Report and Recommendations

NORTHEAST MULTISTATE ACTIVITIES COMMITTEE MEETING

May 20, 2024, 2:00 PM ET Zoom Teleconference

Members: Puneet Srivastava (Maryland-Chair), Jason White (CT-New Haven), Blair Siegfried (Penn State), Chris Smart (NY Geneva), Cindy Fitch (WVU/NEED), Ali Mitchell (NEED-not in attendance) [Non-voting, ex officio: Rick Rhodes (NERA), David Leibovitz (NERA)]

Request to Approve Peer Reviewed Multistate Activities (MAC recommends to NERA)

- NE_TEMP2443: *Biology, Ecology & Management of Emerging Disease Vectors*, 10/2024-09/2029 [Renewal of NE1943, AA: Jason White – Connecticut, New Haven]
  - CT-New Haven has held a longstanding leadership role with this project team.
  - Proposal is focused mainly on mosquitoes and ticks (responsible for more than 17% of all infectious diseases).
  - A large list of approved project participants is already in place.
  - Reviewers were generally positive. Authors responded to all reviewer comments, including refining the approach clarifying the project description, and tying back to CDC priorities.
  - **A motion was introduced to recommend NE_TEMP2443 for NERA approval. The motion was approved unanimously.**
- NE_TEMP2442: *Improving Sustainable Poultry Production through Collaborative Research and Outreach*, 10/2024-09/2029 [Renewal of NE1943, AA: Kumar Venkitanarayanan – Connecticut, Storrs]
  - This project has a legacy of continuous activity dating back to 1978. Project editor Ken Koelkebeck has led the team since 1999.
  - The previous project cycle was highly successful, generating: $20M grants, 284 journal articles, 385 abstracts, 80 proceedings at national meetings.
  - Technical team highlighted a focus on the precision ag element of poultry production, fostering innovative production practices in research and extension.
  - Stakeholder input – one key element is to use access to state-of-the-art production systems which reflect industry collaboration of the project. A twofold benefit: 1.) access to industry systems, and 2.) lack of contemporary facilities housed on LGU campuses, which is a strong justification for the promotion of the Research Facilities Act.
  - Reviews were generally positive. One reviewer called for more detail in the materials and methods sections. Should we encourage multistate teams to provide more granular detail? Are these proposals intended to be broader, with details captured in the reporting of individual participants?
  - **A motion was introduced to recommend NE_TEMP2442 for NERA approval. The motion was approved unanimously.**

MAC Discussion Items

- NE nominations:
    - 2024 agInnovation Award for Excellence in Multistate Research: NE1834 *Genetic Bases for Resistance and Immunity to Avian Diseases* (drafted by AA Bob Taylor, West Virginia)
    - NE nomination for 2024 Multistate Research Fund Impacts writing workshop, a component of NRSP1: NE2335 *Resource Optimization in Controlled Environment Agriculture*
    - **The MAC unanimously agrees upon nomination of NE1834 for the research award, and nomination of NE2335 as the impact writing workshop recipient.**
- The MAC took time to give special thanks to Cindy Fitch for her service on the Multistate Activities Committee. Cindy will be retiring from WVU Extension next month, and this will be her final engagement with the MAC.
    - **Bill Miller, UMass Extension, will be nominated as the NEED representative on the MAC to replace Cindy. Bill will rotate off the NRSP1 Management Committee as the Northeast AA.**
- “Context for reviewing Multistate Research Project Proposals”
    - NERA OED can refine language that is presented to prospective reviewers up front, but also highlight to project teams the level of detail which should be provided.

Administrative Adviser Assignments (activities seeking Administrative Advisers, MAC recommends to NERA)

- NE1938 (intends to renew 9/30/24 as NE_TEMP2438): *Carbon Dynamics and Hydromorphology in Depressional Wetland Systems*
- NE2201: *Mycobacterial Diseases of Animals*
- NE2249: *Sustainable and Inclusive Rural Economic Development to Enhance Housing, Health, Entrepreneurship, and Equity*
- NE2333: *Biological Improvement of Chestnut through Technologies that Address Management of the Species and its Pathogens and Pests*
- NRSP1: *Multistate Research Information Management and Impact Communications Program*
    - **David Leibovitz was nominated to serve as the Northeast AA. The MAC will recommend David’s NRSP1 appointment at the NERA business meeting in June.**
- NRSP8: *Genomic Capacity: Building Applied Genomic Capacity for Animal Industries*

Informational Items

- NERA activities up for Mid-term review in FFY2024
    - NE2140: Sustainable Management of Nematodes in Plant and Soil Health Systems (AA: Anton Bekkerman – New Hampshire)
    - NE2101: Eastern White Pine Health and Responses to Environmental Changes (AA: George Criner – Maine)

- NECC2103: High tunnel specialty crop production (AA: Anton Bekkerman – New Hampshire)
- NEERA2104: Northeast Region Technical Committee on Integrated Pest Management (AA: Margaret Smith – Cornell)

- NERA activities ending 09/30/2024
  - NE1939: Improving the health span of aging adults through diet and physical activity (**fully approved as NE2439**)
  - NE1942: Enhancing Poultry Production Systems through Emerging Technologies and Husbandry Practices (**seeking approval by NERA in June**)
  - NE1943: Biology, Ecology & Management of Emerging Disease Vectors (**seeking approval by NERA in June**)
  - NE1938: Carbon Dynamics and Hydromorphology in Depressional Wetland Systems (**interested in renewal, draft proposal not yet received**)
  - NECC1901: Integrating Genomics and Breeding for Improved Aquaculture Production of Molluscan Shellfish (**interest in renewal TBD**)
  - NE1941: Environmental Impacts of Equine Operations (**elected to not renew**)
- New NERA activities in 2024
  - NE_TEMP1: *Northeast Regional Center for Rural Development*, 10/2024 – 09/2029 (AA: Stephan Goetz) **- currently undergoing peer review**

NE_TEMP2443: Biology, Ecology & Management of Emerging Disease Vectors

Status: Submitted As Final

Duration 10/01/2024 to 09/30/2029
Admin Advisors: [Jason C White]
NIFA Reps:

Non-Technical Summary

Nearly 20 percent of the infectious diseases reported worldwide today are caused by vector-borne pathogens, posing a major threat to farmers, forestry workers, and families living in urban, suburban, and rural areas in the US. With few available vaccines and new vector-borne diseases emerging at a steady rate, minimizing human exposure to arthropod disease vectors (e.g., mosquitoes, sandflies, kissing bugs, ticks) and managing vector populations remain the primary methods for reducing vector-borne disease risk. Our ability to control vector-borne disease threats is limited by our understanding of vector ecology, vector physiology, vector behavior, and the biology of vector-pathogen interactions. Thus, our research will (1) develop and strengthen effective surveillance and monitoring of disease vectors and their associated pathogens at local and regional scales; (2) determine the ecology, physiology, genetics and/or geographic and temporal distributions of historical, extant, and emerging disease vectors and the pathogens they transmit; and (3) discover, develop, and integrate interventions to manage vector-borne pathogen transmission and pesticide resistance. Our team is composed of top entomologists, microbiologists, ecologists, wildlife biologists, and veterinarians who study vector and vector-borne pathogen biology. This work cannot be completed without a multistate effort. In a rapidly changing landscape, the experience and guidance of one region is critical to others. The project will benefit all US residents by understanding, assessing, and mitigating the threat posed by arthropod vectors of health, veterinary, and wildlife importance, and provide for and encourage environmentally sound, scientifically based, and professional control by public health and pest control agencies.

