166 Primordial germ cell-based biobanking of Hungarian indigenous chicken breeds. Bence Lazar*1, Roland Tóth1, Alexandra Nagy2, Mahed Anand3, Krisztina Liptó4, Eszter Patakiné Várkonyi4, and Elen Göcza1, 1NARI, ABC, Godollo, Hungary; 2Veterinary Science University, Budapest, Hungary; 3SZIU, Doctoral School of Animal Husbandry Science, Godollo, Hungary; 4Research Centre for Farm Animal Gene Conservation, Godollo, Hungary.

Nowadays most of the economically important or indigenous chicken breeds are preserved in situ populations, which poses numerous risks, such as epidemics (e.g., avian influenza), environmental disasters, inbreeding and management failure. Therefore, germplasm preservation in poultry is a high priority, although it is faced with difficulties. Embryo cryopreservation is not yet established and semen conservation lacks the ability to preserve the W chromosome and the mitochondrial DNA. Currently, the most promising solution is the cryopreservation of primordial germ cells (PGCs). In this study, by utilizing the unique nature and accessibility of PGCs (they use the vascular system during their migration toward the genital ridge), biobanks for 2 of the indigenous Hungarian chicken breeds (Partridge color, Transylvanian Naked Neck) were established to conserve their genetic resources. Blood samples were collected from each embryo individually, and then the isolated blood, containing the PGCs, was cultured in a PGC selective medium for 3 weeks. After that, PGC samples were collected for DNA, RNA isolation and immunohistochemistry to characterize the quality of the cultured lines. As a next step, parallel vials were frozen from each PGC line, and they have been stored in liquid nitrogen since then. To evaluate the freezing process and to prove the functional integrity and migration ability of PGCs after long-term in vitro cultivation, some of the vials were thawed and the cells were injected into recipient embryos. The cells were labeled with an in vivo fluorescent dye, thus the migration of the injected cells was followed toward the developing gonads, and the ratio of the colonization was analyzed. During the study, 26 individual PGC lines from Transylvanian Naked Neck chicken and 21 lines from Partridge color Hungarian chicken were established with a derivation rate of 46.4% and 31.1% respectively. The PGC lines were frozen and then successfully thawed with a cell viability of around 50% in both lines. The preserved cells were capable of colonizing the gonads of the recipient embryos. This is the first demonstration of a successful PGC-based cryopreservation system applied to Hungarian indigenous breeds and the first initiative in Hungary to establish a biobank based on PGCs.

Key Words: germplasm, cryopreservation, biobanking, primordial germ cells (PGCs), Hungarian indigenous breeds

168 Time course transcriptional analysis of response to Newcastle disease virus infection and heat stress in two genetically distinct inbred chicken lines in Harderian gland tissue. Perot Saelao*1, Ying Wang1, Ali Nazmi1, Rodrigo Gallardo1, David Bunn1, Terra Kelly1, Susan Lamont2, and Huaijun Zhou1, 1University of California–Davis, Davis, CA, 2Iowa State University, Ames, IA.

Biotic and abiotic factors can influence the production potential of both small and large scale poultry flocks. Newcastle disease virus (NDV) and heat stress are 2 major limiting factors that can disrupt physiological processes, and alter the hosts’ ability to mount an effective immune response, which could result in significant economic losses. RNA sequencing enables a comprehensive profiling of the activities of genes at the transcriptional level and evaluates the molecular mechanism of disease response during heat stress. The objective was to identify functionally relevant genes and biological pathways associated with response to NDV infection while under heat stress. Two genetically distinct inbred chicken lines (Fayoumi and Leghorn) were heat treated continuously at 35°C on d 14, then challenged with NDV or PBS at 21 d old. At 2 d post-infection (DPI), 6 DPI, and 10DPI, Harderian gland tissue was collected (4 individuals per line per treatment) and total RNA was then extracted and used to conduct gene expression analysis with an FDR <0.1. Analysis found 9, 38, and 2,867 differentially expressed genes (DEG) for Fayoumi at 2, 6, and 10 DPI with only the PRKCD gene shared across all time points. Leghorn had 18, 3,993, and 50 DEG at 2, 6, and 10 DPI, respectively. Pathway analysis of the 2 lines found an enrichment of immune related pathways in the Fayoumi data set with a significant enrichment in the AGE/RAGE pathway, which is involved in innate immunity and regulating heat stress. These genes and pathways offer potential targets for further investigation into their roles in responding to infection during heat stress. Funding provided by US Agency for International Development (USAID) AID-OAA-A-13-00080.

