

Mini Review

Avianomics: The Beginning at the End

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Article information

Received: September 25th, 2020; Revised: November 13th, 2020 Accepted: November 16th, 2020; Published: November 25th, 2020

Cite this article

Komp M, Bowie R, Teyssier J-R, et al. Avianomics: The beginning at the end. *Adv Food Technol Nutr Sci Open J.* 2020; 6(2): 70-78. doi: [10.17140/AFTNSOJ-6-171](https://doi.org/10.17140/AFTNSOJ-6-171)

PREFACE

The research-teaching nexus is the pinnacle of academic activity. As a scientist and educator, my overall objective is to give students access to cutting-edge research and help them develop high order inquiry skills. In addition, as research and thesis writing frequently involves the development of new complex reading skills associated with comprehension and synthesis of a tremendous volume of information, along with in-depth critical analysis, evaluation, and elegant academic writing style, I decided to assign this editorial to my 2020-Biochemical Nutrition graduate students. As my students have diverse educational and cultural backgrounds and have been exposed to a variety of tech-based learning, we decided to summarize here recent advances in omics research in avian species to facilitate the discussion by asking new questions, which may help open new vistas for future investigations. By following the “scaffolded” approach, my students highlighted progress in Transcriptomics (Komp M and Bowie R), Proteomics (Tabler T and Lee T), Kinomics (Hernandez RM and Zuo B), Epigenomics (Ferver A), Metabolomics (Maynard C and Mullenix G), and Microbiomics (Teyssier JR and Bodle B). I would like to thank my students for their motivation, dedication, determination, perseverance, connection, consistency, and support. I would also like to thank AFTNS-OJ for inviting me to write this editorial and I hope by getting my students involved would promote their curiosity, help them understand complex questions, explore more sources, stimulate their independent thinking, and create research- and question-mindsets.

AVIAN TRANSCRIPTOMICS

The transcriptome of an organism, cell, or tissue refers to the complete set of ribonucleic acid (RNA) species or gene transcripts that are being transcribed at a certain time point. Transcriptomic research aims to compare cells or tissues under defined conditions or disease states to identify changes in gene expression; therefore, giving us a better idea, of which functions different coding or non-coding genes might have, and a better understanding of how they contribute to the phenotype of individuals and their functional adaptations during their lifetime.^{1,2}

There are two main techniques in the field of transcrip-

tomics: microarrays and RNA sequencing. Microarrays are used to measure predetermined sequences, whereas ribonucleic acid (RNA) sequencing captures all sequences through high-throughput sequencing (whole-transcriptome shotgun sequencing).³ These techniques have allowed for a greater understanding of how gene expression changes and identifies trends that cannot be detected by more targeted assays.⁴

In avian research, transcriptomics has had major implications for our understanding of avian diseases, nutrition, growth, and well-being. The use of transcriptomics is also paving the way for future research opportunities and technology developments that will bridge the gap between traditional avian research and ge-

nomics and stimulate more interdisciplinary research.

Transcriptomics in Health and Disease

By using transcriptomics to compare gene expression profiles in healthy and diseased individuals we can attain a greater knowledge of what parts of the genome are or are not being regulated during disease and observe structural alterations, such as mutations. A particular interest in understanding what makes a species resistant or susceptible to a certain infectious disease has further led to several comparative immuned biological studies, which has provided great advancement in the field of immunology. For instance, the use of dual RNA-seq of host and pathogen has led to studies of host and parasite co-evolution. Transcriptomic analysis of chicken lungs characterized the transcriptomic profile of chicken lungs infected with the parasite, *Pasteurella multocida*, and provided valuable information to understand host responses. The study identified an avian *P. multocida* serogroup A strain (PmQ) showing high lethality to chickens and a bovine *P. multocida* serogroup A strain (PmCQ2) with no lethality to chickens.⁵ Analyzing the hardier gland, trachea, and lung tissues, Lamont's group identified several markers associated with Newcastle disease.⁶ Similarly, by using RNA-seq, several differentially expressed genes were identified in woody-breast affected chickens when compared with unaffected counterparts.⁷ Marchesi and co-workers identified several genes associated with white striping in broiler chickens.⁸ Berri's group performed a muscle transcriptome analysis and revealed several biomarkers associated with meat defect in chickens.⁹ By using RNA-seq, several osteochondral genes have been shown to be altered in bacterial chondronecrosis with osteomyelitis (BCO) in chickens.¹⁰