Statement of Issues and Justification

The need as indicated by stakeholders. According to the World Health Organization (WHO), around 17% of the infectious diseases reported worldwide are caused by vector-borne pathogens. In the US, outbreaks of vector-borne diseases are aided by human travel and the introduction of infected vectors into new areas. For example, in 2023, cases of locally acquired malaria were reported in Maryland, Texas, and Florida caused by *Plasmodium vivax* and *Plasmodium falciparum*, leading the US Centers for Disease Control and Prevention (CDC) to issue a national alert. Dengue outbreaks have been reported in Florida, where several serotypes of the virus have been detected in mosquitoes, increasing the risk of severe dengue (Coatsworth et al. 2022). Likewise, autochthonous transmission of leishmaniasis occurs in Texas and Oklahoma (Curtin and Aronson 2021). Expanded geographic distributions of vector-borne diseases endemic to the US, such as Lyme disease and other tick-borne diseases, have been attributed to the spread of vectors into new locations (Eisen and Eisen 2023). According to the CDC, Lyme disease affects around 476,000 Americans each year primarily in the northeast with average treatment costs of $1,200 USD per individual (Hook et al 2022). Thus, vector-borne diseases represent an important risk and burden to the public, including farmers, forestry workers, and families living in urban, suburban, and rural areas in the US.

Invasive vector species threaten to alter the epidemiology and transmission dynamics of existing and new vector-borne diseases, and ultimately the health of humans, companion animals, wildlife, and livestock. Mosquito invaders include the yellow fever mosquito (*Aedes aegypti*) and the Asian tiger mosquito (*Aedes albopictus*), that have become established in the southeastern US and are increasingly reported in California (Metzger et al. 2015). While *Ae. albopictus* is moving northward, additional invasive mosquito species have also arrived. For example, the bush mosquito (*Aedes japonicus*) is a cold weather adapted species that has spread in suburban landscapes along the eastern seaboard, the northwestern US, the Florida panhandle, across the Hawaii volcanoes as well as Canada (Kaufman and Fonseca 2014, Jackson et al. 2016, Peterson et al. 2017), and the Australian *Aedes notoscriptus* has spread into southern California (Paterson and Campbell 2015). Over the last few years, we have witnessed the invasion and spread of the Asian longhorned tick (*Haemaphysalis longicornis*), which was first discovered on an Icelandic sheep in New Jersey in 2017 (Rainey et al. 2018) but was detected in archived records as far back as 2010, remaining misidentified and undetected for nearly a decade, if not longer (Beard et al. 2018). Through increased surveillance, it has since been detected in 19 states: Arkansas, Connecticut, Delaware, Georgia, Indiana, Kentucky, Maryland, Massachusetts, Missouri, New Jersey, New York, North Carolina, Ohio, Pennsylvania, Rhode Island, South Carolina, Tennessee, Virginia, and West Virginia (USDA APHIS, October 2023). US populations of *H. longicornis* are parthenogenetic (clonal) and small infestations can quickly proliferate.. Although humans are not favored hosts of *H. longicornis*, opportunistic feeding is well documented both in the native and invasive ranges (Bickerton and Toledo 2020, Wormser et al. 2020). In East Asia, *H. longicornis* transmits severe fever with thrombocytopenia syndrome virus (SFTSV), an emerging human tick-borne disease recently reclassified as *Dabie bandavirus* (Liu et al. 2015, Luo et al. 2015, Li et al. 2021). Under laboratory conditions, US lineages of *H. longicornis* can transmit the closely related Heartland virus (Rainey et al. 2022a) and Powassan virus (Rainey et al. 2022b), two native pathogenic viruses emergent in parts of the US. While *H. longicornis* is currently not perceived as a major public health threat in the US, this status may change given the enormous densities it can reach in favorable habitats (Bickerton and Toledo 2020, Schappach et al. 2020, González et al. 2023, Rochlin et al. 2023).

The importance of the work and consequences if not done. With few available vaccines and new pathogens emerging at a steady rate, minimizing human exposure to disease vectors and managing vector populations remain the primary methods for reducing vector-borne disease risks. Our ability to properly control vector-borne disease threats is limited by our understanding of vector ecology, vector physiology, vector behavior, and the biology of vector-pathogen interactions. The economic impact of arthropod-borne illness is devastating. Consider Lyme disease, the most prevalent vector-borne disease in the United States. It's been calculated to impose a substantial burden on the U.S. healthcare system, ranging from $712 million to $1.3 billion each year(Adrion et al 2015). This financial strain arises from extensive doctor visits and diagnostic testing, particularly evident in the year following initial diagnosis, as persistent symptoms like fatigue, musculoskeletal pain, and memory issues demand ongoing attention and care. . The total cumulative costs of reported WNV hospitalization cases in the US from 1999 through 2012 were around $800 million USD (about $77 million annually in today's value after inflation adjustment; Staples et al. 2014). The estimated cost per human case of EEE is $3 million in 1995 dollars ($6.2 million after inflation adjustment; Villari et al. 1995). EEE and WNV both threaten the nation's multi-billion-dollar equine industry. The mortality rate of horses infected with WNV is 34%; the rate for those with EEE approaches 100%. For example, in 2000, the estimated loss in New Jersey alone due to equine cases of WNV was $6 million ($11 million in 2024 value). Tourism, which increases human exposure to mosquitoes, is similarly impacted by outbreaks of mosquito-borne disease. The 2015-2017 outbreak of Zika virus, although generally mild in adults, was conclusively linked to severe birth defects, which carry a tremendous economic and emotional burden on affected families. Recent computational analyses estimate that even a mild Zika outbreak could cost the US in excess of $183 million in medical costs and productivity losses, whereas a severe outbreak could cost up to $1.2 billion.

The lessons learned from new and emerging mosquito- and tick-borne disease risks serve as an impetus for bolstering US research and translation to vector surveillance and management (Anyamba et al. 2014). Encouragingly, important advances are being made in areas that include new methods and tools for monitoring and the control of mosquitoes and ticks. However, the budgets for research and abatement programs follow a boom-and-bust cycle reactionary to emerging threats, which weakens the ability to sustain infrastructure necessary for managing vector-borne diseases (Kading et al. 2020). In a report released in early 2018, the CDC estimated that 80% of the nation's vector control organizations lack the critical prevention and control capacities needed to combat the tripling of disease cases from mosquitoes, ticks, and sand flies reported over the past 13 years (CDC 2018). Improved sharing and coordination of research and translation to and standardization of monitoring and control tools are needed for researchers and control professionals to make informed decisions and better utilize limited resources.

The technical feasibility of the research. Our research will focus on critical gaps in our understanding of the vectors of disease agents in the U.S. These include mosquitoes, sandflies, kissing bugs, ticks, and other vectors of human pathogens. We will include research on the biology, spread, and control of other invasive vectors of regional importance. Our project will have three objectives:

1. Develop and strengthen effective surveillance and monitoring of disease vectors (mosquitoes, ticks, etc.) and their associated pathogens at local and regional scales. Under this objective, project participants will leverage and strengthen existing surveillance programs in a coordinated fashion to yield robust comparable data across large geographic scales.

2. Determine the ecology, physiology, genetics and/or geographic and temporal distributions of historical, extant, and emerging disease vectors and the pathogens they transmit. Under changing environmental conditions, these studies will enhance our ability to predict conditions leading to the spread or emergence of existing and novel diseases of public health importance.

