherlands), Hao Bai (College Park, MD; Jorgenson Travel award winner), Colin Kern (UC Davis, Cobb Travel Award Winner)
e. Nine graduate students gave short presentations about their posters.
f. Updates: J Reecy on NRSP8 bioinformatics; W. Warren on chicken genome reference updates
g. 17 NC1170 members presented research updates

Business meeting Sunday January 15, 2017.
Attendees:
(DE) Abasht, Behnam, (TX) Athrey, Giri, (ADOL) Cheng, Hans, (MI) Strasburg, Gale, (AR) Kuenzel, Wayne, (IA) Lamont, Susan J., (USDA) Matukumalli, Lakshmi, (AZ) McCarthy, Fiona, (MN) Reed, Kent, (AR) Rhoads, Douglas, (DE) Schmidt, Carl, (TN) Voy, Brynn, (VA) Wong, Eric A., (CA) Zhou, Huaijun
Administrators: Lamont, Susan (Admin Advisor), Matukumalli, Lakshmi (NIFA/USDA administrator)

1. Convened 11:30 AM in San Diego, CA, Sunset Room, Town and Country Convention Center.
2. USDA Administrative Advisor: Susan Lamont
    a. Project is going well.
    b. The 5-year project terminates on September 30, 2018, the complete project renewal must be submitted by December 1, 2017 and several sections must be submitted earlier than December 1, 2017. Deadlines (these dates start in the fall, one year prior to the project's expiration date, meaning these dates are in 2017 for the NC-1170 renewal):
        i. September 15: Deadline to submit a request to write a proposal in NIMSS and upload the Issues and Justifications section.
        ii. October 15: Deadline to upload the Objectives section in NIMSS.
        iii. November 15: All participants and their AES offices should have submitted completed Appendix E forms into NIMSS.
        iv. December 1: Completed proposal is due in NIMSS in its entirety. Failure to meet this deadline may result in the project not being reviewed and renewed this round.
3. Lakshmi Matukumalli:
    a. RFP for 2017 not yet available. Maybe in February.
    b. Discussed the new peer-review mechanism that was experimented last year. Members discussed advantages and disadvantages of this new method. Overall, there were more positive views than negatives. One person expressed that with the new method proposals get evaluated with more reviewers (5 with the new method vs. 3 with the conventional method), lowing the chance of getting biased ranking of the proposals.
4. 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. 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

    d. Members can expect some partial travel money for students/postdocs to attend PAG, but members need to request.
5. Next meeting of NC1170 will be coordinated with PAG at Town and Country in January 2018.
6. Wayne Kuenzel (AR) discussed the idea of inviting Rob Etches and perhaps a person from either the Jackson Lab or Charles River at a future Poultry Workshop (January, 2018) to determine if there is an interest in the poultry/avian community for utilizing knock-out, knock-in, knock-down chickens in research and whether a company like Jackson Lab or Charles River would be able to provide the necessary tools/lines at an affordable price to labs in the near future. The consensus was that this would be of interest to the group and because this would be a potential business opportunity for a firm such as Crystal Bioscience, they should cover their own travel and attendance costs.
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 2016. 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 92 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 $18,675,963 in funding/grant support that was active during 2016. During the past four years this project has added new participants from Cornell University, Mississippi State University, Pennsylvania State University, University of Tennessee-Knoxville and Texas AgriLife Research, 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.

AZ

AgBase: supporting functional modeling in agricultural organisms.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. During 2016 we continued to provide annotated gene function data to support NC1170 researchers. Via AgBase we provide 1,753,795 Gene Ontology (GO) annotations for 341,743 gene products across 531 species, including poultry species and their pathogens.

Standardized Gene Nomenclature. During 2016 the Chicken Gene Nomenclature Committee (CGNC) biocurators worked closely with NCBI Entrez Curators to ensure that updated gene annotations had revised nomenclature. During 2016 we updated genes in line with the Galgal4 annotations and approved 1,454 chicken gene names. We currently provide standardized nomenclature for 27,237 chicken genes and have a pending grant application to support continued annotation of bird genes. We especially thank avian researchers that have contacted us about chicken gene nomenclature and worked with us to ensure that these names best represent our current understanding of gene function.

Chickspress –developing a tissue specific compendium of gene expression for chicken gene products. 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. The Chickspress genome browser information was also moved from GBrowse to the CoGe genome browser, providing access to more comparative analysis tools and leveraging cyberinfrastructure supported by the NSF funded CyVerse project. Expression information provided by this resources is publicly available and was used as part of the Galgal4 assembly and annotation. This data has also been utilized to investigate avian sex chromosomes.

Host-Pathogen Interaction Database (HPIDB) –curation to support animal health data sets. During 2016 we also continued manual biocuration of host-pathogen interaction data to support network modeling of animal health data sets. The HPIDb now provides 3,733 manually curated molecular interactions, including host-pathogen interactions from 28 different livestock pathogens. These interactions have all been manually curated and pass international QC and curation standards. With collaborators at Mississippi State we are developing tools to support interaction prediction.

CA

Identification of Regulatory Elements in Livestock Species. Regulatory elements play an essential role 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 farm animal genomes. Chicken, pig and cattle are major farm animals in providing the world's food production. Robust functional annotations of their genomes could be leveraged to improve their production efficiency. Recent international FAANG (Functional Annotation of ANimal Genomes) initiative has stimulated such great efforts on livestock species. The overall objective of this study is to functionally annotate regulatory elements in three livestock species. The first key step is to identify regulatory elements in the genomes by integrating RNA-seq, DNase-seq and ChIP-seq data from each tissue. 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 pleen. For chicken, an analysis of 15 DNase-seq data from all issues with two replicates, except hypothalamus (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. Using the two replicates to construct a set of DHS sites that are present in each replicate, we found 29190 sites in cerebellum, 43672 in cortex, 52337 in liver, 64149 in lung, 27433 in muscle, and 63605 in spleen. In addition, 24 ChIP-seq data from all tissues (two replicates) except adipose and muscle (H3K4me3 and H3K27me3 histone modification marks) were generated. For H3K4me3, 19940 peaks were shared between replicates in cerebellum, 9979 in cortex, 6104 in hypothalamus, 5872 in liver, 29891 in lung, and 20323 in spleen. For H3K27me3, 14000 in cerebellum, 7525 in cortex, 16 in hypothalamus, 7642 in liver, 5807 in lung, and 4326 in spleen. Integrative analysis of DHS sites, ChIP-seq, 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.

COH

Identification of genes within the MHC-Y region of chicken chromosome 16. During the past year, our sequencing of the MHC-Y region clones isolated from the Red Jungle Fowl BAC libraries have advanced to completion. Critical to completion was Single Molecule, Real-Time (SMRT) sequencing carried out with USDA NRSP-8 funds in the City of Hope Integrative Genomics Core Facility (Xiwei Wu, Tae Hyuk Kang and Charles Warden) using the new Pacific Biosciences P6-

C4 sequencing chemistry. This method of sequencing provided cohesive and reliable sequence over this region that is filled with genes belonging to several gene families and many, many repeat sequences. The margin between rRNA genes in the NOR and MHC-Y is now clearly defined. Newly determined sequences for five additional clones allowed four distinct MHC-Y contigs to be defined. FISH analyses by Mary Delany, Marla McPherson and Justin Smith of the MHC-Y haplotype in Line 001 (Red Jungle Fowl) provide further evidence that MHC-Y region in RJF is segmented.

Development of a PCR-based method for MHC-Y genotyping. Annotation of the MHC-Y contigs defined in Objective 1 led to efforts focused on ways to develop a simpler method for distinguishing among MHC-Y genotypes. Until now restriction fragment patterns revealed in Southern hybridizations have been used to define MHC-Y haplotypes. While highly reliable, determinations by Southern hybridization are labor intensive and not readily applied in typing large numbers of animals. A number of regions in the RJF MHC-Y sequence were tested as candidate primers for producing patterns of PCR-products that reflect MHC-Y haplotypes previously defined by restriction fragment patterns in Southern hybridization. PCR reaction products from a region upstream of the start of MHC class I-like genes has been identified that produces distinctive patterns when the PCR products are separated on agarose gels and stained with ethidium bromide.

FL

Phosphorylation is a key post-translational modification controlled by kinases, and it regulates essential cellular processes, therefore various anomalies in this modification have been implicated in animal diseases. However, kinases have never been systematically studied in chicken. Therefore, annotation of kinases in chicken is necessary for studying chicken health/disease. To map chicken kinases in tissue we used two different approaches. First, we used kinase domain mapping based on published data and Chickspress database (University of Arizona, http://geneatlas.arl.arizona.edu), which incorporates NCBI and Ensembl gene models with the protein expression data from specimens of multiple tissues from both genders. This bioinformatic approach allowed us to detect chicken kinases in various tissues by using peptide evidence, but it was limited to identification of 7 kinases in spleen and 2 in liver. Second, we utilized active-site directed probes combined with mass spectrometry to identify over 200 ATP- and ADP-binding sites in 140 chicken kinases in spleen and liver combined. This experiment led to identification of novel kinases, 71% of which were previously uncharacterized in chicken. By utilizing this chemical proteomics, which is invaluable in novel enzyme identification, we improved existing annotation of these enzymes, which drastically expands current annotations available via Uniprot and other resources. We compared several sequences of detected kinases to known mouse, rat and human orthologues to understand conservation of these ATP-binding domains. Our study confirmed that chicken proteome contains multiple kinases, some of which have not been previously annotated. We also mapped novel nucleotide-binding lysine residues in these kinases and show that these kinases have a unique abundance in chicken tissue. This information will be made publicly available in collaboration with AgBase (Arizona Univ. and Mississippi State University)

IA

Transcriptome data. Several data sets from RNA seq experiments on chickens were deposited in public databases upon submission of the manuscripts describing those studies.

MN

Working with the University of Minnesota Informatics Institute and the Minnesota Supercomputer Institute we have established an analysis pipeline for data grooming and mapping of RNA-seq data sets. This has facilitated a recent collaborative project with the University of Wisconsin-Madison, “Determining Turkey Selenium Nutrition and Requirements Using Molecular Biology”.

MS

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 1,751,960 GO annotations for 341,748 gene products from 516 species, including more than 40 agriculturally important species and their pathogens (30 November 2016). This information includes
371,046 GO annotations for 63,763 avian gene products.

During 2016 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; last updated June 28, 2016) we provide 2,141 manually curated molecular interactions, including host-pathogen interactions from 57 different livestock pathogen strains. In 2016, we improved the functionality of the database. At present, HPIDB data can be combined with network visualization and ontology functional information to better inform our understanding of infectious diseases. With collaborators at University of North Carolina we are developing tools to support interaction prediction.