Transcriptomics in Well-Being

Transcriptomics has the ability to play a key role in many aspects of avian welfare. For example, a transcriptome analysis reveals that stocking density affects the expression of genes involved in several metabolic pathways such as glycolysis, proteolysis, immune stress, muscle development, cell adhesion, matrix, and collagen.¹¹ Several studies showed different transcriptome profiles in various chicken tissues in response to high ambient temperature.¹²⁻¹⁴ They identified Angiopoietin-like-4 (*ANGPTL4*) gene as a candidate for the improvement of heat tolerance in chickens.

Transcriptomics in Growth, Development and Feed Efficiency

Feed efficiency is a key agricultural and economic trait. Transcriptomic technology could allow for a deeper understanding of residual feed intake among other nutrition related components. A recent study suggested that using transcriptome architectures based on high-quality RNA-Seq data reveals 41 differentially expressed genes associated with residual feed intake. These genes play a role in digestibility, metabolism, stress response, and energy homeostasis.¹⁵ Recently, Yang Lei and co-workers identified key genes and pathways associated with feed efficiency in native chickens.¹⁶ Using high and low feed efficient birds, Kong et al¹⁷ identified several key genes in breast muscle that are involved in anabolic pathways. By comparing the transcriptome between pigeon ovaries before and after ovulation, Xu et al¹⁸ found that the expression of several genes is modulated during different stages of ovulation. By study-

ing six avian species, Wright et al¹⁹ found higher gene divergence on the Z chromosome compared to autosomal chromosomes.

AVIAN PROTEOMICS

Proteomics, in its simplest definition, is the study of the proteome, which encompasses the expression of whole protein profiles in a biological system. Marc Wilkins, in 1994, is credited for developing the concept and coining the term "proteome". Its application today in regard to poultry is, but not limited to, studying the cell, tissue, or organ proteins that are expressed (measured and compared), have protein-protein interactions, or are structural components that may impact bird health, well-being, and growth. In addition to improving bird welfare, its application has extended to improving meat quality to the consumer.

Several techniques are frequently used in proteomics including 2-hybrid systems, 2-dimensional gel electrophoresis, and mass spectrometry. Systems biology is a scientific approach that takes into account the complex relationships among and between genes and proteins and determines how all of these interactions come together to form a functional organism. Proteomic tools can simultaneously probe the properties of numerous proteins and thus are a great aid to the emerging field of systems biology, in which the functional interactions of numerous proteins are studied instead of studying individual proteins as isolated entities. In the field of avian biology, proteomics has been used to study different processes from the development, growth, and function of organs and systems to the interactions of infectious agents and the altered states that they induce in their hosts.

Proteomics in Health, Disease and Meat Quality

The following studies have helped elucidate protein changes involving poultry disease and stress such as ammonia stress,²⁰ ascites,²¹ bacterial, protozoa, and viral infection,²²⁻²⁴ femoral head necrosis,²⁵ woody breast,²⁶ and sudden death syndrome.²⁷ The selection of broilers for high growth performance has resulted in an increase in the occurrence of muscle myopathies. The most common are white striping and woody breast myopathies, which have been estimated to cost the U.S. industry \$200 million or more.²⁸ Work by Kuttappan et al²⁸ helped elucidate that protein metabolism and protein translocation (141 involved proteins) are important contributors to these myopathies. Another incidence is the pale, soft, and exudative (PSE) syndrome that reduces color, water holding capacity, and quality of poultry meat, which deters consumers. Xing et al²⁹ identified proteins related to the glycolysis pathway, calcium signaling, and molecular chaperones that are integral in PSE syndrome. Tenderness is an important – possibly the most important – driving force for consumer purchase decisions. Mekchay et al³⁰ showed three protein spots that were correlated with shear force, play a role in the glycolytic pathway, and are closely related to tenderness. Other meat quality factors like water holding capacity³¹ and flavor^{32,33} have been evaluated for protein biomarkers to improve these areas. These studies not only helped to better understand the protein mechanisms but identified biomarkers for subsequent development of target strategies to reduce or alleviate these issues.