3. Discover, develop, and integrate interventions to manage vector-borne pathogen transmission and pesticide resistance.

Under each of these objectives, we have several sub-objectives to focus our project, which are described in the Methods section. Our objectives are technically feasible, but only if achieved by a coordinated team. Our team is composed of top entomologists, microbiologists, ecologists, wildlife biologists, and veterinarians who study vector and vector-borne pathogen biology, with the required experience and facilities and we are confident we can succeed with the proposed research.

The advantages of doing the work as a multistate effort. This work cannot be completed without a multistate effort. In a rapidly changing landscape, the experience and guidance of one region is critical to others. As a specific example, both *Ae. aegypti* and *Ae. albopictus* are new to California, and California abatement districts have benefited from the experience of those in the east coast states where these species are often the most common mosquito species. Conversely, researchers in Florida and Texas bring to the table experience working with populations affected with dengue and Zika, a set of skills that may be needed more broadly across the US. Finally, the biology that governs range expansion, vector competence, and ecology of different vector species will help to inform surveillance and management programs.

What the likely impacts will be from successfully completing the work. The project will build a highly collaborative network of experts to study existing and newly invasive vector species. The project will benefit all US residents by understanding, assessing, and mitigating the threat posed by arthropod vectors of health, veterinary, and wildlife importance. Our efforts will strengthen the capacity to detect and predict outbreaks of vector-borne diseases and evaluate the effectiveness of existing and novel control interventions under different environmental and social conditions. The project further provides for and encourages environmentally sound, scientifically based, and professional control by public health and pest control agencies. Our proposed research directly builds on a prior multistate project (NE 1943). Since 2019, we have met and exchanged published and unpublished results, have generated novel hypotheses from group participants and discussed coordination in research and sampling efforts. We have produced numerous accomplishments and deliverables and garnered competitive grants from newly formed collaborations. For this revision/replacement, we seek to expand our collaborative network to develop and strengthen a more coordinated region-wide and multi-state research effort.

Related, Current and Previous Work

Some of the key outcomes of the prior NE 1943 project include:

- Conducted active and passive surveillance of tick and tick-borne pathogen distributions at the county level across multiple states.
- Identified the first reported established population of Gulf Coast ticks (*Amblyomma maculatum*) infected with *Rickettsia parkeri* in Connecticut.
- Described the first occurrence of the soft tick *Carios kelleyi* (Cooley and Kohls) from New Jersey, based on larvae collected from big brown bats, *Eptesicus fuscus*.
- Described the regional spread of *Culex coronator*, an invasive mosquito species which has spread throughout the southern U.S. in the last 20 years.
- Conducted population genetic analyses of *Borrelia burgdorferi* in ticks and small mammals and avian malaria pathogen identification in native birds in Delaware.
- Examined environmental determinants of abundance of multiple disease vector species in their northern ranges.
- Conducted mosquito surveillance throughout the fall, winter, and spring to determine when mosquitoes enter and exit from their overwintering dormancy.
- Investigated how urban pollutants, like artificial light at night and higher temperatures associated with heat islands, affect mosquito dormancy in the lab and in the field.
- Evaluated the efficacy of several *I. scapularis* residential control strategies for homeowners, including perimeter treatments with a granular formulation of cyahalothrin, deployment of tick tubes,
- Assessed natural commercial alternatives to synthetic repellents with similar or better properties than DEET, evaluating the repellency of two extracts, CR3 and CR9, derived for newly developed catnip cultivars on two tick species, *I. scapularis* and *H. longicornis*.
- Tested the impacts of catch basin treatments, application of adulticides, and Autocidal Gravid Ovitraps on adult mosquito populations.
- Developed improved monitoring tools that combine internet-connected databases and quality control with the multiple trap types being used by mosquito abatement districts, e.g., B.G. traps, CDC traps, ovitraps, etc.
- Investigated the impacts of active forest management on the risk of exposure to *I. scapularis*.

- Explored the use of RNA interference (RNAi), a new technology that can provide highly specific control of insect pests, as well as established insecticides like *Bacillus thuringiensis israelensis* as new avenues of control of *Culicoides* midges and *Aedes* mosquitoes.

- Conducted research investigating the insecticidal and repellent activities of natural products derived from plants and microbes against mosquitoes, and elucidated the mechanism of action of how a plant-derived natural product binds to a biochemical receptor in mosquitoes that elicits repellent behaviors to facilitate the development of highly mosquito-specific repellents with minimal effects on humans.

- Investigated the effect of a potential novel biocontrol agent (the microsporidian parasite *Edhazardia aedis*) on the immune defense of *Ae. aegypti* mosquitoes.

- Performed research on the drug discovery for flavivirus infections, using in-silico screening approach, to screen compound libraries targeting the active site of viral RNA polymerase.

- Used genomic data to estimate population connectivity between locations and to inform various models aimed at forecasting mosquito and tick distribution and dispersal.

- Developed a next-generation sequencing platform for improved diagnostics for arthropods of medical importance to adapt existing technology for a platform for mosquito- and tick-borne diseases that can be used to identify up to 100,000 targets in a single reaction with a single sample.

- Built a reference database of *Ae. aegypti* mosquitoes worldwide based on microsatellite markers and genome-wide SNPs.

- Conducted research that sought to determine the role of epigenetics in the vectorial capacity of different tick populations, including the variation in methylation of *I. scapularis* collected in Minnesota (high-Lyme disease area) and Texas (low-Lyme disease area).

- Improved the characterization of the male-determining locus (M) in the dengue and Zika vector *Ae. aegypti* by determining the content of the ~160 Kilobase gap in the M-locus.

- Conducted entomological surveys coupled with knowledge attitudes and practices surveys to understand tick borne disease risk and perceptions of ticks and risk in park visitors on Staten Island as well as how ticks are monitored across public health and vector control districts in the USA.

- Developed an active surveillance citizen science project that seeks to understand the economic, environmental, and production factors that influence private forest landowners’ forest management decision-making processes and the implications for tick-borne disease exposure risk in Maine.

- Conducted a scoping review of Lyme disease provider-patient communication.

Objectives

1. 1. Develop and strengthen effective surveillance and monitoring of disease vectors (mosquitoes, ticks, etc.) and their associated pathogens at local and regional scales. Under this objective, project participants will leverage and strengthen existing surveillance programs in a coordinated fashion to yield robust comparable data across large geographic scales.
2. 2. Determine the ecology, physiology, genetics and/or geographic and temporal distributions of historical, extant, and emerging disease vectors and the pathogens they transmit. Under changing environmental conditions, these studies will enhance our ability to predict conditions leading to the spread or emergence of existing and novel diseases of public health importance.
3. 3. Discover, develop, and integrate interventions to manage vector-borne pathogen transmission and pesticide resistance.

Methods

Objective 1: Develop and strengthen effective surveillance and monitoring of disease vectors (mosquitoes, ticks, etc.) and their associated pathogens at local and regional scales.

(1.1) Predict and model the distribution and density of the vectors of public and veterinary health importance in relation to habitat type and climate conditions using surveillance data within a One Health framework.