Phosphorylation is an important post-translational modification controlled by kinases, and it regulates essential cellular processes, therefore various anomalies in this modification have been implicated in animal diseases. However, kinases have never been systematically studied in chicken. Therefore, annotation of kinases in chicken is necessary for studying chicken health/disease. We utilized high-throughput proteomics combined with bioinformatics analysis to identify tissue-specific expression of this important class of enzymes. Specifically, we used kinase domain mapping as well as Chickspress database available at University of Arizona (http://geneatlas.arl.arizona.edu), which incorporates NCBI and Ensembl gene models along with the protein expression data obtained from specimens of multiple tissues and both genders. We identified 81 and 134 active kinases in chicken liver and spleen respectively, majority these kinases have not been previously correctly annotated in NCBI. This information will be made publically available in collaboration with AgBase.

PA

Adiponectin, a hormone secreted from adipose tissue, plays a major role in adipose tissue deposition and energy metabolism. The Ramachandran lab has previously determined that plasma adiponectin levels decreases 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). Metformin, a biguanide anti-diabetic drug, affects glucose and lipid metabolism similar to adiponectin and corrects dysfunctional follicular development in women with polycystic ovarian syndrome. Utilizing real-time quantitative polymerase chain reaction and/or Western blotting, metformin treatment was found to increase the abundance of phospho-extra cellular receptor kinase ½ phospho-acetyl Co-A carboxylase, phospho-adenosine monophosphate-activated protein kinase in theca cells isolated from both prehierarchical and preovulatory follicles in a dose-dependent manner. Both basal and follicle stimulating hormone (FSH)-stimulated steroidogenic acute regulatory protein (STAR) gene expression in granulosa cells isolated from the 9-12mm follicles was found to be significantly decreased by metformin treatment in a dose-dependent manner. Ovarian cortical follicles (400 micrometer in size) were isolated from broiler breeder hen ovaries and treated with metformin (0 or 10 mM) with or without addition of chicken vasoactive intestinal polypeptide (VIP; 1 ng/ml). VIP treatment resulted in a 12-fold increase in STAR abundance that was completely abolished by addition of metformin. It is concluded that metformin treatment is likely to affect steroidogenesis and nutrient metabolism in ovarian follicular cells.

A second goal for the project (directed by ALJ) are to understand the cellular mechanisms responsible for the daily recruitment of a single ovarian follicle into the rapid growth (preovulatory)

stage of development using the commercial laying hen and turkey hen as primary model systems. The regulation of such selection is prerequisite for the approximate daily ovulation, and determines the duration of egg production in both chickens and turkeys. Published data from the laying hen support the proposal that the initiation of cell differentiation within the granulosa cell (GC) layer is initiated at the time of follicle recruitment into the preovulatory hierarchy. This process is characterized by the initial capacity for FSH-induced cell signaling via the protein kinase A/cyclic adenosine monophosphate (cAMP) pathway. Critical consequences of such signaling include the initial capacity for GC steroidogenesis, the initiation of rhythmic clock gene expression and the enhancement of follicle vascularization. The process by which the GC layer from the single follicle selected each ovulatory cycle ultimately escapes inhibitory signaling to initiate FSH-responsiveness remains the focus of our investigations. Recently completed studies of the turkey hen confirm that ovarian follicle dynamics and the regulation of expression for critical endocrine signaling components (e.g., FSHR, STAR, BMP4, BMP6, AMH), together with the mechanisms regulating the initiation of GC differentiation are highly conserved between the two species.

WI

Differential contribution of genomic regions to marked genetic variation and prediction of quantitative traits in broiler chickens. Genome-wide association studies in humans have found enrichment of trait-associated single nucleotide polymorphisms (SNPs) in coding regions of the genome and depletion of these in intergenic regions. However, a recent release of the ENCyclopedia of DNA elements showed that ~80 % of the human genome has a biochemical function. Similar studies on the chicken genome are lacking, thus assessing the relative contribution of its genic and non-genic regions to variation is relevant for biological studies and genetic improvement of chicken populations. A dataset including 1,351 birds that were genotyped with the 600K Affymetrix platform was used. We partitioned SNPs according to genome annotation data into six classes to characterize the relative contribution of genic and non-genic regions to genetic variation as well as their predictive power using all available quality-filtered SNPs. Target traits were body weight, ultrasound measurement of breast muscle and hen house egg production in broiler chickens. Six genomic regions were considered: intergenic regions, introns, missense, synonymous, 5′and 3′ untranslated regions, and regions that are located 5 kb upstream and downstream of coding genes. Genomic relationship matrices were constructed for each genomic region and fitted in the models, separately or simultaneously. Kernel based ridge regression was used to estimate variance components and assess predictive ability. Contribution of each class of genomic regions to dominance variance was also considered. Variance component estimates indicated that all genomic regions contributed to marked additive genetic variation and that the class of synonymous regions tended to have the greatest contribution. The marked dominance genetic variation explained by each class of genomic regions was similar and negligible (~0.05). In terms of prediction mean-square error, the whole-genome approach showed the best predictive ability. All genic and non-genic regions contributed to phenotypic variation for the three traits studied. Overall, the contribution of additive genetic variance to the total genetic variance was much greater than that of dominance variance. Our results show that all genomic regions are important for the prediction of the targeted traits, and the whole-genome approach was reaffirmed as the best tool for genome-enabled prediction of quantitative traits.

Incorporating parent-of-origin effects in whole-genome prediction of complex traits. Parent-of-origin effects are due to differential contributions of paternal and maternal lineages to offspring phenotypes. Such effects include, for example, maternal effects in several species. However, epigenetically induced parent-of-origin effects have recently attracted attention due to their potential impact on variation of complex traits. Given that prediction of genetic merit or phenotypic performance is of interest in the study of complex traits, it is relevant to consider parent-of-origin effects in such predictions. We built a whole-genome prediction model that incorporates parent-of-origin effects by considering parental allele substitution effects of single nucleotide polymorphisms (SNP) and gametic relationships derived from a pedigree (the POE model). We used this model to predict body mass index in mouse population, a trait that is presumably

affected by parent-of-origin effects, and also compared the prediction performance to that of a standard additive model that ignores parent-of-origin effects (the DD model). We also used simulated data to assess the predictive performance of the POE model under various circumstances, in which parent-of-origin effects were generated by mimicking an imprinting mechanism. The POE model did not predict better than the ADD model in the real data analysis, probably due to overfitting, since the POE model had far more parameters than the ADD model. However, when applied to simulated data, the POE model outperformed the ADD model when the contribution of parent-of-origin effects to phenotypic variation increased. The superiority of the POE model over the ADD model was up to 8% on predictive correlation and 5% on predictive mean squared error. The simulation and the negative result obtained in the real data analysis indicated that, in order to gain benefit from the POE model in terms of prediction, a sizable contribution of parent-of-origin effects to variation is needed and such variation must be captured by the genetic markers fitted. Recent studies, however, suggest that most parent-of-origin effects stem from epigenetic regulation but not from a change in DNA sequence. Therefore, integrating epigenetic information with genetic markers may help to account for parent-of-origin effects in whole genome prediction.

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

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, ~1,500 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 35 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 (63, 71, 72, and 15I5,), 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 15I5, and 1 (15.N-*21*) from line 0. Lines 63 and 72 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 63 and 72. 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, efforts will be soon developed to transfer all the lines by 2023.

AZ

Chicken Phenotype Annotation. 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 >15,000 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.

COH

Nothing to report for this objective, except for MHC-Y Genotyping Underway

IA

Iowa State University chicken resource populations. Iowa State University maintained thirteen unique chicken research lines [including highly inbred, MHC-congenic, closed populations; and advanced intercross lines (AIL)] to serve as resources for identifying genes, genetic elements and genomic regions of economic importance; as well as defining unique aspects of chicken genomic architecture. 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 F26) 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) because of defined, distinct responses among lines. 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 or biological materials include H Zhou, UC-Davis (NDV and heat-stress response); C. Schmidt, U Delaware (heat stress), J Womack, Texas A&M (defensins); R Coulombe, Utah State (aflatoxin sensitivity), B Abasht, U Delaware (allele-specific expression); E Wong, Virginia Tech (Eimeria response) and V Kapur, Penn State (virus-embryo assays).

MS

Chinese Painted quail and Beltsville Small White turkeys continue to be selected for parthenogenetic development. For our current populations of these 2 parthenogenetic lines, macroscopic parthenogenetic development is exhibited in approximately 40% of their unfertilized eggs. The most recent research has revealed that the parthenogenetic trait in quail negatively impacts sperm-egg interactions in mated hens. Additionally, albumen pH, calcium, and gas composition is drastically modified by parthenogenetic development.

NC

Gene-editing in Chicken Primordial Germ Cells. The lack of efficient, specific genome editing methods have been one of the major impediments to our ability to produce gene knockouts and targeted mutations in the avian genome. This has significantly limited agricultural scientists to address fundamental questions related to food animal health and disease resistance; however, the CRISPR/Cas system has been used successfully to edit the genomes of prokaryotes to humans, and with a specificity and efficiency unmatched by other genome editing platforms.

As a proof of concept and because of their significance in host defenses against pathogens, a knockout strategy was developed to target iNOS (NOS2) and phox (NOX2), (Gene IDs395807 and 418581, respectively). Specific protospacer adjacent motifs (PAM) sites were selected using CHOPCHOP, a web tool for selecting the optimum target sites for CRISPR/Cas9 (Nucleic Acids Res. 42. W401-W407-2014). Rankings of possible target sequences were checked for off-target sequences in the genome using BLAST. Oligonucleotides of the selected PAM sequences were ligated in to CRISPR/Cas9 vectors and verified by sequencing.

Before targeting PGCs, two avian macrophage cells lines were used to model functional knockout events that resulted through non-homologous end joining DNA repair (NHEJ). MQ-NCSU and HD11 macrophage cells lines were transfected with the specific NOS2 and NOX2 CRISPER/Cas9 constructs generated. The Cas9 protein was fused to a GFP marker and allowed enrichment of Cas9 expressing cells using FACS. Subsequently, the GFP-expressing fraction of cells were cloned by limiting dilution. After 14 days, viable colonies of cells were expanded and cells banked. DNA isolated from individual clonal lines were used to amplify about a 450 bp region flanking the PAM site. Amplification products were gel purified and cloned into plasmid vectors for DNA sequencing. Gentoypes were classified as wildtype, monoalleic or biallelic.