A popular strategy in regulating the host immune system is through feeding probiotics. Luo et al³⁴ offered *Enterococcus faecium* to broilers and identified 42 intestinal mucosal proteins related to metabolism, immune and antioxidant systems.

Proteomics in Well-Being

Heat load is one of the most challenging stressors to poultry from its adverse effects on growth, gut health, well-being, and mortality. Intestinal health and function are paramount to achieving efficient feed utilization, growth, and welfare. O'Reilly et al³⁵ determined the effects of microbial challenge on broiler intestinal proteome.³⁵ They found that the levels of cytoskeleton³⁵ proteins increased over time in the small intestine. Microbial challenges, however, reduced the expression of Villin-1.35 Tu et al³⁶ and co-workers performed proteomic analysis on the hypothalamus of heat-stressed broilers and identified 114 proteins that are mainly involved in cellular processes, metabolism, transport, and cellular defensive response against oxidative stress and toxin export and delivery.

Proteomics in Growth, Development and Feed Efficiency

Proteomics has not only allowed a better understanding of changes in protein processes but has introduced a new era of selecting broiler genetic lines for superior performance. Kong et al³⁷ selected higher feed efficiency birds from a standard flock and demonstrated that birds having a higher feed efficiency had greater breast muscle mitochondria protein expression caused by higher mitochondrial function and oxidative phosphorylation. Phongpa-Ngan et al³¹ separated broilers that had a fast or slow growth rate from the same flock and found broilers with a faster growth rate had a unique protein profile in muscle tissue. In the past decade, the selection of broilers for improved performance has been accompanied by excessive body fat. Wang et al³⁸ identified 33 different proteins that are related to biological oxidation, cell proliferation, cytoskeleton, lipid metabolism, chaperones, protein synthesis, and signal transduction between fat and lean broilers.

AVIAN KINOMICS

Kinomics is mainly used to study the abundance, activity, substrate specificity, phosphorylation status, and other characteristics of kinases and phosphatases in cells or tissues. Protein kinase refers to a class of enzymes that modifies other proteins by chemically adding phosphate groups to them. The protein phosphorylation is one of the important protein post-translational modifications, which plays key roles in the metabolism, transcription, cell cycle regulation, apoptosis, movement, and other cellular processes of most eukaryotic cells.³⁹ Studies have shown that more than 30% of proteins are phosphorylated at any time in cells.⁴⁰ In the human kinome, 518 protein kinases were identified, and 244 kinases were found to be related to the occurrence of diseases through chromosome mapping and other means.⁴¹ Phosphorylation and dephosphorylation are like a molecular switch that could regulate the activation and inactivation of certain proteins. Peptide or protein array screen coupled with mass spectrometry (MS) is widely applied to determine phosphorylation because of the convenience and sensitivity, and proteomics has high-throughput

potential and requires no knowledge of sequence preference of the kinase.^{42,43}

Although kinomics is an emerging powerful technology at large scale, very limited studies have been conducted in avian species.

Kinomics in Health and Disease

By using a chicken-specific kinome array, Kogut and colleagues showed that *Salmonella modulates* host immune signaling pathways in the cecum to establish a persistent infection.⁴⁴ Specifically, they showed that the response is characterized by alterations in the activation of T-cell signaling mediated by the dephosphorylation of phospholipase c-γ1 (PLCG1) that inhibits NF-κB signaling and activates nuclear factor of activated T-cells (NFAT) signaling and blockage of interferon-γ (IFN-γ) production through the disruption of the JAK-STAT signaling pathway.⁴⁴ The same group identified that AMP-activated protein kinase (AMPK) and mechanistic target of rapamycin (mTOR) are key sensors and regulators of immunometabolic changes during *Salmonella infection* in chickens.⁴⁵

Kinomics in Well-Being

Napper and co-workers used the global cellular kinase approach to characterize kinome response within the breast and thigh muscles of heat- and cold-stressed chickens.⁴⁶ In the breast, both cold and heat stress activated calcium-dependent metabolic adaptations. Also within the breast, but specific to cold stress, the activation of ErbB signaling as well as dynamic patterns of phosphorylation of AMPK were observed. In the thigh, cold stress activates innate immune signaling and transforming growth factor-beta (TGFβ), however, heat stress-activated pathways are associated with protein and fat metabolism through adipocytokine and mTOR signaling.