To better understand the interactions between pathogens, vectors, and hosts, it is critical to estimate habitat suitability for a given species. Referred to as species distribution models, multiple quantitative tools identify how environmental variables are most influential in predicting species occurrence, and as such create valuable tools for identifying high risk regions for vector-borne diseases or for predicting future changes in vector or pathogen geographic distributions given projected climate change. This sub-objective will utilize active tick sampling data obtained by drag sampling to associate environmental and climatic features of the landscape with the density of questing ticks or the density of infected ticks. This One Health project will estimate habitat suitability for tick-borne pathogens of human and animal health concern. In addition, we will leverage long-term datasets generated from statewide mosquito surveillance programs to develop descriptive and predictive models on the presence and abundance of mosquito vectors and their associated pathogens. For example, Connecticut maintains a network of 108 fixed mosquito-trapping stations throughout the state providing information that includes mosquito species composition and abundance in the community, seasonal and spatial distribution of mosquito vectors, and prevalence of virus infection in mosquito populations. Data from these efforts will be used to generate information on the habitat suitability of regionally important mosquito vectors and to assess potential risk of arbovirus epidemics by these vectors. We will explore the associations with climatic conditions, socio-economic factors, and land use patterns driving the persistence and abundance of both invasive and native vector species and their associated pathogens. This program of research will provide accurate information on the current and future distribution of medically important mosquito species and the spatial risk of arboviruses which is essential for targeting public health resources effectively. Finally, we will update the North American habitat suitability models for the soft tick *Ornithodoros turicata* (cite) given the threat of African swine fever virus that is spreading in Africa and Asia that emerged in the Caribbean in 2021. We will use multiple species distribution modeling approaches including MaxEnt, generalized linear models, and machine learning. Each approach will be evaluated based on multiple performance metrics. This is a multi-state effort as the updated occurrence data requires reaching out to collections at many institutions across the US.

(1.2): Improve the efficiency of surveillance programs vis-a-vis vector trapping and diagnostics.

Early detection ensures that vector control efforts are directed to the right location at the right time to avert an epidemic. A challenge with proactive surveillance needed for diverse vector and vector-borne disease threats is the need to identify unknown agents of concern (Holcomb et al. 2023). A significant limitation of most existing arbovirus surveillance is the reliance on qPCR for detection, which employs species-specific PCR primers for pathogens chosen a priori by investigators (Luce-Fedrow et al. 2015). With a rapidly changing vector-borne disease landscape, it may not always be possible to predict which pathogens are present as new threats spill over from zoonotic cycles, are introduced to unexpected places, or undergo genetic change (Kilpatrick and Randolph 2012, Vora et al. 2023). The advent of next generation sequencing has facilitated the ability to detect diverse arthropod vectors and both known and unknown (or undescribed) pathogens of concern. For example, new pipelines have been established for high-throughput screening of many tick-borne pathogens using next generation sequencing targeted multiplex PCR amplicon sequencing (Kingry et al. 2020, Hojgaard et al. 2020)). Additionally, metagenomic sequencing of blood fed mosquitoes can reveal diverse human pathogens, a concept known as xenosurveillance (Batson et al. 2021). Finally, metabarcoding cannot only identify the vertebrates that vectors feed on (Fauver et al. 2017) but also identify the species composition of vectors in mixed pools (Mechei et al. 2021). This subobjective will advance surveillance pipelines capitalizing on high-throughput next generation (and further, single-molecule) sequencing to enumerate components of the vector holobiont – that is, the host and all associated microbial constituents that live on or in – identify pools of biting flies and ticks. using metagenomics. In addition, we will explore metagenomic sequencing pipelines for targeted and non-targeted screening of vectors for diverse agents of wildlife disease that include e.g., phlebotomine sand flies and midges of the genus *Culicoides*. Assessment of threats posed to both humans and animals by such vector arthropods properly frames our project in the context of a One Health approach – or the acknowledgement that human and animal health are inextricably linked in a tripartite manner to that of the environment in what is today a rapidly perturbed system. Finally, we will explore new surveillance techniques or modifications to existing traps that improve trap efficacy and improve operational vector control programs.

Objective 2: Determine the ecology, physiology, genetics and/or geographic and temporal distributions of historical, extant, and emerging disease vectors and the pathogens they transmit.

(2.1) Determine the phenology, habitat, and climatic requirements of historical, extant, and emerging vectors, hosts, and pathogens using historical and contemporary datasets.

Predicting when and where vectors, hosts, and vector-borne pathogens are present and interacting in the environment is critical for accurate and effective vector control and vector-borne disease prevention. Climatic and environmental variables that fluctuate across time and/or space can impact each of these factors. Changing temperatures as a result of global warming will result in mild winters and the expansion of vector populations into areas where they were not previously found. Additionally, alterations in land-use, including increased urbanization, will likely affect vector behavior and population dynamics, with downstream changes in disease risk. For this sub-objective, we will conduct field studies to characterize the current activity of vectors, hosts, and pathogens as well as the climatic and environmental predictors most closely associated with their presence and/or activity at a variety of spatial and temporal scales. We will also use historical datasets to gain a comprehensive understanding of how these systems have changed over time and develop predictive models describing how temperature and climatic changes may impact vector and vector-borne pathogen distributions in the near and distant future. Specific studies may examine how abiotic and biotic variables such as temperature, day length, artificial light, land use, vegetation, and stormwater infrastructure, influence the presence/absence, blood feeding behavior, egg-laying, and species composition of arthropod vectors. These studies may also determine how these variables interact with vertebrate host presence, population genetics, behavior, and infection rates.

(2.2) Define physiological, ecological, and behavioral drivers of transmission of vector-borne pathogens of public health importance.

Changing environmental conditions can alter physiological, ecological, and behavioral drivers of transmission of vector-borne pathogens of public health importance. Pathogens, vectors, and hosts are highly responsive to natural environmental variation, to human-induced environmental alterations, and to management interventions, yielding patterns that are often non-linear. Combining and iterating controlled laboratory studies and field studies that query this heterogeneity, therefore, is necessary to interpret and predict patterns of pathogen transmission by vector populations. In this sub-objective, we will complete laboratory and field studies to quantify effects of environmental factors (temperature, humidity, nutrient availability, changes in light:dark cycles), ecological contexts (symbiotic relationships, resource competition, habitat types), and interventions (drugs, pesticides, genetic strategies) on the physiology, behavior, and life histories of vector-host-pathogen interactions. In their studies of diverse vectors, pathogens and hosts, our members will measure vector lifespan, fecundity, circadian activity, host-seeking and feeding behaviors, vector competence, vector genotype x pathogen genotype interactions, and pathogen virulence in the context of variable environmental, ecological and intervention contexts. Further, we will investigate the effects of microbes (human pathogens and environmentally acquired microbiome) on life history traits of vectors that transmit pathogens to vertebrates. Using laboratory assays, field experiments, and field collections from previously established and expanding populations, our group will establish physiological, ecological, and behavioral factors that facilitate vector expansion, adaptation, and pathogen transmission.

(2.3) Evaluate the evolutionary relationships and genetic diversity of vectors and pathogens they transmit.

