



Phenotype and multi-tissue transcriptome response to diet changes in laying hens

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genome (UMD 5.0) and differential expression analysis was used to contrast the effects of AFB₁ exposure in domesticated and wild birds. Transcriptome comparisons identified unique liver profiles in domesticated and wild turkeys with significant variation in the response to AFB₁. Over 5400 genes were significantly affected (FDR pval < 0.05 and |Log₂FC| > 2.0) by AFB₁ treatment with a greater number of transcripts altered in the domesticated birds. These results provide insight into changes in AFB₁ response that have occurred during domestication and selective breeding for production and will help elucidate ways to improve poultry resistance to AFB₁. Supported in part by Agriculture and Food Research Initiative Animal Genome competitive grant 2013–67015–21241 of the USDA National Institute of Food and Agriculture.

Key Words: turkey, aflatoxin, RNA-seq, transcriptome

MT46 Genetic diversity and LD size between commercial and native chickens in Korea. D. Seo*, D. Lee, N. Choi, S. Jin, P. Sudrajat, S. H. Lee, and J. H. Lee, *Division of Animal and Dairy Science, Chungnam National University, Daejeon, South Korea.*

Native chickens have low productivity than commercial broilers. However, these chickens become more popular due to the unique texture and taste characteristics. Genetic diversity and linkage disequilibrium (LD) size were investigated in this study for identifying genetic relationships in order to give basic information for the native chicken populations. 600K high-density SNP array was used using 187 native chicken samples from 14 different chicken lines. The results of genetic diversity indicated that most of the native chicken lines were independently clustered with commercial native chickens. The r^2 values of LD size in each population were calculated and the results indicated that most of the native chicken populations have higher LD values than that of the commercial native chicken populations. With further analysis, these results can provide valuable information for the breeding strategy using the native chicken breeds.

Key Words: genetic diversity, linkage disequilibrium, native chicken, commercial native chicken

MT47 Mapping QTLs affecting Marek's disease by selective genotyping in F6 of full-sib intercross population. E. Lipkin^{*1}, J. Smith², D. Burt², M. Soller¹, and J. Fulton³, ¹*Dept. of Genetics, Silberman Life Sciences Institute, The Hebrew University of Jerusalem, Jerusalem, Israel;* ²*The Roslin Institute and Royal (Dick) School of Veterinary Studies, The University of Edinburgh, Easter Bush, Midlothian, UK;* ³*Hy-Line International, Dallas Center, IA, USA.*

Marek's disease (MD), a lymphoma caused by an avian herpesvirus, is a major disease affecting the poultry industry. One way to reduce MD prevalence is genetic selection. Genetic progress can be accelerated by using genetic markers associated with the disease. The aim of the present study was to map QTLs, genes and mutations affecting chicken MD susceptibility. A full-sib intercross population was generated by two partially inbred commercial White Leghorn layer lines differing in genetic resistance to MD. At the F6 generation, a total of 1,615 chicks from five independent families were phenotyped for MD resistance. Phenotypic tails from the five families were used in a GWAS based on survival test. Moving average of -LogP and Log drop were used to define QTL regions (QTLRs). A total of 159 QTLRs were found among the five families, averaging 32 QTLs per family and ranging from 23 to 41. Overlap and proximity were used to condense the list to 138 QTLRs shared among families. RNASeq and bioinformatics analyses of the QTLRs identified differentially expressed and functional candidate genes, and protein Loss of Function (LoF) mutations. Putative quantitative traits genes (QTG) and candidate causative nucleotides (QTN) were identified. Markers from the candidate genes and the LoF mutations were used

to genotype 9,391 males with MD progeny tests from eight lines across 16 generations. Association analysis confirmed most of the QTLs found in F6. An independent study based on selective DNA pooling, reported elsewhere at this meeting, confirmed most of the QTLs found in the F6 population.

Key Words: poultry, animal health, genome-wide association, complex trait, infectious disease

MT48 Copy number variation in SOX6 contributes to chicken muscle development. X. Zhang*, *Department of Animal Genetics, Breeding and Reproduction, College of Animal Science, South China Agricultural University, Guangzhou, Guangdong, China.*