Key Words: host immune response, genomics, RNA-Seq
169 Capture array genomic analysis of the causative region for wingless-2, a developmental syndrome in the chicken. Ingrid Youngworth* and Mary Delany, University of California–Davis, Davis, CA.

Research on developmental disorders using well-characterized animal models improves our understanding of relevant pathways for both animal and human systems. A mutation in chicken, wingless-2 (wg-2), is an embryonic lethal characterized by absence of wings, truncated legs, craniofacial defects, and kidney malformations. The mutation is autosomal recessive and heterozygous carriers are normal. To facilitate genetic and genomic research of wg-2, carrier birds were backcrossed repeatedly into a highly inbred line and then maintained as a closed congenic inbred population. Mapping and genotyping determined that the causative mutation resides within a 227-kb region of chromosome 12. Recently a capture array was created to specifically target sequence from the candidate region and flanking DNA. The captured DNA was sequenced at high depth of coverage in 18 mutants, 2 homozygous normal siblings, and 4 carrier parent birds. This comparative data set allows for identification of that subset of variants linked to and potentially causative of the wg-2 mutation as well as distinguishing authentic variants from artifacts. One to 2 thousand single nucleotide polymorphisms (SNPs) were found in the 300 kb captured region for each individual. A large number of the SNPs are novel, not previously reported (as per the NCBI dbSNP database). An analysis of the genotypic differences of these SNPs between mutant, carrier, and wild type birds indicates that the region retaining the causative wg-2 mutation is now less than 120 kb in size. Approximately 500 informative and therefore potentially causative SNPs remain in this region, along with 2 confirmed genes that contain 106 of these SNPs. Further details on the variants in the region and their priority for likely contribution to the phenotype will be discussed. This research will contribute to understanding an important human congenic disorder as this multi-system syndrome is phenotypically similar to human tetra-amelia, which is also autosomal recessive, characterized by lack of limbs, and exhibits urogenital abnormalities. Continued work on wg-2 therefore enhances an invaluable genetic resource useful for elucidating human and vertebrate development pathways with specific attention to the chicken. Supported by USDA-NIFA Animal Genome Research Program (NRSP-8).

Key Words: SNP, genetics, mutation, wingless-2, breeding

170 The effects of feeding a diet high in methyl donors on Japanese quail. Chelsea Phillips*, Roselina Angel, and Christopher Ashwell, North Carolina State University, Raleigh, NC, University of Maryland, College Park, MD.

Much speculation surrounds the parental influence or epigenetic effects on progeny performance. Several genomic modifications have been shown to be inherited from parent to offspring including methylation of DNA in the form of 5-methylcytosine. Changes in DNA methylation leads to changes in gene expression and ultimately phenotypes, which can affect the health and growth of the bird. The addition of methyl side chains to DNA inhibits the access of transcription machinery to DNA for the transcription process. Dietary nutrition has been shown to impact the health and growth of the bird. The addition of methyl side chains for the transcription process. Dietary nutrition has been shown to impact the health and growth of the bird. The addition of methyl side chains for the transcription process. Dietary nutrition has been shown to impact

171 Comparison of fecal bacteria richness and functional gene prediction between broiler and layer chickens. Liliana Nolasco Isaula*, Justin Dobbs, and Rosemary Walzem, Texas A&M, Bryan, TX.