Vector-borne diseases represent a growing public health concern and are expanding in distribution within the US. The ability of vector-borne pathogens to widen their host range is dependent on genetic adaptations that allow them to infect new vector and host species, while their geographic expansion is linked to the capacity of their vectors to establish into new areas. Thus, pathogen evolution, spread, and transmission dynamics are tightly connected with the evolution and adaptation of their vectors. Likewise, pathogens can influence epigenetic mechanisms, such as DNA methylation, histone modifications, and alter small RNA expression, within vectors (De et al. 2021). These epigenetic modifications can in turn result in nucleotide changes, increasing mutation rates, or variation in gene expression (Ord et al. 2023). In this sub-objective, we will characterize and analyze the genetic diversity of vector and pathogen populations to gain a more complete understanding of their movement patterns, evolution, and overall population structure. In addition, we will explore the co-evolution of pathogenic microorganisms with their vectors, and the molecular determinants of vector-pathogen adaptations. To accomplish these aims, we will deploy Next-Generation Sequencing (NGS) pipelines to sequence pathogen genomes and develop high resolution markers (SNPs, rad-SEQ) to genotype more complex microorganisms or vectors and define epigenetic changes within vector populations (WBGS and Chip-Seq). Genetic data will be analyzed by phylogenetic approaches and population genetic methods in order to understand the origins, movement, population dynamics, and gene flow of emerging vectors and pathogens. Differences in epigenetic markers and small RNA expression will be used to identify areas in the genome and chromatin that correlate with vectorial differences in arthropod populations (e.g., northern versus southern *Ixodes scapularis* populations). This information will allow us to track patterns of pathogen and vector emergence, spread, and persistence and identify genetic and molecular changes that facilitate the transmission of vector-borne pathogens of public health importance. Newly developed CRISPR/Cas9 technologies allow for the methylation and demethylation of differentially methylated regions (DMRs) in humans. Although this technology is not available in vector studies yet, the identification of DMRs and genetic variants potentially influencing vectorial capacity (such as loci associated with host detection, vector immune responses, and others) can result in targets that can disrupted with these technologies to decrease vector-borne pathogen transmission. Likewise, the identification of genes or loci associated with bacterial or viral pathogenicity can lead to the discovery of targets to knock-out virulence for the development of vaccines, which has been explored with the tick-borne pathogen *F. tularensis* and *Anaplasma phagocytophilum*. These studies are expected to unlock translational insights for future vector-borne disease surveillance and control.

Objective 3: Discovery, development, and integration of interventions to manage vector-borne pathogen transmission and pesticide resistance.

(3.1) Screening of natural products for new chemical control tools with novel modes of action (insecticides/acaricides, repellents).

Insecticides presently used for mosquito and tick control are limited in their modes of action. The overuse of these control agents has led to the well-documented evolution of resistance in several mosquito and tick species of relevance to public health. Thus, a broader diversity of chemical control tools with novel modes of action is needed to mitigate resistance and limit transmission of mosquito and tick-borne pathogens. Natural products have been—and continue to be—an important resource and inspiration for developing biologically active chemicals with novel modes of action for controlling insect vectors and pests. First, we will exploit the diverse chemistry of plants and microbes to discover novel active ingredients and formulations for mosquito and tick control. Plant and microbe extracts will be screened for insecticidal, acaricidal, and repellent bioactivities against medically relevant mosquito (e.g., *Aedes aegypti*) and tick (e.g., *Ixodes scapularis*) species. Once extracts with potent insecticidal, acaricidal, and/or repellent activities are identified they will be fractionated using GC/MS and LC/MS approaches to identify active compounds. In addition, extracts and active compounds will be tested in conjunction with current control tools (e.g., pyrethroids) to identify potential novel natural product synergists. Thus, our proposed research is likely to lead to highly novel chemical tools for enhancing control of mosquito and tick vectors and their transmission of deadly human pathogens. Second, we will examine the degree of “avoidance proximity” to mosquitoes of plant derived repellents obtained from commercially harvestable plants in the Lamiaceae herb Family. Specifically, we will test GC/MS and LC/MS characterized oil and extracts from various mechanically harvestable cultivars of *Nepeta cataria* (catnip) with mixtures of mint, basil, and sage against *Ae. aegypti* and *Anopheles quadrimaculatus.* Additionally, we will use NGS (RNAseq) to investigate the mode of action of commercially available plant-derived acaricides.

(3.2) Discovery of novel control targets by investigating vector-microbe interactions and molecular/physiological mechanisms.

Reducing transmission of arthropod-borne pathogens can be achieved through limiting vector populations, disruption of acquisition of the pathogen by the arthropod, or disruption of transmission of the pathogen from arthropod to host. Each of these processes are governed by complex molecular, biochemical, physiological, or behavioral mechanisms that are often poorly understood. Accordingly, we aim to identify these mechanisms and their associated components to guide development of strategies for disrupting these processes in arthropod vectors.

First, blood feeding behaviors of arthropods typically occur within the tissues of the host, and so are difficult to impossible to directly observe. Electropenetrography (EPG) has been used for decades to study the probing behaviors of plant feeding insects (Backus et al., 2019). This technique can measure electrical signals associated with probing by passing a current through a wired insect attached to the EPG system through a host during probing. EPG can therefore be used to indirectly measure mouthpart movements, salivation events, and ingestion of fluids that occur when an arthropod vector takes a blood meal. We will use EPG to record electrical waveforms and correlate them with specific feeding behaviors. Subsequent experiments will then use molecular techniques (RNA interference, RNAi) and feeding disruption experiments to identify the specific activities associated with the different waveform types. This data will enhance our understanding of arthropod probing behaviors and allow conceptualization of new strategies for disrupting blood feeding and/or pathogen transmission by arthropod vectors.

Second, basic physiological and biochemical processes govern arthropod vector survival, feeding, and host seeking. A more thorough understanding of these mechanisms will identify novel targets for arthropod vector control. Accordingly, we will use RNAi and CRISPR technology in pharmacological and biochemical studies to explore intercellular communication, excretory physiology, sensory physiology, and seasonal physiology of tick and mosquito vectors. Intercellular communication in insects, particularly through extracellular vesicles (EVs), is poorly understood; however, recent evidence suggests that EVs play important roles in insect immunity, arthropod feeding, RNA interference, and pathogen transmission (Tessetto, et al. 2017, Atayde, et al. 2015, Vora, et al. 2018), and understanding the roles EVs play in arthropod physiology may inform development of new control approaches (Oliva Chavez et al. 2021, Butler et al. 2023). EVs can also be exploited to discover novel vaccine candidates for vaccine development. Similarly, excretory, sensory, and seasonal physiology are vital for arthropod vector survival. These processes rely on specific molecular components to function properly, and these molecular components can be identified using RNAi, CRISPR, and pharmacological techniques, and then the ramifications of limiting the expression or function of these components can be assessed in biochemical and physiological assays. For example, TRPA1 channels are involved in critical physiological processes in some arthropod species including sensing of noxious chemicals and excessive heat. Further characterization of the role of TRPA1 in arthropod vector physiology may show it to be an ideal target for limiting pathogen transmission or vector populations.

(3.3) Develop countermeasures against flavivirus infection and transmission.

Flaviviruses are important human and animal epidemic diseases pathogens, such as the well-known Dengue virus (DENV), West Nile virus (WNV), Zika virus (ZIKV), Japanese encephalitis virus (JEV) and Yellow fever virus (YFV)(Best, SM. 2016). All these five flaviviruses mentioned above also belong to mosquito-borne viruses which can cause dengue-like fever diseases. This objective is to develop small molecule-based drugs for treating Flavivirus infections by targeting viral RNA dependent RNA polymerase (RdRp) which is a key enzyme for viral RNA replication(Barrows *et al.* 2018). Especially, the novelty of this proposed research is to develop common drugs that can be effective for all or several different flaviviruses based on the finding of a conserved three aspartic acids, D535, D665 and D666 (DDD) motif in the active sites of viral RdRp, which has an electrostatic negative charged binding surface. Therefore, this conserved triple-D motif is an excellent common drug target against flaviviruses.