EPIGENOMICS

Epigenomics is the study of the complete set of epigenetic (modifications that alter gene expression that cannot be attributed to the modification of the primary deoxyribonucleic acid (DNA) sequence) modifications on the genetic material of a cell, known as the epigenome. DNA methylation and histone modification are among the best-characterized epigenetic processes.⁴⁷ Several approaches, including histone modification assays, Chip-Chip and Chip-Seq, DNA methylation assays, bisulfite sequencing, and chromatin accessibility assays, are used.

Epigenomics in Health and Disease

Deoxyribonucleic acid methylation has been investigated in relation to disease states in chicken, specifically Marek's disease, comparing two lines, Fayoumi and Leghorn. This study confirmed that variation in DNA methylation leading to changes in gene regulation likely confers disease resistance and it was observed that DNA methylation was highest in internal exons.⁴⁸ Immune related genes, such as TLR4, was found to be both differentially methylated and expressed in the two lines and further demon-

strates the potential regulatory role of DNA methylation in the control of gene expression. Marek's disease infection of two lines, with differing resistance, was also analyzed for histone post-translational modifications (HPTMs). Differentially enriched regions were seen between the susceptible and resistant lines, with the HPTM profile being modulated after infection around immune response genes.⁴⁹⁻⁵¹ Epigenetic modifications were also observed in the immune underlying mechanisms in chicken susceptible to *Salmonella enteritidis*.⁵²

Epigenomics in Well-Being

Epigenetic studies have been conducted in heat-stressed chickens and showed alterations in the methylation level of CpG site in the promoter of the brain-derived neurotrophic factor (*BDNF*) gene. Modifications of histone H3 lysine 9 (*H3K9*) and methylation of histone H3 lysine residue 27 (*H3K27*) in the promoter of hypothalamic brain-derived neurotrophic factor (*BDNF*) were also observed during thermotolerance acquisition on day 3 post-hatch.^{9,53} In addition to postnatal experience and genetics, it has been reported that chicken behavior can be modulated by epigenetic mechanisms.⁵⁴

Epigenomics in Growth, Development and Feed Efficiency

It has been shown that environmental and nutritional factors can influence embryonic development and the adult phenotype, at least partially, *via* epigenetic mechanisms and adaptation. For instance, Tzschentke and Basta have shown that temperature variation during the prenatal period influences hypothalamic neuronal thermosensitivity during the postnatal stage.⁵⁵ Similarly, nutritional programming affects offspring phenotypes. Rao et al⁵⁶ showed that 4 week-old chicks from mothers fed low-protein diets had a higher body and breast muscle weights confirming that maternal nutrition can affect offspring phenotypes.

METABOLOMICS

Metabolomics is the emerging field of metabolome analysis that characterize, identify, and quantify metabolites in biological (cell, tissues fluids) samples. As metabolites play a crucial role in the maintenance and growth of organisms, they are considered as the "canaries" of the genome and the intermediate phenotypes. Their application in livestock in general and poultry, in particular, might become a cornerstone for the "next-generation phenotyping" approaches. An increasing number of studies have integrated metabolomics in poultry.

Metabolomics in Health and Disease

Le Roy et al⁵⁷ established a high-resolution proton nuclear magnetic resonance-based metabolic atlas and identified several metabolites in different tissues in chickens. By using ultra-performance liquid chromatography/tandem mass spectrometry, Aggrey's group identified several metabolites associated with fatty acids metabolism and beta-oxidation in *Eimeria acervulina*-infected chickens.⁵⁸ Ma and coworkers have shown that *Salmonella enterica* infection altered the intestinal metabolome in chickens.⁵⁹ Dridi's group identified

a total of 108 known metabolites in chicken breast, with 60 having significant mean intensity (42 higher and 18 lower) in woody breast-affected compared to unaffected muscles.⁶⁰ These differentially abundant-metabolites were associated with purine nucleotide degradation and *de novo* biosynthesis, sirtuin signaling pathway, citrulline-nitric oxide cycle, salvage pathways of pyrimidine DNA, IL-1 signaling, iNOS, Angiogenesis, PI3K/AKT signaling, and oxidative phosphorylation.⁶⁰