Discontinuation of antibiotic growth promoters requires new strategies to maintain intestinal health in poultry. Intestinal microbiota can confer host protection from pathogenic bacteria. The evolution of a healthy gut microbiota in different commercial chicken lines remains poorly documented. We sought to analyze and compare the evolution of bacterial richness (i.e., α-diversity, AD) and computationally estimated functional capacity (CEFC) in Hubbard-Hubbard broiler and Hy-Line layers chickens sharing the same environment and diet. We hypothesized that AD would increase with age, and that broilers and layers would acquire different CEFC. Fertile eggs were hatched together and chicks placed (n = 5 per strain) in the same rearing environment with new litter. Three non-medicated diets were used during the 7-week trial. Starter diet was provided from d 1 to 14 (22% crude protein, 3050 kcal/kg ME), grower diet from d 15 to 35 (20% crude protein, 3100 kcal/kg ME), and finisher diet from d 35 to 49 (18% crude protein, 3300 kcal/kg ME). Bacterial composition of freshly voided feces was determined for each bird at 1, 3, 5 and 7 weeks of age by 16S rRNA gene sequencing and CEFC prediction by PICRUSt estimation. Alpha-diversity estimated by Chaol and Shannon indexes gave similar outcomes; values for Chaol are presented. At 1 week fecal microbiota of broilers and layers were similar in number and evenness (broiler = 2739, layer = 2641). Chaol values dropped at wk 3 following transfer to the grower diet (broiler = 2036, layer = 2178) by AD restoration at wk 5. However, the magnitude of restoration differed in layers (44% increase) and broilers (12% increase) so that AD was greater in layers than broilers (p-value 0.016) at wk 5. Despite similar Chaol values at wk 7, the CEFC of the microbiota differed between the broiler and layer strains, with 22 probable bacteria functions being greater in layers than in broilers. Additionally, in layers, the finisher diet resulted in significant increases in 10 bacterial functions when compared with CEFC estimated during starter and grower periods. In conclusion, AD increases to near peak at 1 week post hatch and can be affected by diet composition. In this study, fecal AD of layers was more responsive to diet than was that of broilers. Future studies are
required to evaluate if these AD and CEFC differences correlate with fecal microbial metabolites and diet composition in both chicken strains.

**Key Words:** microbiota, broiler, layer, 16S rRNA

172 The relationship between the microbiome in different sections of the gastrointestinal tract of broiler chickens fed a corn versus a rye based diets. Mikayla Baxter*1, Juan Latorre2, Si Hong Park3, Xiaolun Sun1, Billy Hargis1, Guillermo Tellez1, and Steve Ricke2, 1University of Arkansas, Prairie Grove, AR, 2University of Arkansas, Fayetteville, AR.

Poultry diets rich in non-starch polysaccharides such as rye decrease digestibility and performance and increase gut permeability and digesta viscosity. The purpose of this study was to evaluate the microbiome in chicks consuming a rye or a corn-based diet from hatch to 10 d of age. At 10 d of age, birds were euthanized and samples of duodenum, upper and lower ileum were collected for microbiome analysis. The 2 cecal sacs were collected separately as right or left ceca. Each cecum was further separated in 3 sections: top, middle, and bottom. Bacterial genomic DNA was extracted from the samples, and the V4 region of 16S rRNA gene were amplified. Amplicons were sequenced on Illumina MiSeq, and microbial communities were analyzed by using QIIME. In the duodenum, there was no difference between the 2 treatments at phylum and family level. In the upper ileum corn-fed chicks had a higher percentage of Cyanobacteria at the level of the phylum and Aerococcaceae at the level of the family ($P < 0.05$). In the lower ileum, rye-fed chicks had a significantly higher amount of Firmicutes than the corn-fed birds. At the level of the family, corn-fed chicks had higher amounts of Bacteroidaceae, Chitinophagaceae, Isosphaeraceae and Prevotellaceae than rye-fed chicks. The top of the left and right ceca had higher amounts of Actinobacteria in rye-fed birds. In the bottom left ceca, rye-fed chicks had a higher percentage of Actinobacteria and Proteobacteria; however, there were no differences between treatments in the right bottom ceca. At the family level at each ceca location, rye-fed chicks had a higher percentage of Lactobacillaceae and Clostridiaceae and corn-fed chicks had a higher percentage of Ruminococcaceae. In the top left ceca, the middle right ceca and top right ceca rye-fed birds also had significantly higher percentage of Peptostreptococcaceae than corn fed birds. The middle and lower sections of the right ceca and the middle of the left ceca also had a higher percentage of Coriobacteriaceae in corn-fed chicks. Corn fed chicks had higher percentage of Ruminococcaceae; however, rye-fed chicks had higher percentage of Lactobacillaceae. It is evident from this experiment that diet drastically effects the microbiome at the phylum and family level.

**Key Words:** microbiome, gastrointestinal tract, diet, broiler

173 Effect of DNA isolation methodology on gene expression of *Clostridium perfringens* alpha toxin in qPCR. Whitney Briggs*, Revathi Shanmugasundaram, Kim Wilson, and Lisa Bielke, OARDC, The Ohio State University, Wooster, OH.