To accomplish this objective, we will use structure-based approaches and incorporate Artificial Intelligence (AI) to conduct drug design and development. The major methods will include as follows. (1). Building a consensus RdRp structure for virtual compound screening from all five protein sequences of RdRps. Then, conduct In-Silico compound screening (Murgueitio et al. 2012) against the Triple-D motif. (2). In vitro evaluation of the compounds that have activity to inhibit flavivirus infections in a cell-based system (such as Vera E6 cells) in a 96-well plate. Plaque reduction assay and Quantitative RT-PCR (qRT-PCR) will be used for identifying the potent compounds (IC50s) against flavivirus infections. (3). Structure-based design and modification of compounds to increase potencies. (4). Inhibition control in mosquitoes. Because the mosquitoes are the intermediate hosts, the compound inhibition activity in the mosquitoes will be evaluated using the larval-based approach. (5). Pre-clinical evaluation of compounds including In vivo activities, toxicities and pharmacokinetic (PK) studies in mice. (6). Artificial Intelligence (AI) will also be used for drug design and development.

(3.4) Examining the integration of new tools with conventional control, e.g., SIT and Bti.

Initial efforts will focus on surveying for current and potential control tool integrations. Subsequently, one or more of the identified integrations will be selected for additional characterization. We anticipate that initial efforts will be laboratory and field cage based work, and progressing toward field activities. Key goals include the identification of potential synergism and antagonism between the integrated tools, which can affect safety, efficacy, cost and public acceptability.

(3.5) Develop high throughput methods to detect insecticide resistance in *Culex* vectors of West Nile virus that can be combined with virus surveillance

Insecticide resistance (IR) in mosquitoes can arise from disorganized, misinformed, or otherwise inefficient applications of insecticides. Insecticide resistance management (IRM, Roiz et al. 2018) requires cohesive and productive collaborations among academia and publicly funded mosquito control programs tasked with control. Professional mosquito control programs across the NE US but specifically in NJ, have signaled clear interest in assessing the existence, extent, and effect on their operations of insecticide resistance (IR) in their local mosquito populations. Significant levels of organophosphate and pyrethroid resistance were detected in NJ populations of *Culex pipiens* and *Aedes albopictus* (Burtis et al. 2021). However, county superintendents are often unclear what to do with the results, since they often take weeks to months to become available and refer to a small subset of populations. Furthermore, insecticide resistance in some species of potential or realized public health importance is not being examined. For pyrethroid insecticides, currently the go-to class of insecticides for urban mosquito control, IR commonly arises from target site mutations that modify the voltage-gated sodium channels (VGSC) making them refractory to the insecticide. Resistant mosquitoes often "faint" before recovering, which is why IR is called knockdown resistance (kdr) in mosquitoes. Prior work has identified knockdown resistance (kdr) mutation (L1014F) in NJ *Cx. pipiens* including an homozygote excess indicating strong selection (Johnson and Fonseca 2016). We propose to develop assays to detect point mutations that result in IR to pyrethroids in *Cx. pipiens* and *Cx. restuans,* the latter a often-neglected enzootic vector of West Nile virus (WNV). Tests will be optimized so they can be combined with WNV analysis. High infection rates with WNV in disturbed wetland sites with high populations of *Cx. restuans* suggest *Cx. restuans* may enable the introduction of WNV to urban environments where both it and *Cx. pipiens* contribute to transmission, potentiating disease risk (Johnson et al. 2015, 2016). Unlike *Cx. pipiens,* which is primarily an urban mosquito, *Cx. restuans* is likely exposed to toxins and insecticides from agricultural practices. Difficulties in correctly differentiating *Cx. restuans* mosquitoes from *Cx. pipiens* have left the spatiotemporal mechanisms underlying the epidemiology of West Nile virus (WNV) in the northeastern United States largely unresolved.

Measurement of Progress and Results

Outputs

- Increased capacity to predict vector distribution and density to operationalize better risk assessment (Objs. 1, 2)
- Recommendations for improved vector surveillance practices by local, regional, state and national entities (Obj. 1)
- Parameter estimates connecting vector physiology, behavior, and ecology to the transmission of human and animal pathogens (Obj. 2)
- Epigenomes and sequence variations in vectors of public health importance (Obj. 2)
- EV derived antigens that can be used for vaccine development, qPCR assays to detect IR associated mutations in the voltage-gated sodium channels of Culex pipiens and Cx. restuans (Obj. 3)
- New, natural product-based chemical tools for controlling mosquitoes and ticks (Obj. 3)
- Discovery of novel biochemical targets for guiding new chemical and/or genetic control strategies (Obj. 3)
- Identification of small molecules that can inhibit flavivirus infections (Obj. 3)

Outcomes or Projected Impacts

- An interactive and dependent network of scientific expertise to deal with new mosquito-, tick-, and other vector-borne disease outbreaks.
- Develop, test, implement, and encourage novel control (management) interventions that reduce transmission of human and animal diseases using environmental sound and scientifically-based approaches.
- Identification of novel plant and microbial sources as sources of pesticides and synergists
- Identification of novel biochemical targets and physiological mechanisms in mosquitoes and ticks

Milestones

Outreach Plan

Several of the universities represented in this multistate group of researchers have formal extension programs while some do not. However, most researchers and all institutions interact closely with health officers, mosquito and tick professionals or the public at large. Many of us conduct a variety of outreach activities and create documents or web-based information.

We have identified four objectives to guide our outreach plan. First, a needs assessment will be conducted to identify key gaps in vector education, training, and evaluation for various audiences. This will involve surveying different stakeholder groups to assess their current knowledge, identify areas requiring more education, and determine preferred methods for receiving information. Focus groups and interviews will also gather qualitative data on specific needs. The findings will inform the development of tailored educational programming, training, and appropriate evaluation metrics. Second, outreach initiatives will promote awareness and understanding of vectors among students and the public. Strategies may include integrating vector biology and prevention into K-12 curriculum through engaging lesson plans, hosting public webinars on emerging vector-borne diseases, and establishing community science projects that empower community members to participate in vector surveillance. Third, specialized educational programming will be developed to build capacity among professionals on the frontlines of vector-borne disease. This includes in-person workshops, short courses, online modules, and field training for veterinarians, public health officials, and clinical health workers. The focus will be on advancing knowledge of vector biology and vector-borne diseases as well as integrated vector management strategies encompassing surveillance, prevention, and control methods. Pre- and post-assessments will evaluate changes in knowledge, attitudes, and competencies. Fourth, we will examine the usefulness of free and fee-for-service tick-testing programs as a conduit to educate the public on vector-borne pathogens, assess the usefulness of different outreach approaches, and increase the public’s familiarity with science and the scientific pursuit.

Additionally, we will hold an annual meeting to promote cooperation among participants, which can lead to enhanced research opportunities. Results of our meetings and activities will be available to all interested parties via the NIMSS website. All publications, both refereed and non-refereed, will be listed on NIMSS. Informational meetings will be held to brief appropriate state and county mosquito control personnel on our progress, particularly via oral presentations at national, regional, and local meetings (e.g., Entomological Society of America, Society for Vector Ecology, American Society for Tropical Medicine and Hygiene, American Mosquito Control Association), regional (Northeastern Mosquito Control Association) that are designed to bring together scientists and mosquito control practitioners.

Organization/Governance

This multistate research project will be established in accordance with the format suggested in the “Manual for Cooperative Regional Research”. One person at each participating institution or agency will be designated, with the approval of the institution’s or agency’s director, as the voting member of the Technical Committee. Other individuals and interested parties are encouraged to participate as non-voting members of the committee. There will be elections of a Chair and a Chair-elect. Officers are to be elected for overlapping three-year terms to provide continuity. Administrative guidance will be provided by an assigned Administrative Advisor and a CSREES Representative.