Sequence analysis of two targeted sequences for iNOS (NOS2) resulted in monoallelic and biallelic genotypes in MQ-NCSU cells. In all cases of a homozygous knock-out of NOS2, the sequence was either in-frame or generated a new start site for the correct transcription of a truncated protein. Functional induction of nitrous oxide production in such cases was no different than that observed for wild-type cells. New CRISPR/Cas9 targets will need to be generated and tested in the cells lines before use in PGCs.

Sequence analysis of a targeted locus in NOX2 in MQ-NCSU and HD11 macrophages also resulted in monoallelic and biallelic genotypes in both cells lines. Stimulation of reactive oxygen species (ROS) production was tested in the biallelic knockout genotypes. In all cases, ROS production was inhibited. In the monoallelic cell lines, ROS production was roughly half of that produced by the wild-type cells and demonstrated a gene-dosage effect for NOX2 function. Therefore, the CRISPR/Cas9 constructs for NOX2 is a suitable candidate for attempting a gene knockout in PGCs.

Culture of Primordial Germ Cells. Work with primordial germ cells continues. We have adapted the culture of PGCs from our serum-based system to a serum free system (Stem Cell Reports. 2015 Dec 8;5(6):1171-82). Previously, frozen stocks of PGCS cultured using serum were readily adapted to the serum free culture conditions.

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

ADOL

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 (JM/102W strain to help promote large homogenous gonal tumors), which resulted in ~100 tumor samples. To date, most samples have been screened to generate three primary datasets: (1) whole genome sequencing analysis, (2) whole transcriptome sequencing analysis, and (3) cytogenetic analysis to identify MDV integration sites. Thus far, characterization of 26 MD tumors with multiple algorithms has repeatedly identified ~300 somatic single nucleotide variants (SNVs) and ~50 non-synonymous mutations per tumor sample. Frequently mutated genome targets shared between tumors include tumor suppressor genes (TSGs) containing loss-of-function mutations. Transcriptome analysis shows differential gene expression and deregulation of many cancer-associated genes and pathways (e.g., MAPK, cell cycle,

apoptosis). Collectively, these data demonstrate how somatic mutations in MD tumors contribute to tumor genesis and progression, and made aid in genomic selection for MD resistance.

Identifying the molecular basis for MD vaccine synergy. Marek's disease (MD) vaccines utilize protective synergism, a phenomenon where the protective efficacy of two vaccines in combination is greater than either vaccine when administered alone. A key example is the bivalent MD vaccine of serotype 2 (SB-1) and serotype 3 HVT (FC-126). Despite this widespread usage, the biological mechanism of the synergistic effect has never been elucidated. We hypothesized that a combination of SB-1 and HVT would alter replication pattern of either SB-1 or HVT or both via competitive or cooperative effects. After confirming protective synergy of SB-1 and HVT vaccines, we measured the replication patterns of both viruses at 1, 5, 10, and 14 day post infection (dpi) using qPCR of birds that were vaccinated with HVT only, SB-1 only, or both. The replication patterns of SB-1 and HVT were demonstrated to be different in term of tissue tropism and time. SB-1 replicated to the highest levels in spleen, bursa, and thymus, respectively, while HVT showed replication only in bursa at 1 dpi and could not be detected at any other time point or tissue type. Moreover, replication patterns of either SB-1 or HVT in birds given bivalent vaccine did not change from birds given SB-1 or HVT monovalent vaccines suggesting that synergism is not a result of replication pattern changes through cooperative or competitive actions. Currently, we are screening cytokines to determine if unique or enhanced expression of one or more might account for protective synergism.

Influence of Marek's disease on immune response and core gut flora in commercial layer lines. Enhancing Marek's disease (MD) genetic resistance is desirable to augment current vaccinal control in commercial poultry. There has been interest to understand the influence of the gut microbiome on the immune system during MD. In the present study, 14 birds from control and Marek's disease virus (MDV; JM strain) infected treated groups were sampled at 4 and 8 days post infection (dpi), and splenic and cecal contents were screened using high throughput sequencing. Data analysis of splenic RNA at 4 and 8 dpi resulted in 401 and 1,446 differentially expressed genes ($P > 0.05$), respectively, after Bonferroni correction for multiple testing. Pathway analysis indicates genes involved in the immune response, defense response, and cytokine production are altered by viral infection. At day 8, which corresponds to the beginning of the latent phase of MDV infection, pathways with chick development and cell cycle are also highly upregulated. 209 genes were found to be common on between 4 and 8 dpi and predominately involved immune system responses. Furthermore, the bacterial populations in the ceca also responded to MDV infection, with a shift in community structure (ANOSIM, R= 0.664, $P = 0.0001$). Although there were no differences in total number of operational taxonomic units (OTUs), there was enrichment of *Lactobacillus* spp. associated with MDV infection, suggesting the presence of lactate and acidic pH in the ceca of infected birds. These results provide insights into host immune and gut microbiome response to MDV infection for improved MD control strategies.

Comparative transcriptome analysis of genetically divergent lines of chickens in response to Marek's disease virus challenge at cytolytic phase. To augment current control measures of MD, one of the continuous efforts in MD research is to advance the understanding on genes that confer genetic resistance to MD. This project was conducted to expand the knowledge on what genes, in addition to the reported ones, also possibly contributing to the genetic resistance. Total RNA samples were extracted from spleen tissues taken from euthanized line 6 and 7 chickens at 5-day post MDV challenge. The cDNA libraries were prepared and subjected to deep sequencing on a HiSeq2500 platform. Bioinformatics analyses resulted in identification of a total of 203 genes that dysregulated in expression in response to MDV challenge. Of those, 153 genes were reported in one or up to 7 similar studies; the other 50 genes were identified for the first time.

Identifying coding and non-coding genes differentially expressed between ALV-like spontaneous tumors and normal bursal tissues to elucidate genetic and epigenetic mechanisms underlying the tumorigenicity in chickens. It has been known that some of the experimental and commercial lines of chickens develop spontaneous ALV-like tumors while the birds were free of detectable oncogenic pathogens like exogenous ALV. Although the incidence of the spontaneous ALV-like tumors generally speaking is relatively low, it, however, creates hardship for management and economic loss due to condemnation. Reported observations suggested that the non-oncogenic subgroup E ALV and MDV-2 (SB-1) can boost the incidence each individually or in combination in the susceptible chickens.

Up to date, there is no clear understanding as to what makes some chickens develop such tumors while others are free of the disease under mutually equal or same environment and management conditions. There is even little information or any knowledge to go with in formulating measures to prevent or to reduce the incidence of the disease from happening in those lines of chickens. This project was developed to gain some understanding on what coding and noncoding genes may be possibly involved by examining differential expression profiles between the tumor and non-tumor tissues.

By next-gen sequencing of mRNA and small RNA samples of the spontaneous ALV-like tumors and non-tumor tissues, a total of 1,981 differentially expressed coding and 50 noncoding genes were identified (fold change > 2). Preliminary analyses showed the differentially expressed coding genes and the target genes of the non-coding genes are enriched in key pathways, including Wnt signaling pathway, MAPK signaling pathway and mTOR signaling pathway, which have been shown to implicate in a wide range of human tumors.

AR

Identification of genes affecting ascites susceptibility. AR- in collaboration with other investigators at AR and at DE continued to investigate the genetics of ascites. Multiple GWAS in different generations identified a region around 60 Mbp on GgaZ that is associated with male susceptibility and a female specific region on Gga2. Selected matings suggest that the heterozygotes are more susceptible. Marker based breeding is underway to better understand the contributions of this region. Heart RNAs have been purified from altitude challenged birds with and without hypertrophy, and ambient pressure birds for RNAseq analysis of hypobaric affects on gene expression in the heart. A reanalysis of SNPs has been completed for a region on Gga9 that has been associated with ascites, based on VNTRs. That work has been published. Current work is examining Allele Specific Expression (ASE) for candidate genes in this region. A very large dataset of whole genome sequencing from selected pooled DNAs has been generated. Those sequences are currently being utilized to identify copy number variations (CNVs) differentially represented in ascites phenotypes. The data have also been utilized for genotype-by-sequencing and has identified a new gene on Gga2 that is currently under focus.

Bacterial chondronecrosis with osteomyelitis and lameness in broilers. AR- collaborators have identified Staphylococcus agnetis as involved in bacterial chondronecrosis with osteomyelitis (BCO) leading to lameness. The genome and initial characterization as well as microbiomes of the bone and blood associated with BCO has been published. The parameters for the infection of BCO have been defined, and we have tested a number of pre- and pro-biotics for reducing the spread of the infection have been tested and published. Recent work has indicated that the bacterium can be easily communicated from broilers through contact or the air. We have also initiated a phylogenomic analysis of related Staphylococci isolates from cattle to understand the genetic differences that appear to be critical for infection of broilers.

Feed efficiency of broilers. AR- in collaboration with other investigators at AR, shotgun proteomics was conducted using in-gel trypsin digestion and tandem mass spectrometry on breast muscle samples obtained from pedigree male (PedM) broilers exhibiting high feed

efficiency (FE) or low FE phenotypes. Over 1800 proteins were identified, of which 152 were different ($P < 0.05$) by at least 1.3 fold and ≤ 15 fold between the high and low FE phenotypes. Data were analyzed for a modified differential expression (DE) metric (Phenotypic Impact Factors or PIF) and interpretation of protein expression data facilitated using the Ingenuity Pathway Analysis (IPA) program. In the entire data set, 228 mitochondrial proteins were identified whose collective expression indicates a higher mitochondrial expression in the high FE phenotype. Pathway enrichment analysis also identified mitochondrial dysfunction and oxidative phosphorylation as differentially expressed canonical pathways in high FE.

Genes and proteins involved in the stress response of broilers. AR- in collaboration with other investigators at AR and the University of Missouri have completed studies on the neuroendocrine regulation of stress in broilers and have focused upon one of the major receptors involved in stress known as the vasotocin 1a receptor (V1aR). Results strongly suggest that in addition to stress the V1aR functions in the neural regulation of food intake. Completed studies have shown that when a potent neuropeptide, neuropeptide Y (NPY) well known to stimulate food intake, is administered to birds by central brain administration, the result is a robust increase in food intake. When the V1aR is effectively blocked by a specific antagonist of the avian V1aR the result is a significant augmentation of food intake above the effect of NPY. A second publication detailed diencephalic and septal structures containing the avian V1aR involved in the regulation of food intake. Another set of experiments examined two separate structures in the brain known as circumventricular organs, to determine their involvement with a physical stressor, osmoregulation and a psychogenic stressor, immobilization. Three genes were found differentially expressed in two circumventricular organs, dependent upon the stressor. The two CVOs were located in the anterior region of the hypothalamus.