Copy number variations (CNVs), covering a large number of functional genes, were associated with the complex diseases and phenotypic diversity. In previous study, 5 individual variation of White Rock Resistance (WRR) chicken (CN = 1, CN = 3) and two individual variation of Xinghua (XH) (CN = 3) chicken were found in the CNP13 region (chr5:10500294–10675531) which overlaps with *SOX6*. It has been shown that *Sox6* plays a key role in fast-twitch muscle fibre differentiation in the zebrafish. However, the role of *SOX6* on chicken skeletal muscle development is still unknown. In current study, Acucopy and CNVplex were used to characterise the CNVs phenotypes in XH and WRR chickens. Real-time quantity PCR (RT-qPCR) was used to verify the candidate copy number polymorphisms (CNPs) and to detect the relative expression of genes. The overexpression and knockdown of *SOX6* in QM-7 cells were used to study the function of *SOX6* in skeletal muscle development. As a result, 15 CNPs were significantly related to different traits on genome level, of which, 12 were associated with growth traits, 4 were with carcass traits, and 6 were with meat quality traits. Five WRR (CN = 1, CN = 3) variant individuals and 2 XH (CN = 3) variant individuals were found in the CNP13 region (chr5:10500294–10675531) which overlaps with *SOX6*. *SOX6* was expressed in 16 tissues of XH and WRR chicken, with higher expression levels in the chest, leg muscle, pituitary, heart, cerebellum and kidney. Notably, the expression of the *SOX6* mRNA was associated with *SOX6* copy number variation. Interestingly, bioinformatics analysis of *SOX6* protein showed that the amino acid sequence (265–579 aa) region, coded by overlapping partial CNV region, is a disordered region. Moreover, the QM-7 cells were significantly decreased in G1 phase and arrested in S phase after overexpression of *SOX6*. The *SOX6* is highly expressed during the QM-7 cell differentiation, the same as muscle different marker genes *myogin* and *MYHC* are. Surprisingly, after the knockdown of the *SOX6*, the expression levels of *IGFIR1*, *MYF6*, *SOX9*, *SHOX* and *CCND1* were significantly down-regulated, indicating that *SOX6* can influence these genes to promote the proliferation of muscle cells.

Key Words: chicken, CNV, *SOX6*, skeletal muscle, disorder region

MT49 Phenotype and multi-tissue transcriptome response to diet changes in laying hens. M. Brenet^{1,2}, A. Rau³, C. Désert^{1,2}, M. Boutin^{1,2}, K. Muret^{1,2}, S. Leroux⁴, D. Esquerre⁵, C. Klopp⁶, D. Gourichon⁷, F. Pitel⁴, T. Zerjal³, and S. Lagarrigue^{*1,2}, ¹*INRA, UMRI1348, PEGASE, St Gilles, France;* ²*Agrocampus-Ouest, UMRI1348, Rennes, France;* ³*INRA, AgroParisTech, Université Paris-Saclay, UMR GABI, Jouy-en-Josas, France;* ⁴*INRA/INPT ENSAT/INPT ENVT, GenPhySE, Castanet Tolosan, France;* ⁵*INRA, Plateforme GENOTOUL, Castanet-Tolosan, France;* ⁶*INRA, SIGENAE, Castanet-Tolosan, France;* ⁷*INRA, PEAT, Nouzilly, France.*

Adaptation to feed changes in laying hens is particularly important to promote innovation, in selection schemes and in dietary solutions, for the sustainability of the egg-production sector. In Eu-

rope and USA poultry feed is rich in cereals, while Asian countries privilege by-products to soybean meal, resulting in low energy diets. In this study, we investigated the effects of a sub-optimal low energy diet on different traits and multi-tissue transcriptomes of brown egg layers of 2 divergent lines selected for low (R-) and high (R+) residual feed intake. The 2 diets had a similar protein content, while the energy content was reduced by 15% as compared to the standard diet (2450 Kcal versus 2800 Kcal). The R+ and R- hens were fed *ad libitum*, with the standard diet until 17 wk of age; half of the birds were fed with the low energy feed until 33 wk of age when a subset of birds was slaughtered for tissue sampling (8 per line and diet). Food intake was increased in response to the sub-optimal diet whereas egg number was unchanged showing that birds were able to adjust their energy intake by modifying feed intake. Nevertheless, hens fed the low energy diet had a higher feed efficiency ($p\text{-val} < 5\%$) and a higher residual feed intake ($P\text{-value} < 1\%$). No diet $\times$ line interaction was observed for these traits. PolyA+ RNA from different tissues were sequenced resulting in 90 M reads per sample. After bioinformatics treatment and differential analysis, we observed in liver and adipose tissue only few differentially expressed genes (DEG) between diets (16 and 21 respectively). In contrast, we observed in blood 1179 DEG out of 17123 expressed genes with 463 and 716 over and under expressed in the suboptimal diet compared to the standard diet. No diet $\times$ line interaction was observed in the three tissues. GOBP term enrichment revealed that under expressed DEG in blood were associated with glucose catabolism, cholesterol biosynthesis, mitotic cell cycle and protein catabolic process. Taken together, these results indicate an adaptation of birds to diet changes by increasing feed intake to maintain egg production, and a tissue-specific response with a limited role of metabolic tissues as liver and adipose tissue compared to the blood.