Since 1983, polymerase chain reaction (PCR) has been used for multifarious purposes, and advanced assays such as quantitative PCR (qPCR) have been developed. In cases of necrotic enteritis, standard methods for evaluating infection severity include body weight gain and lesion scoring. Some research has adopted the task of measuring the quantity of *Clostridium perfringens* (CP) by extracting bacterial DNA from intestinal digesta and subjecting it to qPCR. But, to measure the CP quantity from the intestine, the DNA has to have a high level of purity due to the high sensitivity of qPCR and the presence of PCR inhibitors in the samples. Many methods for bacterial DNA extraction have been published, but the success of extraction protocols are dependent on sample source, presence or absence of bacterial cell wall and the chromosomal or plasmid location of the target gene. To determine a sound DNA extraction method for ileal samples, we spiked ileal digesta samples with 10$^6$ cfu of CP and subjected the digesta to 4 extraction protocols. To evaluate the success of each protocol, we measured the final DNA concentration and assessed the purity by the absorbance ratios given at the wavelengths of 230, 260 and 280nm. Low ratios are associated with contamination possibly due to remaining extraction detergents or protein carryover, both of which can have an impact on the sample’s performance in qPCR. The averaged final DNA concentrations for extraction methods 1–4 were 101.38, 354.25, 29.12, and 380.07ng/µL respectively. Averages for the A$260/280$ and A$260/230$ ratios were 2.18/1.47, 2.13/2.19, 1.41/0.50 and 2.05/2.05 respectively. Each method had an individual standard curve developed using a 10-fold dilution from a concentrated extraction sample. Regression coefficient values ($R^2$) were 0.99, 0.94, 0 (no replication) and 0.99 respectively, with the efficiency percentage ($E\%\)$ values being 109.10, 86.70, 0, 89.40% respectively. These variations in concentration, purity, $R^2$ and $E\%\$ highlight the challenges in DNA extraction from intestinal samples. In conclusion, variable DNA extraction methods yielded different quantifiable DNA quality and $cpa$ detection standard curves, and digesta conditions and source should be considered when selecting qPCR protocols.

**Key Words:** qPCR, DNA purity, DNA extraction, alpha toxin, ileal digesta

174 Evaluation of broiler chicken myogenic stem cell population heterogeneity and skeletal muscle fiber morphometrics. Oscar Tejeda*, Jeanine Arana, Allan Calderon, and Jessica Starkey, Auburn University, Auburn, AL.

Myogenic stem cells play a key role in mediating post-hatch skeletal muscle growth in broilers through their donation of nuclei to existing muscle fibers which increases DNA content and ultimately enhances the myofibrillar protein synthesis potential of those fibers. Information regarding myogenic stem cell populations and muscle fiber size distributions in modern broiler strains of both sexes is scarce. Therefore, we evaluated myogenic stem cell populations and skeletal muscle fiber size in 2 functionally different muscles (*pectoralis major, PM* and *biceps femoris, BF*) from 2 broiler chicken strains, Red Ranger (RR; S&G Poultry, Clanton, AL) and Ross 708 × Ross 708 (ROSS; Aviagen Group, Huntsville, AL). Male and female broilers from both strains (n = 80 birds per strain) were penned separately (20 birds per pen) and fed a common corn-soybean meal-based diet. At 43 d of age, 8 birds per sex per strain were euthanized and samples from the PM and BF muscles of each bird were collected and stored for subsequent analysis by cryohistological immunofluorescence staining and fluorescence microscopy. Cryosections from each muscle from each bird were immunofluorescence stained to detect Myf-5, MyoD, and Pax7-expressing myogenic cells. Myogenic stem cell populations (Myf-5+, MyoD+, Pax7+, Myf-5+:MyoD+, MyoD+:Pax7+, and Myf-5+:MyoD+:Pax7+) were enumerated and the cross-sectional area (CSA) of each fiber in the representative digital image was determined. ROSS broilers exhibited a 27% larger population of Myf-5+:MyoD+:Pax7+ stem cells compared with RR ($P = 0.04$). Within both the PM and BF muscles, fiber CSA was similar among the 2 strains and sexes ($P > 0.05$). Mean fiber CSA was greater in the PM compared with the BF muscle ($P = 0.005$). The Myf-5+ and Pax7+ population sizes were similar among muscles ($P > 0.05$). Both nuclear density ($P = 0.003$) and the number of Myf-5+:MyoD+...
cells ($P = 0.03$) were greater in the BF compared with the PM muscle. Overall, we observed that the major differences in broiler myogenic stem cell populations were among the 2 functionally different muscles and not among the 2 different sexes or broiler strains.