AZ

Training workshops and community support via AgBase. We continue to provide training, outreach and support for poultry researchers via AgBase to ensure that they are able to better leverage their functional genomics data to understand key economic traits for poultry. During 2016, the AgBase resources were visited by 18,506 different researchers, with 30.2% of these visitors from the US (includes visitors from 48 states). AgBase was cited in 65 publications during 2016, and in more than 600 publications overall.

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.

For inbred line study, tissues at 2, 6, and 10 dpi were collected to focus on NDV study under heat stress. RNA isolated from trachea, lung, and Harderian glands were used to generate 192 libraries for the sequencing. Tissues at 4 hours, 9 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. RNA-seq data from both treated and non-treated at both lines at 2 or 3 time points (depend on NDV focus or heat stress focus) have been analyzed and genes and signal pathways have been identified. Further bioinformatics analysis will be done and manuscript is under preparation.

For Hy-Line Brown commercial layer study, 564 chicks were challenged with LaSota strain NDV with heat stress treatment at 2 weeks of age. Virus titer in tears at 2, 6 dpi and antibody response at 10 dpi in serum and 13 heat stress related parameters in blood at 4 hours, 6 and 9 days post-heat and body weight were measured in all birds. Birds were genotyped using the 600K SNP chicken panel and genome-wide association study between these phenotypes and genotypes were analyzed using AsReml and Gensel. Genetic parameters for these phenotypes were estimated and genomic regions affecting disease resistance, heat stress parameters and growth were identified. Manuscripts will be prepared next year.

COH

Defining the function of MHC-Y class I-like proteins. During the past year we were also able to advance understanding of the structure of the MHC-YF class I-like molecules. With considerable effort, some expert advice on modification of our protein expression system Bjorkman and Stadtmueller), and excellent support for mass spectrometry determinations (Gabriel Gugiu) we now reliably produce MHC-Y heavy chain and beta 2-microglobulin proteins of sufficient quantity and quality for high quality mass spectrometry determinations of the ligands bound by different MHC-Y class I isoforms.

This work is advancing quickly providing insights into both the nature of the ligands bound by MHC class I-like molecules and how the inherent polymorphism of these molecules is displayed.

CU

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. The relationship between the metabolic and reproductive axis in broiler breeder hens was examined 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. As previously reported, 120 genes were differentially expressed with a > 2 fold change ($p<0.05$; FDR< 0.05); 51 genes were up-regulated and 69 genes were down-regulated in FF compared to RF. The technique of qPCR was used to validate some of the RNA seq data and mRNA for IGF-I was elevated in FF hens ($p<0.03$), GHR mRNA was increased in FF ($p<0.05$) and the IGF-I binding proteins, particularly IGFBP2, which is regulated by feed intake in chickens, were dramatically elevated ($p<0.01$) in RF hens. There were no significant differences in the abundance of two forms of liver vitellogenin (VT1 and VT2). In the RNAseq results, the vitellogenins are very abundantly expressed in the liver of both FF and RF broiler hens. Of note however, is that the liver weight is 2.3 fold greater in FF broiler breeder hens compared to RF hens, suggesting that the larger size could account for increased availability of yolk precursors necessary for increased numbers of follicles in FF hens. Plasma concentrations of thyroid hormones were evaluated and plasma T3 was significantly elevated in FF hens as compared to RF hens. Furthermore, plasma cholesterol and triglycerides were also significantly elevated in the plasma from FF hens.

Occludin (OCLN), a tight junction protein, mediates transfer of yolk material to the oocyte surface. OCLN may be a key regulator of yolk accumulation and follicle growth, however, the expression and regulation of OCLN in granulosa cells during various stages of follicle development is unknown. OCLN expression in granulosa cells of 3-5 mm follicles of FF hens

was lower compared to RF hens and yolk weights were higher in the FF group. This suggests that feeding level regulates occludin expression and hence, may have a role in the excessive yolk accumulation in FF broiler breeder hens.

DE

Metabolomics Study of Wooden Breast Disease in Commercial Broiler Chickens. This study was conducted to characterize metabolic features of the breast muscle (pectoralis major) in chickens affected with the Wooden Breast myopathy. Live birds from two purebred chicken lines and one crossbred commercial broiler population were clinically examined by manual palpation of the breast muscle (pectoralis major) at 47–48 days of age. Metabolite abundance was determined by gas chromatography/mass spectrometry (GC/MS) and liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) using breast muscle tissue samples from 16 affected and 16 unaffected chickens. Muscle glycogen content was also quantified in breast muscle tissue samples from affected and unaffected chickens. In total, levels of 140 biochemicals were significantly different (FDR < 0.1 and fold-change A/U > 1.3 or < 0.77) between affected and unaffected chickens. Glycogen content measurements were considerably lower (1.7-fold) in samples taken from Wooden Breast affected birds when compared with samples from unaffected birds. Affected tissues exhibited biomarkers related to increased oxidative stress, elevated protein levels, muscle degradation, and altered glucose utilization. Affected muscle also showed elevated levels of hypoxanthine, xanthine, and urate molecules, the generation of which can contribute to altered redox homeostasis. In conclusion, our findings show that Wooden Breast affected tissues possess a unique metabolic signature. This unique profile may identify candidate biomarkers for diagnostic utilization and provide mechanistic insight into altered biochemical processes contributing to tissue hardening associated with the Wooden Breast myopathy in commercial chickens

Hepatic Gene Expression Analysis of Wooden Breast Disease in Chickens. Recent research on Wooden Breast Disease (WBD) has focused on understanding the molecular contributors of breast muscle damage, fibrosis, and lipidosis resulting from WBD. The current study was conducted to explore the differentially expressed (DE) genes in the liver of chickens affected by the Wooden Breast myopathy. Live birds, from a purebred line of commercial broiler chickens, were clinically examined by manual palpation of breast muscle at 47 days of age. Liver tissue samples from 4 affected and 4 unaffected birds were used in high throughput mRNA sequencing (RNA-seq) using Illumina HiSeq 2500 platform. After aligning sequence reads to the chicken reference genome Galgal4) using TopHat, differential gene and transcript expression analysis was performed using Cuffdiff. The 113 significant differentially expressed genes were used for functional annotation clustering with DAVID Bioinformatics Resources. In affected birds, 71 of these genes were upregulated and 42 were downregulated. The most prominent differentially expressed gene pathways included transmembrane, extracellular matrix receptor interaction, and oxidoreductase. In conclusion, this study shows breast muscle is not the sole tissue affected by WBD, indicating further physiological complexities involved in the occurrence of this muscle abnormality in commercial chickens.

Pathology of Wooden Breast Disease in Modern Broiler Chickens: A Histologic and Ultrastructural study. Wooden Breast Disease (WBD) is a novel muscle disorder in the poultry industry observed to frequently affect the breast muscles of high-yielding modern broilers. Characterized by extreme stiffening of the breast muscles upon palpation of the pectoral region, WBD is known to result in significant economic loss in the poultry industry, and may potentially cause behavioral alterations and reduced welfare in birds. To examine tissue changes associated with onset and pathogenesis of this disorder, a time-series experiment was conducted using chickens from a high- breast-muscle-yield, purebred commercial broiler line. Birds were raised for a period of six weeks, and breast muscles sampled on a weekly basis from selected birds and processed for light and transmission electron microscopy. Histologic presentation indicated presence of focal single-myofiber degeneration and hyalinization in the second week, preceding inflammatory reaction that started in the third week. Lesions in the

fourth week were generally characterized by multifocal to diffuse muscle fiber degeneration and necrosis accompanied by increased inflammatory cell infiltration. Lesions in the fifth and sixth week were characterized by diffuse muscle fiber damage, fibrosis, fatty infiltration including granulomatous tissue encompassing lipid droplets, and irregular myofiber regeneration. Ultrastructural examination showed fibrosis with dense regular collagen fibers, irregular Z-discs, myofibril splitting, displacement and degeneration, including mitochondrial degeneration. This study therefore demonstrates that WBD exhibits an early onset in modern broilers and appears to assume a progressive course with acute inflammatory phase occurring in the earlier stages and chronic inflammation and fibrosis in the later stages of the disease course.

GA

Molecular mechanisms associated with dietary methionine deficiency. Methionine (MET) is the first limiting amino acid in a typical poultry diet. Restriction of dietary MET affects protein biosynthesis, feed efficiency and body composition. The molecular mechanisms that underlie such restrictions remain to be elucidated. We studied the molecular mechanisms that underlie dietary MET restrictions in broiler chickens from hatch until 10 days of age in the Pectoralis major muscle using next generation sequencing. Cobb 500 male chickens (n=900) were fed a diet deficient in Met+Cys (0.77% in 0-10 days starter phases) or the deficient diet supplemented with either LMET, DLMET or MHA-FA (equimolar comparison). Fold change of ≥1.5 and false discovery rate of ≤0.05 were used as criteria for differentially expressed genes (DEGs). The major biological processes were immune response, regulation of immune response and regulation of T cell activity. The gene ontology molecular functions were signal receptor activity, transmembrane signaling, and receptor activity. Most significant cellular component was related to extracellular matrix, collagen type IX trimer, proteinaceous extracellular matrix and components of membrane. Among the major pathways inhibited or activated during dietary MET deficiency in chickens were inflammatory bowel disease (IBD), hematopoietic cell lineage, T cell receptor signaling pathway, Cytokine-cytokine receptor interaction, and B cell receptor signaling pathways. Inflammatory bowel disease is characterized by chronic inflammation of the gastrointestinal tract due the dysregulated immune system. Differential expression of TLR and ILs, STAT4 and AP1 elicit cascade of events that leads to IBD. When the LMET was compared with either DLMET or MHA-FA isomers, the differential pathways were dilated cardiomyopathy, hypertrophic cardiomyopathy and adrenergic signaling in cardiomyocytes. Among the differentially expressed genes between DLMET and HMA-FA were NFA1P2, HMGB2, DCP1B, glycoprotein Ib, and GP1BB.

mRNA analysis of genes in the protein biosynthesis and ubiquitin-proteosome pathways in meat-type chickens under heat stress. Growth is significantly reduced in poultry under heat stress and several strategies have been suggested to remedy the effects of heat stress with varying success. Molecular mechanisms that underlie protein biosynthesis of poultry under heat stress would allow for strategies to mitigate the effects of heat stress. We investigated the immediate and long term transcriptomics changes in chickens under heat stress. Forty-eight Cobb500 male birds were divided into two groups and raised under either constant 25oC or 35oC from 14-26 days of age in individual cages and fed ad libitum on a diet containing 21% CP and 3100kcal ME/kg. Five birds per treatment at 1 and 12 days after heat treatment were euthanized and the Pectoralis (P.) major was sampled for gene expression analysis. mRNA expression of key genes in the avian target of rapamycin (avTOR) and the ubiquitin-proteosome pathways representing protein synthesis and breakdown, respectively were studied. Feed intake and growth were reduced in the heat stressed birds compared to the controls, whereas FCR and rectal temperature were similar among the two groups. The P. major mRNA expression analyzed by the delta delta Ct method indicated that, after a day of heat stress, avTOR and EIF4E expressions as well as UBE21, UBE3A, PSMC1, PSMD1 and FBXO32 were reduced in the stressed birds compared to controls suggesting that both protein synthesis and protein breakdown were significantly reduced. However, after 12 days of heat stress, there was an increased mRNA expression of UBE21, UBE3A, PSMC1, PSMD1 and FBXO32 compared to the controls suggesting a significant increase in protein ubiquitination and degradation.