Note: This proposal has purposefully been written to define the project in broad research terms. This is intended to best position the Multistate Project to cast a broad net to secure an expanded membership, and therefore expanded opportunities for community building and collaboration. First, an expansion of membership will be sought geographically so that the Multistate Project can become a truly national project. Second, the intended expansion will be aimed at securing participants beyond the traditional agricultural experiment station and USDA-ARS mosquito biologists to involve mathematical modelers, community ecologists, and others with special skills regardless of whether they study mosquitoes and/or ticks.

Literature Cited

Atayde VD, Aslan H, Townsend S, Hassani K, Kamhawi S and Olivier M, Exosome secretion by the parasitic protozoan *Leishmania* within the sand fly midgut. *Cell Rep* 13: 957-967 (2015).

Backus, E.A., Cervantes, F.A., Guedes, R.N.C., Li, A.Y. and Wayadande, A.C. 2019. AC–DC electropenetrography for in-depth studies of feeding and oviposition behaviors. *Annals of the Entomological Society of America*. 112, 236–248. https://doi.org/10.1093/aesa/saz009.

Batson, J., Dudas, G., Haas-Stapleton, E., Kistler, A.L., Li, L.M., Logan, P., Ratnasiri, K., Retallack, H. 2021. Single mosquito metatranscriptomics identifies vectors, emerging pathogens and reservoirs in one assay. *Elife*. 27, 10:e68353.

Beard, C. B., Occi, J., Bonilla, D. L., Egizi, A. M., Fonseca, D. M., Mertins, J. W., … Halperin, W. (2018). Multistate infestation with the exotic disease-vector tick *Haemaphysalis longicornis* - United States, August 2017-September 2018. *MMWR*, 67(47), 1310–1313. https://doi.org/10.15585 /mmwr.mm6747a3

Bickerton, M., McSorley, K., Toledo, A., 2021. A life stage-targeted acaricide application approach for the control of *Haemaphysalis longicornis*. *Ticks Tick Borne Dis*. 12, 101581. https://doi.org/10.1016/j.ttbdis.2020.101581

Bickerton, M., Toledo, A., 2020. Multiple pruritic tick bites by Asian longhorned tick larvae (*Haemaphysalis longicornis*). *Int. J. Acarol.* 46, 373–376. https://doi.org/10.1080/01647954.2020.1805004

Burtis, J. C. *et al.* NEVBD Pesticide Resistance Monitoring Network: Establishing a Centralized Network to Increase Regional Capacity for Pesticide Resistance Detection and Monitoring. *Journal of Medical Entomology* 58, 787-797, doi:10.1093/jme/tjaa236 (2021).

Butler LR, Gonzalez J, Pedra JHF, Oliva Chavez AS. 2023. Tick extracellular vesicles in host skin immunity and pathogen transmission. *Trends Parasitol*. 39(10):873-885. doi: 10.1016/j.pt.2023.07.009.

Coatsworth H, Lippi CA, *et al*. 2022. A molecular surveillance-guided vector control response to concurrent dengue and West Nile virus outbreaks in a COVID-19 hotspot of Florida. *Lancet Reg Health Am.* 11:100231. doi: 10.1016/j.lana.2022.100231.

Curtin JM, Aronson NE. 2021. Leishmaniasis in the United States: Emerging Issues in a Region of Low Endemicity. *Microorganisms*. 9(3):578. doi: 10.3390/microorganisms9030578.

De S, Kitsou C, Sonenshine DE, *et al*. 2021. Epigenetic Regulation of Tick Biology and Vectorial Capacity. *Trends Genet*. 37(1):8-11. doi: 10.1016/j.tig.2020.09.012.

Diuk-Wasser, M.A., VanAcker, M.C., Fernandez, M.P. 2021. Impact of Land Use Changes and Habitat Fragmentation on the Eco-epidemiology of Tick-Borne Diseases. *J Med Entomol*. 58(4):1546-1564.

Eisen L, Eisen RJ. 2023. Changes in the geographic distribution of the blacklegged tick, *Ixodes scapularis*, in the United States. *Ticks Tick Borne Dis.* 14(6):102233. doi: 10.1016/j.ttbdis.2023.102233.

Fauver, J.R., Gendernalik, A., Weger-Lucarelli, J., Grubaugh, N.D., Brackney, D.E., Foy, B.D., Ebel, G.D. 2017. The Use of Xenosurveillance to Detect Human Bacteria, Parasites, and Viruses in Mosquito Bloodmeals. *Am J Trop Med Hyg.* 97(2):324-329.

González, J., Fonseca, D.M., Toledo, A., 2023. Seasonal dynamics of tick species in the ecotone of parks and recreational areas in Middlesex County (New Jersey, USA). *Insects* 14, 258. https://doi.org/10.3390/insects14030258

Holcomb KM, Khalil N, Cozens DW, Cantoni JL, Brackney DE, Linske MA, Williams SC, Molaei G, Eisen RJ. 2023. Comparison of acarological risk metrics derived from active and passive surveillance and their concordance with tick-borne disease incidence. *Ticks Tick Borne Dis*. 14(6):102243.

Johnson, B. J., Robson, M. G. & Fonseca, D. M. 2015. Unexpected spatiotemporal abundance of infected Culex restuans suggest a greater role as a West Nile virus vector for this native species. *Infect Genet Evol* 31, 40-47, doi:10.1016/j.meegid.2015.01.007.

Johnson , B. J. and Fonseca, D. M. 2016. Insecticide resistance alleles in weland and residential populations of the West Nile virus vector Culex pipiens in New Jersey. *Pest Manag Sci.* 72:481-488.

Kading, C. R., L. W. Cohnstaedt, K. Fall, G. L. Hamer. 2020. Emergence of arboviruses in the United States: The boom and bust of funding, innovation, and capacity. *Tropical Medicine and Infectious Disease*. 5:E96.

Li, A., Liu, L., Wu, W., Liu, Y., Huang, X., Li, C., Liu, D., Li, J., Wang, S., Li, D., Liang, M., 2021. Molecular evolution and genetic diversity analysis of SFTS virus based on next-generation sequencing. *Biosaf. Health* 3, 105–115. https://doi.org/10.1016/j.bsheal.2021.02.002

Liu, K., Zhou, H., Sun, R.-X., Yao, H.-W., Li, Y., Wang, L.-P., Mu, D., Li, X.-L., Yang, Y., Gray, G.C., Cui, N., Yin, W.-W., Fang, L.-Q., Yu, H.-J., Cao, W.-C., 2015. A National assessment of the epidemiology of severe fever with thrombocytopenia syndrome, China. *Sci. Rep.* 5, 9679. https://doi.org/10.1038/srep09679

Luce-Fedrow, A., Mullins, K., Kostik, A.P., St John, H.K., Jiang, J., Richards, A.L. 2015. Strategies for detecting rickettsiae and diagnosing rickettsial diseases. *Future Microbiol*. 10(4):537-64.