**Key Words:** broiler chicken, myogenic stem cell, muscle fiber cross-sectional area, satellite cell, muscle

### 175 Mechanisms for programming reduced adiposity in broiler chicks through hen dietary n-3 long-chain polyunsaturated fatty acids.

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Omega-3 long chain polyunsaturated fatty acids (n-3 LCPUFA) and their metabolites activate nuclear receptors that control lipid metabolism and adipose development. We previously reported that enriching the hen diet in n-3 LCPUFA found in fish oil (FO) significantly reduced adiposity without compromising growth in broiler chicks (Beckford et al., Poultry Science, Vol. 95, E-Suppl. 1). The objectives of the current study were as follows: (1) determine if hen FO altered hepatic lipid catabolism; (2) define effects of hen diet on chick adipose fatty acid profile; and (3) discover molecular effects of hen diet on chick adipose proteomes. Cobb 500 broiler chicks (n = 30/diet) were hatched from breeder hens fed diets containing fat (5%) from either fish oil (FO) or corn oil (CO) for 4 weeks and fed a CO-based diet after hatch, until 7 or 14 d of age. QPCR was used to characterize liver gene expression. Mass spectrometry was used to determine the effects of diet on adipose fatty acid profiles (GC-MS) and proteomes (LC-MS/MS). Mixed model ANOVA and least squares means were used to identify effects of maternal diet and age × diet interactions on chick phenotypes (SAS v9.4, Cary NC). Diet did not significantly affect expression of genes associated with de novo lipogenesis or fatty acid oxidation (carnitine palmitoyl transferase 1 (CPT1), acyl-CoA oxidase 1 (ACOX1) and fatty acid synthase (FASN)) in liver. Hen dietary fish oil significantly enriched chick adipose tissue in eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) species relative to CO-chicks at 7 but not 14 d of age ($P < 0.05$). Proteomics identified 95 known proteins that differed in abundance between FO and CO adipose tissue. KEGG pathway enrichment analysis of differentially abundant proteins indicated that hen FO feeding significantly suppressed glycolysis (n = 5 proteins) and altered the cytoskeletal architecture of adipocytes and lipid droplet proteins (n = 11 proteins). In combination with our previous findings, these results suggest that hen FO programs reduced adiposity by promoting differentiation of adipocytes with reduced capacity for fatty acid uptake and storage. Exploration into potential epigenetic effects of hen diet is ongoing.

**Key Words:** extracellular vesicles, amniotic fluid, layer embryo, nanoparticle tracking analysis

### 177 RNA sequencing analysis of the shell gland region of oviduct in laying hens challenged with infectious bronchitis virus.


RNA-Sequencing has been rapidly adopted for profiling of the transcriptome to study gene regulation, tissue development and disease. In this study, brown egg laying hens were challenged with IBV T strain and shell gland tissue was collected at d 10–11 post infection. RNA-Sequencing was performed on shell gland tissue samples collected at 5 and 15 h post-oviposition time from infected and control groups of hens. Functional analysis was performed in Cytoscape plugins ClueGO using Gene Ontology (GO) terms in biological process, molecular function and cellular component specific for Gallus gallus. Comparing the samples at 5 and 15 h time points, 1953 and 1678 differentially expressed genes (adjusted P-value <0.05; fold change >1.5) were identified, respectively. There was no significant effect (adjusted P-value >0.05) of virus challenge on the genes involved in eggshell formation and the immune system. There was a significant effect of time points on the expression of genes involved in eggshell formation. The 2 most enriched GO terms appearing at the 5-h time point were T-cell homeostasis and signal release. The most enriched genes at the 5-h time point in T-cell homeostasis were ACE, P2RX7 and SLCL13A2. Similarly, the most enriched genes in GO term signal release were C2CD4C, P2RX7 and SYT15. At the 15 h time point, the 2 most enriched GO terms were regulation of calcium ion dependent exocytosis and response to organonitrogen compound. The most enriched genes in regulation of calcium ion dependent exocytosis were LOC472491, RMS1, SYT16 and TC2N. Similarly the most enriched genes in response to organonitrogen compound were AGTR2, BAIAP2L2, CISH, GNRRH, HTR1B and INHHB. This study provides detailed descriptions of the shell gland genes that are overexpressed when the eggshell is in the oviduct but not in the shell gland or when

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Expression of avian beta-defensins and liver-expressed antimicrobial peptide 2 mRNA in young broilers infected with Campylobacter. Javier Garcia*, 1, J. Byrd2, and Eric Wong1, 1Virginia Tech, Blacksburg, VA, 2USDA, ARS, Southern Plains Agricultural Research Center, College Station, TX.