Multi-generational imputation of SNP marker genotypes and accuracy of genomic selection. Availability of high-SNP genotyping platforms provided unprecedented opportunities to enhance breeding programs in poultry and to better understand the genetic basis of complex traits. Using this genomic information, genomic breeding values (GEBVs), which are more accurate than conventional BVs. The superiority of genomic selection (GS) is possible only when high-density SNP panels are used to track genes and QTLs affecting the trait. Unfortunately, even with the continuous decrease in genotyping costs, only a small fraction of the population has been genotyped with these high-density panels. Most often a larger portion of the population is genotyped with low-density and low-cost SNP panels and then imputed to a higher density. Accuracy of SNP genotype imputation tends to be high when minimum requirements are met. Nevertheless, a certain rate of genotype imputation errors is unavoidable. Thus, it is reasonable to assume that the accuracy of GEBVs will be affected by imputation errors; especially, their cumulative effects over time. To evaluate the impact of multi-generational selection on the accuracy of SNP genotypes imputation and the reliability of resulting GEBVs, a simulation was carried out under varying updating of the reference population, distance between the reference and testing sets, and the approach used for the estimation of GEBVs. Using fixed reference populations, imputation accuracy decayed by about 0.5% per generation. In fact, after 25 generations, the accuracy was only 7% lower than the first generation. When the reference population was updated by either 1% or 5% of the top animals in the previous generations, decay of imputation accuracy was substantially reduced. These results indicate that low-density panels are useful, especially when the generational interval between reference and testing population is small. As the generational interval increases, the imputation accuracies decay, although not at an alarming rate. In absence of updating of the reference population, accuracy of GEBVs decays substantially in one or two generations at the rate of 20% to 25% per generation. When the reference population is updated by 1% or 5% every generation, the decay in accuracy was 8% to 11% after seven generations using true and imputed genotypes.

IA

Selection signatures in African ecotypes and Northern European breeds assessed using a 600K SNP chip. Understanding the genetic strategies that indigenous, non-commercial breeds have evolved to survive in their environment could help to elucidate molecular mechanisms underlying biological traits of environmental adaptation. We examined chicken DNA from diverse breeds and climates of Africa and Northern Europe for selection signatures that have allowed them to adapt to their indigenous environments. Selection signatures were studied using a combination of population genomic methods that employed FST, iHS, and runs of homozygosity procedures. All the analyses indicated differences in environment as a driver of selective pressure in both groups of populations. The analyses revealed unique differences in the genomic regions under selection pressure from the environment for each population. The African chickens showed stronger selection towards stress signaling and angiogenesis, while the Northern European chickens showed more selection pressure toward processes related to energy homeostasis. The results suggest that chromosomes 2 and 27 are the most divergent between populations and the most selected upon within the African (chromosome 27) and Northern European (chromosome 2) birds. Examination of the divergent populations have provided new insight into genes under possible selection related to tolerance of a population's indigenous environment that may be baselines for examining the genomic contribution to tolerance adaptions.

Heat stress and immune stimulation impact on lymphoid organ transcriptome. Although decreased circulating antibody levels, increased pathogen shedding, and other immunosuppressive effects of heat stress have been reported in chickens, the mechanisms underlying these reduced immune responses need to be better understood to improve poultry heat tolerance and immune capabilities. RNA-sequencing (RNA-seq) was used to provide a transcriptome-wide measure of the effects of exposure to high temperatures in three chicken immune tissues (spleen, bursa and thymus) from two genetic lines (a heat tolerant inbred

Fayoumi line and a heat-susceptible outbred broiler line). At 22-days-old, chickens were exposed to acute heat stress and/or lipopolysaccharide (LPS) in four treatment groups (Thermoneutral + Saline, Thermoneutral + LPS, Heat + Saline, and Heat + LPS). Responses to bacterial LPS were included to model endotoxemia and investigate heat stress interactions with a pro-inflammatory stimulus. Indexed cDNA libraries from individual tissue samples (n = 3-4 libraries/tissue/treatment/line) were sequenced using the Illumina HiSeq 2500. Differential expression analyses identified 1,300-2,500 significant genes in each tissue, with greater responses in spleen and bursa in Fayoumi, and in thymus in broiler. Pathway analyses further revealed distinct responses in each tissue and each genetic line. Expression changes in cell adhesion, cell signaling, and immune pathways provide insight into the effects of heat stress on immune tissues and targets to improve heat tolerance and disease resistance in chickens.

Gut microbiome of laying hens altered by exposure to high ambient temperature. We characterized the changes in chicken cecal microbiome during a 4 week heat stress experiment with mature layer hens during active egg production. Eighty hens were randomly split into 2 groups: cyclic daily high ambient temperature or thermoneutral temperature. Eight birds from each group were euthanized to harvest samples at each of 5 time points during heat treatment: day 1 (acute), and weekly in weeks 1 through 4 (chronic/adaptation phase). 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. The cecal content microbiome was found to be altered after 2 weeks of cyclic heat exposure.

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) conducting NDV challenge studies and associated genetic and genomic analyses on local African chickens in Tanzania and Ghana, (5) genomic, molecular and cellular characterization of the host-pathogen interactions between chickens and avian pathogenic E. coli, (6) genomic analyses of chickens that survived high pathogenic avian influenza outbreaks.

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.

I examined impact of resource population on ability to fine map QTL using a model species, i.e. the brown Norway rat. We were able to fine map regions of selection to areas less than 1kb in length, and often to regions of a gene (regulatory vs. coding), and thereby uncover the modes of gene action for complex traits. This degree of fine mapping has never been achieved previously in any species. We showed that the ability to fine map was a result of critical design features in the resource population. a) They were replicated, allowing the ability to separate signal (fixation due to selection) from noise (fixation due to random drift); b) They resulted from a MAGIC cross that resulted in small HB that pinpoints SS to discrete portions of a gene; c) They are derived from a long-term, bi-directionally selected population, that magnified the phenotypic and genomic differences between lines for increased power; and d) Within family selection was used to develop the lines, which maximized the effective population size and minimized the rate of inbreeding because all families contributed at least one individual to the next generation. Maximizing the effective population size also minimizes the buildup of new HB.

Importantly fixation of alleles can result from the combined effects of random genetic drift and selection . Without replication, the majority of allele fixations were due to random genetic drift. This problem cannot be fixed statistically by increasing stringency for testing. For example, when analyzed by replicate, and GWp<.001, 3,698 genes were detected in Replicate-1 and 3876 genes were detected in Replicate-2, yet only 1,060 genes were verified across replicates at this GWp. Only ~29% of genes identified by a single replicate were reproducible across replicates (71% failure rate). Further, increasing the stringency by decreasing GWp <.0001, resulted in 3,100 genes detected in Replicate-1 and 3,041 genes detected in Replicate-2; yet only 761 verified across replicates at this Gwp, i.e. only ~24% were reproducible, which is less than that at the lower stringency GWp. In the extreme, if only the top 1000 SS in the first and second replicates were chosen, these were associated with, respectively, 177 and 184 genes, but only 9 of these genes were verified across repliactes, which translates to a >95% failure rate. The lack of reproducibility between replicates was due to the confounding effects of random genetic drift, not to incorrectly setting the critical Type 1 error rate. Without replication these types of SS would largely give non-reproducible results even at high stringencies.

MD

Project: Epigenetics and Avian Diseases

Chicken gga-miR-103-3p Targets CCNE1 and TFDP2 and gga-miR-130a targets HOXA3 and MDFIC, both miRNAs Inhibit MDCC-MSB1 Cell Migration. Marek's disease (MD) is a highly contagious viral neoplastic disease caused by Marek's disease virus (MDV), which can lead to huge economic losses in the poultry industry. Recently, microRNAs (miRNAs) have been found in various cancers and tumors. In recent years, 994 mature miRNAs have been identified through deep sequencing in chickens, but only a few miRNAs have been investigated further in terms of their function. Previously, gga-miR-103-3p was found downregulated in MDV-infected samples by using Solexa deep sequencing. In this study, we further verified the expression of gga-miR-103-3p among MDV-infected spleen, MD lymphoma from liver, noninfected spleen, and noninfected liver, by qPCR. The results showed that the expression of gga-miR-103-3p was decreased in MDV-infected tissues, which was consistent with our previous study. Furthermore, two target genes of gga-miR-103-3p, cyclin E1 (CCNE1) and transcription factor Dp-2 (E2F dimerization partner 2) (TFDP2), were predicted and validated by luciferase reporter assay, qPCR, and western blot analysis. The results suggested that CCNE1 and TFDP2 are direct targets of gga-miR- 103-3p in chickens. Subsequent cell proliferation and migration assay showed that gga- miR-103-3p suppressed MDCC-MSB1 migration, but did not obviously modulate MDCC-MSB1 cell proliferation. In conclusion, gga-miR-103-3p targets the CCNE1 and TFDP2 genes, and suppresses cell migration.