Metabolomics in Growth, Development and Feed Efficiency

Mignon-Gasteau group showed that ileal, caecal, and serum metabolome explains approximately 75-76% of the variability of the apparent metabolizable energy and digestive efficiency in chickens.⁶¹ Analyzing serum metabolome, Metzler-Zebeli et al⁶² observed a distinct profile between two divergently selected chicken lines for residual feed intake and feed efficiency. Supplementation with Magnolia Bark extract or *Bacillus subtilis* modulates the intensity of several intestinal metabolites associated with amino acids, fatty acids, peptides, and nucleosides.⁶³ Peng and co-workers have shown that 39 and 68 metabolites were significantly changed from E14 to E19 and from E19 to hatch, respectively in chicken embryos, indicating a potential role of the metabolome in chicken development.⁶⁴ Similarly, Wu et al⁶⁵ studied the hepatic metabolic changes during postnatal liver maturation in breeder roosters and identified several differentially abundant metabolites involved in carbohydrate, protein, amino acid, lipid, cholesterol, nucleic acid, and vitamin metabolisms.

MICROBIOMICS

The microbiome is defined as a characteristic microbial community in a reasonably well-defined habitat, which has distinct physicochemical properties as their theater of activity.⁶⁶ The study of this microbial community is known as microbiomics. Until early 2000, the study of the microbiome was only relying on the culturable microorganism, which represents a very low portion of the bacterial population as low as 1%. In the microbial research field, the two last decades have been marked by the development of high-throughput DNA-based sequencing methods, new bio-informatics developments, and analysis methods. That methodological progress has allowed the detection and quantification of unculturable bacteria and access to an important amount of data on genome diversity. The study of the microbiome has revealed bacteria to be implicated in numerous functions for the human organism, but the microbiome research has also a wide range of applications in food science, biotechnologies, and agriculture.⁶⁶

Microbiomics in Health and Disease

As described above, gut health, function, and integrity are essential for the overall well-being and health of birds. Consequently, the gut microbiota, which contains tremendous numbers of bacterial species, has recently been extensively characterized in some avian species including chickens, turkeys, and ducks.⁶⁷⁻⁶⁹ The influence of viral and bacterial infections on gut health, integrity, and homeostasis have been also studied. For instance, Experimental infection of young chickens with H9N2 virus caused alterations in the intestinal

microbiota composition with increased phylum Proteobacteria.⁷⁰ In a study conducted by Li et al,⁷¹ in addition to increased phylum proteobacteria, Firmicutes was decreased in H9N2 virus-infected chickens. Hird and colleagues reported that the cloacal microbiome in wild ducks is specie-dependent and varies by influenza A virus infection status.⁷² Similarly, the effect of Newcastle and bursal disease viruses on avian microbiota has been investigated.^{73,74}

Microbiomics in Growth, Development and Feed Efficiency

Several studies investigated the effect of diet on growth and gut microbiome in regard to diversity and composition. The inclusion of xylanase and β -glucanase enzymes in diet enhance the growth of lactic acid bacteria, which adhere to the gut epithelium and compete with pathogens for its colonization while decreasing lumen viscosity.⁷⁵ Supplementation with phytase promotes the abundance of *Aeromonadaceae* and *Flavobacteriaceae* in the crop, and *Lactobacillus sp.*, *Clostridium leptum* and *Enterococcus sp.* in the ileum and enhances growth performances in chickens.⁷⁶ Costa et al⁷⁷ have shown that the administration of different antibiotic growth promoters induces specific changes in the cecal microbiota in broilers. Wang et al⁷⁸ showed that probiotics modulate gut microbiota and improves meat flavor in chickens. By performing 16S ribosomal ribonucleic acid (rRNA) sequencing, Jurburg et al⁷⁹ monitored the chicken fecal microbiome at different ages and identified three successional stages characterized by dominant *Streptococcus* and *Escherichia* taxa from 1 to 3 d post-hatch, followed by *Lachnospiraceae* and *Ruminococcus*-like species from 4 to 10 d post-hatch, and a final stage with dominant *Candidatus Arthrobacter* and *Romboustia* from day 10 (Figure 1).