Key Words: poultry, functional genomics, RNA-seq, adaptation, diet

MT50 Addition to the chicken W-chromosome specific repetitive landscape. A. Saifitdinova, S. Galkina*, A. Dyomin, M. Kulak, E. Koshel, and E. Guginskaya, *Saint-Petersburg State University, Saint-Petersburg, Russia.*

In many species, sex determination is genetic and often accompanied by the presence of dimorphic sex chromosomes in the karyotype. Recent progress in genomic sequencing gave the information about the gene content of sex chromosomes which allowed to reveal their origin from ordinary autosomes and to trace their evolutionary history. Unlike other sequenced sex chromosome chicken W chromosome does not contain genes specifically expressed in reproductive tissues. At the same time female-specific W chromosome in birds as well as mammalian male-specific Y chromosome is characterised by the degeneration of gene content and the accumulation of repetitive DNA. Despite the best efforts chicken W chromosome assembly includes only 7 Mb of expected 55 Mb, because tandem repeats complicate the analysis of genomic data and assembling. Repetitive DNA occupies more than two thirds of the chicken W chromosome. In the composition of chicken W chromosome three major repetitive families *XhoI*, *EcoRI* and *SspI* have been characterised. Recently we identified (GGAAA)_n tandem repeat and assigned it by FISH into two sites in chromosome W. Here, we performed a structural analysis of the borders between assembled scaffolds and extended flanking areas enriched with (GGAAA)_n. For this, we used sequences of BAC clones CH261-114G22 (AC182258.2) and CH261-75N4 (AC175832.2) co-localised with the repeat by high resolution FISH on lampbrush chromosomes and the last version of W chromosome assembly. We studied also the distribution of (GGAAA)_n short stretches in the chicken genome. The potential role of homopurine sequences as regulators of tissue-specific gene expression is discussed. Financial and technical support: RFBR

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Key Words: poultry and related species, sex determination, genome assembly

MT51 MicroRNAs associated with high rate of egg production in chicken ovaries. U. Gaur*, N. Wu, M. Yang, and D. Li, *Sichuan Agricultural University, Chengdu, Sichuan, China.*

Chicken (*Gallus gallus*) is an important agricultural and avian-model species, which is a major source of protein worldwide. However, a role for miRNAs in chicken ovarian development has not hitherto been reported clearly. The characteristics of ovarian tissue are highly related with reproductive and economic traits of chicken, so it is necessary to identify and characterise the miRNA in ovarian tissue. In this study, we performed the first miRNA analysis of low and high egg production chicken ovarian tissues at 300 days of age using high-throughput transcriptome sequencing. By comparing low egg production (LP) chickens with high egg production chickens (HP), 17 significantly differentially expressed miRNAs were found ($P < 0.05$), including 11 known and 6 novel miRNAs. We found that all 11 known miRNAs were mainly involved in pathways of reproduction regulation, such as steroid hormone biosynthesis and dopaminergic synapse. Additionally, expression profiling of randomly selected six differentially regulated miRNAs were validated by quantitative real-time polymerase chain reaction (qRT-PCR). Some miRNAs such as gga-miR-34b, gga-miR-34c and gga-miR-216b were reported to regulate processes such as proliferation, cell cycle, apoptosis and metastasis and expressed differentially in HP chicken ovaries, suggested they have an important role in ovaries development and reproductive management of chicken. Furthermore, we uncovered a significantly up-regulated miRNA: gga-miR-200a-3p, which is ubiquitous in reproduction regulation related pathways. This miRNA might play a special central role in the reproductive management of chicken.

Key Words: microRNA, chicken, ovary, Illumina sequencing, qRT-PCR

MT52 Genome wide association study of complex traits in response to Newcastle Disease Virus in chickens. K. Rowland*¹, H. Zhou², R. Gallardo³, T. Kelly^{2,3}, A. Wolc^{1,4}, and S. J. Lamont¹, ¹*Iowa State University, Department of Animal Science, Ames, IA, USA;* ²*University of California-Davis, Department of Animal Science, Davis, CA, USA;* ³*University of California-Davis, School of Veterinary Medicine, Davis, CA, USA;* ⁴*Hy-Line International, Dallas Center, IA, USA.*

Newcastle disease (ND) causes up to 80% mortality in chickens in developing countries where velogenic NDV strains are endemic. Genetic improvement of disease resistance, complementary to vaccination, has the potential to be an important tool to reduce the impact of NDV. We hypothesise that many genes regulate NDV response in chickens. Our specific objective was to identify genetic markers associated with NDV resistance (reduced viral load, high antibody titer) so these markers can be applied in a genetic selection program benefiting areas of endemic NDV challenge. The experiment was replicated across three hatches from 150 dams of a commercial egg-laying line, Hy-Line Brown. We inoculated with NDV, La Sota strain, on day 21 of age by an ocular-nasal route. Virus load was estimated from viral mRNA level in lachrymal fluid by qRT-PCR. Systemic antibody response to NDV in serum was measured by ELISA. Genomic DNA was genotyped via Affymetrix 600K chicken SNP array. Analyses of viral RNA and antibody levels confirmed response of challenge groups to the virus and lack of response in control groups. ASReml estimated heritabilities of 0.34, 0.34, 0.105, 0.117, 0.19, and 0.05 for hatch weight, day 31 body weight, 0 and 10 days post-infection (dpi) antibody levels, and 2dpi