Moreover, we also found the expression of gga-miR-130a in MDV-infected and uninfected spleens. Subsequently, proliferation and migration assays of MDV- transformed lymphoid cells (MSB1) were carried out by transfecting gga-miR-130a. The target genes of gga-miR-130a were predicted using TargetScan and miRDB and clustered through Gene Ontology analysis. The

target genes were validated by western blot, qRT-PCR, and a dual luciferase reporter assay. Our results show that the expression of gga-miR-130a was reduced in MDV-infected spleens. Gga-miR-130a showed an inhibitory effect on MSB1 cell proliferation and migration. Two target genes, homeobox A3 (HOXA3) and MyoD family inhibitor domain containing (MDFIC), were predicted and clustered to cell proliferation. Results indicate that gga-miR-130a regulates HOXA3 and MDFIC at the protein level but not at the mRNA level. We conclude that gga-miR-130a can arrest MSB1 cell proliferation and migration, and target HOXA3 and MDFIC, which are both involved in the regulation of cell proliferation. Collectively, gga-miR- 103-3p and gga-miR-130a plays a critical role in the tumorigenesis associated with chicken MD.

Coding and linc RNAs in differentiation of chicken embryonic stem cells to spermatogonia stem cells. Regulation of crucial lncRNAs involved in differentiation of chicken embryonic stem cells (ESCs) to spermatogonia stem cells (SSCs) was explored by sequencing the transcriptome of ESCs, primordial germ cells (PGCs) and SSCs with RNA-Seq; analytical bioinformatic methods were used to excavate candidate lncRNAs. We detected expression of candidate lncRNAs in ESCs, PGCs and SSCs and forecasted related target genes. Utilizing bioinformatics tools, function and protein-protein interactions of target genes were analyzed. Based on RNA Sequencing result, we further explored the specific regulating mechanisms of Hedgehog (HH) signaling in this process. In in vivo, siRNA- Indian Hedgehog (IHH) could stably express in fertilized chicken embryos and significantly down-regulate the IHH expression. With real-time quantitative PCR and western blot, we identified that integrin α6 and integrin β1 expression was significantly suppressed along with IHH inhibition in vivo. We found that Hedgehog signaling pathway positively regulates the differentiation of ESCs to male germ cells through signal transduction by IHH.

A decision analysis model for KEGG pathway analysis. A decision analysis model is developed that accounts for dependence among pathways in time-course experiments and multiple treatments experiments. This model introduces a decision coefficient-a designed index, to identify the most relevant pathways in a given experiment by taking into account not only the direct determination factor of each Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway itself, but also the indirect determination factors from its related pathways. Meanwhile, the direct and indirect determination factors of each pathway are employed to demonstrate the regulation mechanisms among KEGG pathways, and the sign of decision coefficient can be used to preliminarily estimate the impact direction of each KEGG pathway. The simulation study of decision analysis demonstrated the application of decision analysis model for KEGG pathway analysis. Our results showed that the decision analysis model can provide the promising and more biologically meaningful results. Therefore, the decision analysis model is an initial attempt of optimizing pathway analysis methodology.

MI

Project: Influence of thermal challenge on turkey muscle development and meat quality. This project, conducted in collaboration with Ohio State University and the University of Minnesota, is studying the effect of thermal challenge on: 1) cultured turkey muscle satellite cells; and 2) posthatch turkey poults by characterizing changes in transcriptional profiles, muscle microstructure, and meat quality characteristics. In addition, one of the aims of the project is the use of thermal manipulation of embryos to enhance thermotolerance in the birds and thereby enhance turkey meat quality in the market age birds.

Exposure of newly hatched turkey poults to hot or cold thermal stress often results in detrimental effects on breast muscle growth and development. Typical changes include increased lipid deposition and damage to muscle ultrastructure, leading to inferior meat quality with consequent economic losses to producers and processors. Likewise, exposure of market-weight turkeys to acute heat stress immediately prior to harvest, frequently results in a high incidence of inferior meat quality characterized by pale color, reduced marinade uptake and water-holding capacity, and poor protein functionality. Thermal manipulation of embryonic

development has met with some success as a strategy to improve thermotolerance of broilers. We hypothesized that exposure of turkey eggs to a mild heat challenge at a critical developmental stage would alter muscle growth and development, thereby setting the stage for improved thermotolerance of the growing bird. Eggs from RBC2 (slow-growing) and F (fast-growing) turkey lines were exposed to a control temperature of 38C throughout 28 days of incubation or 12h of 39.5C between days 21-25. Following hatch, birds were brooded at temperatures of 31C, 35C (control), or 39C for 3d, followed by brooding at 35C until 14d of age when the birds were sacrificed. Breast muscle (P. major) was collected for analyses including muscle weight and fiber diameter. Results from this preliminary study suggest that mild thermal manipulation of turkey eggs results in changes to muscle ultrastructure that may be associated with altered thermotolerance in the growing bird.

MN

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.

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 AFB1. We have completed analysis of a liver RNAseq database from an AFB1 challenge of 16wk wild and domestic turkeys conducted at our collaborating institution (Utah State University, RA Coulombe). We are currently working to examine the intestine (primary site of absorption), and spleen (site of secondary immune response) transcriptomes in the challenge experiment.

Antibiotic-free alternatives to improve health and performance in commercial turkeys. The goal of this project is to advance our understanding of the interactions between the turkey gastrointestinal microbiome and host during maturation and microbiome modulation. We seek to change the paradigm by which alternatives to antibiotics are developed, using systematic and science-grounded approaches. First, we are examining host-microbiome relationships that occur during turkey development and gastrointestinal microbiome modulation. Second, we are dissecting the turkey intestinal microbiome relative to its capacity to enhance growth and development.

TN

Project: Dietary manipulation of adipose development in broiler chicks. Commercial broiler chickens accumulate excess adipose tissue due to inadvertent consequences of genetic selection for rapid growth. Feed converted into fat is effectively wasted, increasing costs to producers. The metabolic influence of excess adipose tissue may also impair lean tissue growth and contribute to fertility and skeletal issues in broiler-breeders. To be most efficient, strategies to control fatness in broilers should focus on limiting the initial deposition of excess adipose tissue, which begins shortly after hatch. Based on previous transcriptomic, metabolomic and functional studies, our lab works under the premise that specific fatty acids and other lipid

mediators play important roles in the regulation of adipogenesis and adipocyte lipid metabolism in broilers. The hypothesis guiding our current research is that the lipid component of the diet, either in chicks or in hens, can be manipulated to limit fatness in broilers.

In 2016, we tested this hypothesis by manipulating the source of fats in the diets of chicks during the first few weeks after hatch and evaluated the effects on adiposity and other metabolic phenotypes, and on the expression of genes involved in lipid metabolism and adipocyte differentiation. Providing fats from flax, fish and other sources rich in omega-3 polyunsaturated fatty acids (PUFA) reduced adipose mass and/or adipocyte size relative to diets in which fat was provided as lard or omega-6 PUFA and induced corresponding changes in gene expression. In a separate study, we tested the hypothesis that the maternal (hen) diet could me used to program reduced adiposity in chicks by enriching the diet in fish oil (FO), which provides eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), relative to corn oil (CO). Chicks hatched from broiler-breeder hens that were fed diets containing FO had a significant reduction in adiposity compared to those from hens fed CO. Expression profiling (QPCR) and proteomics (LC-MS/MS) identified developmental and metabolic pathways that may underlie these effects of diet.

VA

Spatial profile of PepT1 mRNA in the yolk sac and intestine during the embryonic and post-hatch periods. The yolk sac and small intestine are two important organs responsible for the digestion and absorption of nutrients during the embryonic and post-hatch periods, respectively. The peptide transporter PepT1 is expressed in both the yolk sac and small intestine and plays an important role in the transport of amino acids as short peptides. Thus, PepT1 can serve as a marker for absorptive cells. The objective of this study was to profile the spatial transcriptional patterns of PepT1 mRNA in the yolk sac and small intestine from embryonic and post-hatch broilers using the RNAscope assay for in situ hybridization. The distribution of PepT1 mRNA was investigated at embryonic (e) days 11, 13, 15, 17, 19 and day of hatch (DOH) in the yolk sac and at e19, DOH, D1, D4 and D7 in the small intestine. PepT1 mRNA was expressed in the endodermal cells of the yolk sac. Cells expressing PepT1 mRNA were barely detectable at e11, increased from e11 to e13, e15 and e17, and then decreased from e17 to DOH. In the small intestine, PepT1 mRNA was expressed in the enterocytes that line the villi. There was a rapid increase in the number of cells expressing PepT1 mRNA from e19 to DOH, with the number of PepT1 expressing cells relatively constant from D1 to D7. These results show that absorptive cells express PepT1 mRNA in the yolk sac and small intestine in a development-specific pattern.

Identification of stem cells in the intestinal crypts and the yolk sac. A single layer of cells lines the intestinal villi, which include absorptive enterocytes, secretory cells and enteroendocrine cells. These differentiated cells arise from a stem cell population located in the crypts. In mammals, stem cells express the Lgr5 (Leucine-rich repeat-containing G-protein coupled receptor 5) and Olfm4 (Olfactomedin) genes. Olfm4 has been found to be expressed at higher levels than Lgr5 in stem cells and has emerged as a more robust marker for intestinal stem cells. The objective of this study was to identify stem cells in both the yolk sac and intestinal crypts. Using the RNAscope assay for in situ hybridization, we have identified cells in the intestinal crypt of chickens that express both Lgr5 and Olfm4 mRNA, which presumably represent a population of stem cells. In the yolk sac, cells expressing Lgr5 mRNA, but not Olfm4 mRNA, were detected around the blood vessels and are presumably stem cells. Thus Lgr5 and Olfm4 are markers of stem cells in the intestine, whereas only Lgr5 is a stem cell marker in the yolk sac.

Cellular response to Eimeria infection. Avian coccidiosis is a disease caused by the intestinal protozoan Eimeria. Lesions in the intestinal mucosa cause reduced feed efficiency and body weight gain, which is likely due to changes in expression of intestinal nutrient transporters and effects on the immune system. The objective of this project was to compare the expression of nutrient transporters and host defense peptides in the small intestine of Ross chickens and the

more disease-resistant Fayoumi chickens in response to an E. maxima challenge. The Fayoumi 5.1 line has been reported to be less affected by E. maxima challenge than the Fayoumi M15.2 line. In our study, however, we did not see a difference in weight gain depression between Fayoumi lines 5.1 and 15.2. The Ross and Fayoumi lines showed common downregulation of the cationic (bo,+AT) and anionic (EAAT3) amino acid transporters, which would result in a decreased uptake of important amino acids such as lysine and glutamate. There was also common downregulation of the host defense peptide LEAP2. Using in situ hybridization, LEAP2 mRNA was found to be expressed in the enterocytes lining the villi of both control and E. maxima challenged Ross chickens. The villi in the E. maxima challenged chickens were shorter than the villi of control chickens and may partially account for the observed decrease in LEAP2 mRNA expression after Eimeria challenge. The Fayoumi 15.2 line also showed enhanced mRNA expression of avian beta defensins (AvBD) 6, 10, 11, 12 and 13 in comparison to Ross chickens and Fayoumi line 5.1.