CONCLUSION

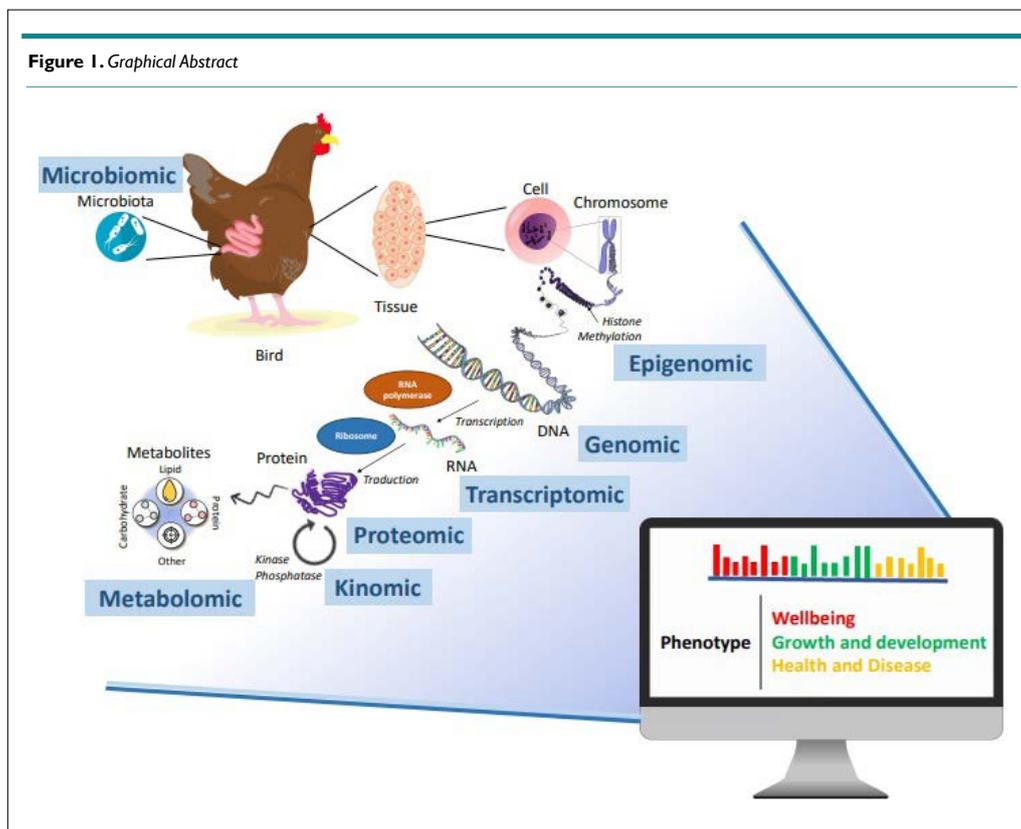
Challenges and Perspectives

From ontogenesis to homeostasis, the phenotypes are shaped by multi-complex interactions and networks between genes, proteins, and microbiome and their interaction with their surrounding environment (nutrition, environment, stress, etc.).

In poultry, multi-omics analysis revolutionized avian researches and revealed unprecedented interesting data. However, exploring the full extent of the abovementioned interactions requires careful considerations of study design to efficiently generate optimal data integration. For instance, past omics studies used a variety of tissues and mixed cell populations to attain the differential expression profile, which might mask the cellular specific information. In the future, integrative single-cell omics is warranted.

Development of portable technology would help omics studies in an on-site field, which would minimize the time between harvesting and sequencing, but can also facilitate the workflow in countries where sequencing facilities are scarce or where export of tissues is a hampering factor. If the technology of such is incorporated, rapid identification of the virus or pathogens could be achieved, which in turn could help to minimize the spread and outbreak of infection.

Despite their descriptive nature due to lack of mechanistic studies, the multi-omics studies have the potential to change



the future of poultry production and sustainability from dietary, environmental, management, and breeder guidelines by identifying key markers and their networks (gene, protein, metabolite, etc.) for precision selection and personalized nutrition approach under appropriate conditions and adaptations.

CONFLICTS OF INTEREST

All authors declare that there is no conflict of interest in conducting this review or in publishing its results.

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