Campylobacter is a human foodborne pathogen that is commonly found in the gastrointestinal tracts of chickens. Avian beta-defensins (AvBD) and liver-expressed antimicrobial peptide 2 (LEAP2) are host defense peptides that serve as part of the host’s innate immune system. In this study, the objective was to evaluate the mRNA abundance of AvBD and LEAP2 during a Campylobacter infection in the intestines of young broilers. One hundred and 52 broiler chicks were divided into 4 groups of 38 birds each. On day of hatch, all chicks within one group were challenged with 1 of 3 doses of Campylobacter jejuni: Low (10 6), Medium (10 7), and High (10 8) colony-forming units. On d 7 and 14, 6 chicks from each group were randomly selected and subjected to necropsy. Tissue samples from the duodenum, jejunum, ileum and ceca were collected. Total RNA was extracted and gene expression was determined using real-time PCR. Tissue- and age-specific differences in gene expression were observed for AvBD and LEAP2 (P < 0.05). On d 7, AvBD-10 mRNA abundance in the ileum and LEAP2 mRNA abundance in the ceca were lower in the 10 6 and 10 7 groups compared with the control. On d 14, mRNA abundance of AvBD 1, 6, 8, 10, 11, 12, and 13 in the ileum and ceca were greater in the 10 6 group than the control. Interestingly, mRNA abundance for AvBD 6, 8, and 11 was greater in the 10 6 group than the 10 5 group in the ileum and ceca. These results suggest that AvBDs play a role in modulating a Campylobacter infection. Additionally, at a low dose of Campylobacter the immune system is able to mount a strong response leading to enhanced AvBD expression, but at higher doses the immune system may become overwhelmed and there is downregulation of the AvBD.

Key Words: Campylobacter, zinc transporter, LEAP2, avian beta-defensins, broiler

Molecular mechanisms of synergistic enhancement of chicken innate immunity and barrier function by butyrate and forskolin. Kelsy Robinson*, 1, Hong Li2, Long Zhang3, Ryan Arsenault4, Lakshmi Sunkara5, Brian Cougar1, and Glenn Zhang6, 1Oklahoma State University, Stillwater, OK, 2Henan Agriculture University, Henan, Zhengzhou, China, 3Sichuan Agricultural University, Chengdu, Sichuan, China, 4University of Delaware, Newark, DE, 5USDA, East Lansing, MI.

Removal of medically important antibiotics from poultry production has created an urgent need for effective antibiotic alternatives. Dietary modulation of host defense peptide (HDP) synthesis has arisen as a promising solution. HDPs are capable of killing bacteria directly, modulating the immune response, and improving barrier function. Recently, we revealed a significant synergy between butyrate, a short chain fatty acid, and forskolin, a plant labdane diterpene molecule, in the enhancement of HDP gene expression and barrier function both in vitro and in vivo. To investigate the molecular mechanisms of the butyrate-forskolin synergy, chicken HD11 macrophages were treated with 2 mM butyrate, 5 µM forskolin or the combination, followed by analyses of cellular transcriptome and kinome, respectively. Transcriptome analysis confirmed a significant synergistic induction (false discovery rate ≤0.05 and fold change ≥1.5) of multiple HDP and barrier function genes. For example, tight junction protein claudin-10 and mucin-5B gene expression levels were minimally altered by either butyrate or forskolin, but displayed a synergistic 2.22-fold and 2.55-fold increase, respectively, in response to both butyrate and forskolin. Additionally, several anti-inflammatory cytokines such as IL-10 and IL-19 were significantly induced in HD11 cells in response to a combination of butyrate and forskolin, without showing an increase in the expression of major proinflammatory cytokines such as IL-1-β and TNF-α. Chicken-specific kinome peptide array also revealed an enrichment (P < 0.05) of multiple signaling pathways such as MAPK and tight junction pathways in HD11 cells treated with 2 mM butyrate and 5 µM forskolin for 4 h. Overall, these results indicate the capacity of butyrate and forskolin to enhance host immune and barrier function without triggering inflammation, reinforcing their potential as alternatives to antibiotics.

Key Words: host defense peptide, butyrate, RNAseq, kinome peptide array, immunity