Brief Impact Statements

ADOL

- Candidate genes for MD drivers of oncogenesis have been identified.
- HVT and SB-1 MD vaccines appear to replicate differently with respect to time after vaccination and organ, which may explain their interaction to enhance vaccinal protection.
- MDV induces specific immune responses that alter the colonization of the gut microbiome.
- A total of 50 genes were identified for the first time, which may contribute to genetic resistance to MD.
- Identification of the relatively large number of coding and non-coding genes dysregulated in expression between the spontaneous ALV-like tumors and non-tumor tissues study provided the first piece of genomic evidence in elucidating the tumorigenic mechanism of ALV-like tumors attributable to genetics and epigenetics of the susceptible chickens.

AR

- 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.
- Providing additional insight into the fundamental molecular landscape of feed efficiency (FE) in breast muscle of broilers as well as further support for a role of mitochondria in the phenotypic expression of FE.
- The avian vastotocin receptor 1a (ViaR) is not only involved in the regulation of stress but also the regulation of food intake.

AZ

- Our work provides fundamental annotation information (both structural and functional) that underpins functional modeling of genomic data sets. This enables poultry researchers to more accurately identify genes involved in the systems they are studying.

CA

- 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.
- ChIP-seq assays developed and other omic data generated for regulatory elements annotation will be important for animal genome community.

COH

- The MHC-Y sequence determinations are having an impact in the study of MHC-Y gene function by a) providing sequences sufficient for development of new methods for assigning MHC-Y haplotypes, b) defining at the nucleotide level the relative positions of MHC-Y and the NOR, c) providing an overview five types of genes found in MHC-Y (distinctive MHC class I-like genes, distinctive MHC class II β chain genes, numerous c-type lectin-like genes, leukocyte receptor genes and zinc finger protein genes), d) revealing high content of several types of repetitive sequences, and e) providing data suggesting that MHC-Y region is inherently segmented.
- Development of a simplified method of MHC-Y that makes it feasible to MHC-Y genotype large numbers of birds in variousexperimentally challenged populations
- Definition MHC YF isoform variability and ligands bound is providing evidence that MHC class I-like molecules represent yet one more, so far unique, utilization of the MHC class I fold.

CU
- These findings provide insight into hormonal mechanisms of the integrated metabolic and reproductive axis in broiler breeder hens.

DE
- Our studies demonstrate that Wooden Breast Disease exhibits an earlier onset in modern broilers than when detectable by clinical examination, and that the disease appears to assume a progressive course with acute vasculitis, lipid deposition and myodegeneration occurring in the earlier stages followed by a chronic fibrotic phase.
- Our findings show that Wooden Breast affected tissues possess a unique metabolic signature. This unique profile may identify candidate biomarkers for diagnostic utilization and provide mechanistic insight into altered biochemical processes contributing to tissue hardening associated with the Wooden Breast myopathy in commercial chickens.
- Our results from the hepatic gene expression study have shown WBD to be a much more physiologically complex disease than previously thought and studies on major organs, other than muscle, will be necessary to determining the ultimate cause of Wooden Breast Disease.

FL
- Chicken is an important agricultural species as one of the most important sources of animal foods worldwide. To further the research on chicken health, the enzyme annotation is necessary as this class of proteins often represents a key part of many regulatory and signaling pathways. Phosphorylation is an important post-translational modification controlled by kinases and it regulates essential cellular processes. Moreover, this modification as well as kinases regulating it, have been implicated in animal diseases. Utilizing functional genomics data to understand disease in poultry requires the availability of functional information for genes. Our work adds to this foundational knowledge that helps effective use of investments made in sequencing the chicken genome that is critical for applying “omics” approaches.

GA
- Multi-generational imputation of SNP marker genotypes and accuracy of genomic selection
  - Imputed genotypes provide a viable alternative, even after several generations, as long the reference and training populations are appropriately updated to reflect the genetic change in the population.
- Dietary methionine deficiency seems to be associated with:
  - Muscular inflammation
  - Compromised immunity
  - Inflammation in the digestive system
- Molecular basis of heat stress
  - Initial molecular response under heat stress is to reduce both protein synthesis and

degradation.
- When heat stress persists, protein breakdown is elevated.

IA

- 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 cecal microbiome.
- Genomic regions associated with apparent resistance to high pathogenic avian influenza differed between H5N2 and H7N3 outbreaks, thus requiring a more diversified strategy for improving resistance to AI

IN

- 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. Through differentiated populations, and re-sequencing analysis, many of the genes associated with economic traits can be identified. These include genes associated with animal behavior, competition, animal wellbeing, as well as disease traits, such as Marek's Disease and Avian Influenzia. Critical to being able to fine map is replication, to date there are no replicated selected line in poultry and use of genomic data to detect SS in non-replicated populations is unable to differentiate differences due to random genetic drift and will result in a large number of false positives, regardless of the level of stringency applied.

MD

- By deep sequencing analysis, Song and colleagues found chicken gga-miR-103-3p targets CCNE1 and TFDP2 and gga-miR-130a targets HOXA3 and MDFIC, both miRNAs Inhibit MDCC-MSB1 Cell Migration, suggesting that miRNA plays important roles in MD. In addition, Song and his collaborators identified some cytokine receptors and several immune genes were upregulated. 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. The results provide new insight regarding the mechanisms of ILT etiology.
- The chick embryo has a long and distinguished history as a major model system in developmental biology. We explored genomics and epigenetics studies in chicken stem cells and found a lot regulatory elements in chicken ESC, PGC and SSC. We do believe that in combination with classical techniques, the chicken is now one of the most versatile experimental systems available for variety of research and human health.
- Today, from genomics and epigenetics studies, many researches just listed a lot pathways and so-called networks from significant expressed gene list. They are not systems biology, but belong to prediction. How to select most important pathway is a big challenge. Our method can be used for the selections functional pathways in KEGG, DAVID and IPA.

MI

- Thermal challenge poses a multi-dimensional threat to poultry production systems, with direct impacts on meat production and quality. The information derived from these studies will be used to develop more effective breeding, nutritional, and management strategies that can be used by poultry breeders and growers to advance the production of consistent, high quality muscle food products.

MN

- Our efforts are focused on projects that directly impact poultry health and production. Extreme temperature variations threaten the quality of poultry muscle as a healthy, high quality food product. 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. Likewise, AFB1 causes annual industry losses estimated in excess of $500 M. Increasing innate resistance to AFB1 could result in numerous health benefits. Transformational improvements in AFB1 resistance require a multidisciplinary approach to identify protective alleles with potential to reduce disease. 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. The gastrointestinal health of an animal is key to its successful growth and development. Elimination of subtherapeutic antibiotics for growth promotion and health in poultry will leave a critical void. This project will improve our mechanistic understanding of host-microbiome interactions in the avian host, and identify feasible approaches towards modulating the turkey intestinal microbiome resulting in enhanced health and performance.

MS
- Leveraging functional genomics data to understand disease in poultry requires the availability of functional information for genes. Our work adds to this foundational knowledge that helps effective use of investments made in sequencing the chicken genome that is critical for applying “omics” approaches. During 2016, the AgBase resources were visited by 11,813 different researchers, with 36.6% of these visitors from the US (includes visitors from all 50 states and the District of Columbia), while 518 different researchers visited HPIDB, of which 23% of these visitors were from the US (includes visitors from 27 states and the District of Columbia)A description of active kinases in chicken tissues forms the foundation for understanding the role of phosphorylation in regulation of genome expression in this agriculturally important animal. In 2016, HPIDB related work resulted in the publication of 3 refereed abstracts.
- Our work has revealed that the parthenogenetic trait in quail reduces fertility and hatchability in mated hens. Also, parthenogenetic development in the avian egg alters albumen ionic composition. Therefore, it is possible that parthenogenesis within the poultry industry is negatively impacting reproductive performance.

NC
- A strategy for gene editing in avian cells was developed. If at all possible, the function of the gene-editing targets should be tested in cell culture before gene editing in PGCs.
- The culture of primordial germ cells in serum-free conditions has applications in germ plasm preservation, gene-editing in poultry, and as means for assisted reproduction of endangered species.

PA
- Results of these studies of adiponectin and energy balance are intended to help direct the genetic selection of broiler breeder hens, which inherently are predisposed to producing double ovulations. Mechanisms regulating follicle growth, selection and differentiation are directly related to determining sequence length, thus reproductive potential.

TN
- This study suggests that the chick genome can be programmed in a way that reduces fat deposition, and raises questions about the role of the hen diet in epigenetic control of chick metabolism.

VA
- We have identified and are studying the ontogeny of stem cells in the yolk sac and small intestine. Because stem cells are precursors of absorptive cells necessary for nutrient

uptake, this knowledge will enhance our understanding of growth and development of the embryonic and post-hatch chick.

- We continue to investigate the changes in gene expression during an Eimeria challenge. We have shown that the host defense peptide LEAP2 is downregulated following Eimeria challenge and may play an important role in mediating an Eimeria infection.

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Presentations Published in Symposium Proceedings:

1. Anthony, K.A., Hadley, J.A., Johnson, A.L., Ramachandran R., Diaz, F.J. 2016. Vasoactive Intestinal polypeptide plays a role in ovarian cortical follicle steroidogenesis in broiler breeder hens. Presented at the 49th Annual Meeting of the Society for Study of Reproduction, San Diego, CA.
2. Ghanem, K and A.L. Johnson. 2016. Hen Ovarian Prehierarchal Follicles Demonstrate a Differential Sensitivity to Follicle Stimulating Hormone, *In Vivo*. Society for the Study of Reproduction Annual Meetings, SanDiego, CA.
3. Johnson, A.L. 2016. Hormonal Mechanisms Associated with Cyclic Recruitment of Hen Ovarian Follicles. Symposium presentation at the 11th International Symposium on Avian Endocrinology (ISAE), Niagara-on-the-Lake, Canada.
4. Hadley, J.A., Ramachandran, R. 2016. Adiponectin and metformin affects chicken ovarian follicular cell metabolism. Presented at the 11th International Symposium on Avian Endocrinology (ISAE), Niagara Falls, Canada.
5. Hadley, J.A., and Ramachandran, R. 2016. Adiponectin and metformin affects chicken ovarian follicular granulosa and thecal cell metabolism. Symposium presentation at the 11th International Symposium on Avian Endocrinology (ISAE), Niagara-on-the-Lake, Canada.
6. Song, J. Epigenetics and infection disease. The Middle East and South Asia Conference on Epigenetics and Genomics of Infectious Diseases, MESA, March 7-8 2016. (Conference Proceeding).

7. Song, J. The Current and Future of Epigenetics in Poultry Health, the 26th World Poultry Congress Proceeding. September 5-9, 2016 Beijing, China.

Books and Chapters in Books:

1. Johnson, A.L., Woods, D.C. and Truman, A.M. 2016. Granulosa Cell Tumors. In: Encyclopedia Of Cancer, M. Schwab, Ed., fourth Ed., doi:10.1007/978-3-642-27841-9_2508-2.
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Technical Reports

1. Al-Rubaye, A.A., Koltes, D., Kwon, Y.M., and Rhoads, D.D. BCO incidence protection with Anpario supplement: incidence of lameness with and without Staphylococcus challenge, microbiome, and TER. Anpario, plc. August 2016.

PhD Dissertations and Master Theses

1. Ferreira, V. C. 2016. Application of propensity score to learn causal relationships involving genetically correlated traits. M.S. Thesis. University of Wisconsin-Madison.
2. Fleming, D. S. 2016. Examination of the genomic architecture of divergent poultry populations that underlies adaptation, tolerance, and resilience to environmental stressors. Ph.D. Dissertation. Iowa State University.
3. Hamad, S. 2016. Developmental gene expression of host defense peptides in immune organs and the small intestine of turkey poults (Meleagris gallopavo). MS Thesis. Virginia Tech.
4. Hayden A. 2016. Identification of Biomarkers Associated with Rous Sarcoma Virus-induced Tumors in Two Divergently Selected Chicken Lines. Master Thesis; University of Arkansas.
5. Howard, S.J. 2016. Effects of Dietary Fatty Acids on Adipose Development in Young Broiler Chicks. Sarah J. Howard, M.S., Thesis, University of Tennessee.
6. Lemcke, RA. 2016. Investigating the Reproductive Role of Anti-Mullerian Hormone via AntiMullerian Hormone Receptor, Type II in the Hen. M.S. Thesis, Cornell University.
7. Su, S. 2016. Cellular events during coccidial infection in chickens. PhD Dissertation. Virginia Tech.
8. Van Goor, A.G. 2016. The genomics of heat stress and immune response in chickens. Ph.D. Dissertation. Iowa State University.
9. Weixuan Fu, 2016. Linkage disequilibrium and recent selection signatures in commercial broilers. University of Delaware.
10. Zhang, S. 2016. Physiological and biochemical aspects of methionine isomers and precursors in broilers. PhD Dissertation, Virginia Tech.

Funding and Leveraging

Grant	Funding
Adapting chicken production to climate change through breeding. USDA-NIFA-AFRI; PI: Schmidt; CoPIs: Lamont, Rothschild, Persia, Ashwell	$4,700,000
Antibiotic-free alternatives to improve health and performance in commercial turkeys. USDA-NIFA-AFRI. 2016-2018; (co-I w/ Johnson (PI), Baumler, Knights, and Noll	$464,000
Antistress compounds as effective tools for addressing chronic stress. ABI; 07/2016-05/2017; PI: Kuenzel; CoPIs: Kumar, Christensen, Kang.	$50,000
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
Assembly and annotation of the Timber Rattlesnake genome. National Science Foundation; 1/2014-12/2015; PI: Beaupre; coPI: Rhoads	$250,000
BCO incidence protection with Anpario supplement: incidence of lameness with and without Staphylococcus challenge, microbiome, and TER. Anpario Corporation; 2/2016-9/2016; PI: Rhoads; coPIs: Kwon, Koltes	$35,121
Confidential-Not for distribution outside of NC1170 CA no PI reported	NA
Verification of Allele Specific Expression in Chicken Embryonic Brain and Liver by using Minor Allele Finder. Delaware INBRE Core Center Access Award August 2016- January 2017, Supported by the NIH NIGMS IDeA Program (P20 GM103446); PI: Behnam Abasht	$3,960
Development of colonization resistance in chicks NIH-NIFA Dual purpose with Dual benefits. 2015-67015-22930. A. Baumler, H. Zhou	NA
Development of Methods for Knockout Chickens: CRISPR-Cas Genome Editing To Understand Foodborne Pathogen-Host Interactions in Poultry, USDA- National Institute of Food and Agriculture, 2015-2017; PI: Koci; Co-PIs: Petitte, Hassan	$100,000
Effect of AFB1 on immune tissues of turkeys from diverse genetic backgrounds. USDA-UMN Multi-State Project, 2016-2018. Strasburg	$94,221
Egyptian Ministry of Education 2016; PI: Aggrey, S. E.	$7,000
Follicle Selection and Development in Chickens. USDA NIFA Foundational Program 2017- 2020; PI: Johnson.	$499,745
Follicle Selection and Differentiation in the Avian Ovary. NSF, 2014-2017(IOS-1354713); PI: A.L. Johnson.	NA
Genome wide identification and annotation of functional regulatory regions in livestock species USDA NIFA 2015-67015-22940 H. Zhou, P. Ross, I. Korf	NA
Genomics for improving animal production USDA NIFA National Need Training Grant 2014-38420-21796 H. Zhou, J. Murray, P. Ross.	NA
Genotype-based selection for ascites susceptible and resistant broilers. USDA Animal Health; 7/2014-8/2016; PI: Anthony; coPI-Rhoads	$22,000
High throughput characterization of gene transcript variants by full-length single-molecule sequencing to improve farm animal genome annotation. USDA NIFA 2016. P. Ross, H. Zhou. J. Medrano	NA

Improving food security in Africa by enhancing resistance to disease and heat in chickens; Feed the future innovation lab for genomics to improve poultry, USAID AIDOAA-A-13-00080; PI: H. Zhou; CoPIs: D. Bunn, R. Gallardo, S. J. Lamont, J. Dekkers etc	$6,000,000
Improving the Efficiency of Livestock Production Using Genetic Selection. USDA-National Needs training grant; PI: Lamont (original PI: Spurlock); coPI: Rothschild, Garrick	$262,500
Industry funding. Aviagen Limited, EW Group, Hy-Line, International, Iowa Egg Industry Center Lamont, Dekkers, et al.	NA
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. 2014- 2018. PD: G.M. Strasburg; co-PDs: Kent Reed, Sandra Velleman, and William Atchison.	$975,000
Marker Assisted Selection for Ascites Resistance in Broilers. NIFA-AFRI; 11/2014-10/2017; PI: Rhoads; coPI: Anthony, Kong, Schmidt	$467,000
MHC-Y-directed immune responses during colonization of chickens by Campylobacter USDA NIFA 2016. Miller	NA
NIH-NIAAA P60 AA007611. Genomics and epigenomics of alcohol preference; PI: Muir; coPI: Feng Zhou	$750,000
NLRP3 Inflammasome activation and lameness in chickens. USDA Animal Health; 7/16-6/19; PI: Dridi; CoPI: Rhoads	$45,000
PACBIO P6-C4 Sequencing of Chicken Chromosome 16 BAC Clones USDA NRSP-8 funding. Miller	
Polymorphic MR1-like Molecules in Immunity to Microbial Pathogens, Caltech-City of Hope Biomedical Initiative with Pamela Bjorkman. Miller	NA
Regression of Rous Sarcoma Virus-Induced Tumors in Arkansas Regressor Chickens –Mechanisms and Implications for Tumor Treatment. ABI; 07/2016-05/2019; PI: Anthony; coPI: Kong, Dridi, Greene.	$150,000
Replacement Autoclave for Ferritor Hall. ABI; 7/2016-6/2017; PI: Rhoads	$47,170
Role of mitochondrial hormone receptors in cell bioenergetics; relevance to metabolic syndrome. ABI; 07/2016-05/2019; PI:Bottje; coPI: Kong, Dridi, Rochelle, Hakkak, Baum	$150,000
Strategies to enhance de novo biosynthesis of methionine in organic chicken. USDA-NIFA 3/14-2-18; PI: Aggrey, S.E.	$500,000
Targeting Mitochondrial Health in the Prevention of Cancer-Cachexia Induced Muscle Atrophy. ABI; 07/2016-05/2017; PI: Greene; coPI: Kong, Washington.	$30,000
The biological mechanisms that underlie dietary methionine in the mitigation of the effects of heat stress in the broiler chicken. Evonik Nutrition and Care GmbH, Germany 2016 (10/16-12/18). PI: Aggrey, S.E; CoPIs: R. Rekaya and W. K. Kim	$365,000
UMD-UMB Research and Innovation Grant 2015-2017	NA
US Poultry, Cattle and Swine Genome Coordinators Funds for farm animal ENCODE Aviagen Limited National Pork Board CA no PI reported	NA
US-UK Collaborative Research: Host Resistance to Avian Pathogenic E. coli. USDA-NIFA-AFRI/BBSRC; PI: Lamont; coPIs: Wolc, Kaiser, Stevens, Vervelde	$499,999
USDA AFRI Competitive grant ARZT-3013680: Enabling network analysis of host-pathogen interactions. 2015-2017. McCarthy	NA

USDA AFRI Competitive grant ARZW-06024: Knowledge representation resources for agricultural researchers. 2012-2016. McCarthy	NA
USDA National needs fellowship for enhancing animal production: Addressing national need in poultry production. USDA-NIFA-NNF. 2016-2021. Strasburg	$241,000
USDA-NIFA Grant No. 2016-67015-25027) 03/01/2016 - 02/29/2020 Title: Genome-wide Identification and Functional Validation of Genes Causing Susceptibility to Wooden Breast Disease in Commercial Broiler Chickens My role; PI: Behnam Abasht; CoPIs: Jack C.M. Dekkers, Sandy G. Velleman and Carl J. Schmidt	$500,000
USDA, AFRI, award no. 2012-67015-19419, Enhancing genetic resistance to Marek's disease in chicken via allele-specific expression screens and genome-wide selection. PI: Cheng; coPIs: John Dunn (ADOL), Bill Muir (Purdue U.), Sudeep Perumbakkam (ADOL), and Frans van Sambeek (Hendrix Genetics, The Netherlands.	$499,960
USDA, AFRI, award no. 2013-67015-21330, Genome biology of Marek's disease: Viral integration and genome alterations in genetically resistant and susceptible stocks. PI: Cheng; coPIs: Mary Delany (UC Davis) and Bill Muir (Purdue U.).	$499,997
Total	**$18,675,963**