Luo, L.-M., Zhao, L., Wen, H.-L., Zhang, Z.-T., Liu, J.-W., Fang, L.-Z., Xue, Z.-F., Ma, D.-Q., Zhang, X.-S., Ding, S.-J., Lei, X.-Y., Yu, X., 2015. *Haemaphysalis longicornis* Ticks as reservoir and vector of severe fever with thrombocytopenia syndrome virus in China. *Emerg. Infect. Dis.* 21, 1770–1776. https://doi.org/10.3201/eid2110.150126

Mechai, S., Bilodeau, G., Lung, O., Roy, M., Steeves, R., Gagne, N., Baird, D., Lapen, D.R., Ludwig, A., Ogden, N.H. 2021. Mosquito Identification From Bulk Samples Using DNA Metabarcoding: a Protocol to Support Mosquito-Borne Disease Surveillance in Canada. *J Med Entomol.* 58(4):1686-1700.

Oliva Chávez AS, Wang X, Marnin L, Archer NK, Hammond HL, Carroll EEM, Shaw DK, Tully BG, Buskirk AD, Ford SL, Butler LR, Shahi P, Morozova K, Clement CC, Lawres L, Neal AJO, Mamoun CB, Mason KL, Hobbs BE, Scoles GA, Barry EM, Sonenshine DE, Pal U, Valenzuela JG, Sztein MB, Pasetti MF, Levin ML, Kotsyfakis M, Jay SM, Huntley JF, Miller LS, Santambrogio L, Pedra JHF. 2021. Tick extracellular vesicles enable arthropod feeding and promote distinct outcomes of bacterial infection. *Nat Commun.* 12(1):3696. doi: 10.1038/s41467-021-23900-8.

Ord J, Gossmann TI, Adrian-Kalchhauser I. 2023. High Nucleotide Diversity Accompanies Differential DNA Methylation in Naturally Diverging Populations. *Mol Biol Evol*. 4;40(4):msad068. doi: 10.1093/molbev/msad068.

Rainey T, Occi JL, Robbins RG, Egizi A. Discovery of *Haemaphysalis longicornis* (Ixodida: Ixodidae) parasitizing a sheep in New Jersey, United States. *J Med Entomol* 2018;55:757–9. https://doi.org/10.1093/jme/tjy006

Raney, W.R., Herslebs, E.J., Langohr, I.M., Stone, M.C., Hermance, M.E., 2022a. Horizontal and Vertical Transmission of Powassan Virus by the Invasive Asian Longhorned Tick, *Haemaphysalis longicornis*, Under Laboratory Conditions. *Front. Cell Infect. Microbiol.* 12. https://doi.org/10.3389/fcimb.2022.923914

Raney, W.R., Perry, J.B., Hermance, M.E., 2022b. Transovarial Transmission of Heartland Virus by Invasive Asian Longhorned Ticks under Laboratory Conditions. *Emerg. Infect. Dis.* 28, 726–729. https://doi.org/10.3201/eid2803.210973

Rochlin, I., Egizi, A., Narvaez, Z., Bonilla, D.L., Gallagher, M., Williams, G.M., Rainey, T., Price, D.C., Fonseca, D.M., 2023. Microhabitat modeling of the invasive Asian longhorned tick (*Haemaphysalis longicornis)* in New Jersey, USA. *Ticks Tick Borne Dis.* 14, 102126. https://doi.org/10.1016/j.ttbdis.2023.102126

Roiz, D. *et al.* Integrated *Aedes* management for the control of *Aedes*-borne diseases. *PLoS neglected tropical diseases* 12, e0006845, doi:10.1371/journal.pntd.0006845 (2018).

Schappach, B.L., Krell, R.K., Hornbostel, V.L., Connally, N.P., 2020. Exotic *Haemaphysalis longicornis* (Acari: Ixodidae) in the United States: Biology, Ecology, and Strategies for Management. *J. Integr. Pest Manag.* 11. https://doi.org/10.1093/jipm/pmaa019

Staples, J.E., Shankar, M.B., Sejvar, J.J., Meltzer, M.I., Fischer, M. 2014. Initial and long-term costs of patients hospitalized with West Nile virus disease. *Am J Trop Med Hyg*. 90(3):402-9. doi: 10.4269/ajtmh.13-0206.

Tassetto M, Kunitomi M and Andino R, Circulating immune cells mediate a systemic RNAi-based adaptive antiviral response in *Drosophila*. *Cell* 169: 314-325 e313 (2017).

Tufts, D.M., Goodman, L.B., Benedict, M.C., Davis, A.D., VanAcker, M.C., Diuk-Wasser, M. 2021. Association of the invasive *Haemaphysalis longicornis* tick with vertebrate hosts, other native tick vectors, and tick-borne pathogens in New York City, USA. *Int J Parasitol*. 51(2-3):149-157.

Tufts, D.M., Sameroff, S., Tagliafierro, T., Jain, K., Oleynik, A., VanAcker, M.C., Diuk-Wasser, M.A., Lipkin, W.I., Tokarz, R. 2020. A metagenomic examination of the pathobiome of the invasive tick species, *Haemaphysalis longicornis*, collected from a New York City borough, USA. *Ticks Tick Borne Dis*. 11(6):101516.

Vora A, Zhou W, Londono-Renteria B, Woodson M, Sherman MB, Colpitts TM, Neelakanta G and Sultana H, Arthropod EVs mediate dengue virus transmission through interaction with a tetraspanin domain containing glycoprotein Tsp29Fb. *Proc Natl Acad Sci U S A* 115: E6604-E6613 (2018).

Wormser, G.P., McKenna, D., Piedmonte, N., Vinci, V., Egizi, A.M., Backenson, B., Falco, R.C., 2020. First Recognized Human Bite in the United States by the Asian Longhorned Tick, *Haemaphysalis longicornis*. *Clinical Infectious Diseases* 70, 314–316. https://doi.org/10.1093/cid/ciz449

Land Grant Participating States/Institutions
KY,MD,ID,PA,FL,DE,ME,CT,NJ,MN,TX,KS,OH,NE

Non Land Grant Participating States/Institutions
Indiana Univ of PA, USDA-ARS/Washington

Participation

Participant	Is Head	Station	Objective	Research						Extension	
				KA	SOI	FOS	SY	PY	TY	FTE	KA

Combined Participation

Combination of KA, SOI and FOS	Total SY	Total PY	Total TY
Grand Total:	**3.80**	**1.20**	**0.80**
211	0.1	0.1	0.1
721	0.2	0	0
722	0.2	0	0
0	0.03	0	0
721	0.03	0	0
722	0.03	0	0

721	0.01	0	0.1
721	0.01	0	0.1
721	0.01	0	0.1
722	0.01	0	0.1
722	0.01	0	0.1
722	0.01	0	0.1
903	0.01	0	0.1
721	0.27	1	0.5
721	0.27	1	0.5
721	0.27	1	0.5
311	0.1	0	0
721	0.05	0	0
722	0.05	0	0
721	0.1	0	0
721	0.5	0	0
722	0.1	0	0
721	0.1	0	0
721	0.1	0	0
721	0.25	0	0
722	0.05	0	0
722	0.05	0	0
721	0.03	0	0
721	0.03	0	0
722	0.03	0	0
311	0.05	0	0
312	0.05	0	0
312	0.1	0	0
311	0.05	0	0
312	0.05	0	0
722	0.1	0	0
721	0.1	0	0
721	0.1	0	0
311	0.15	0.1	0.1

Program/KA	Total FTE
Grand FTE Total:	**0.52**
721	0.03
721	0.03
722	0.03
721	0.07
722	0.01
722	0
721	0.03

Reviewer 1:

The objectives and reasons for research plan are laid out in a plausible manner. Some sections (e.g., flavivirus drug development, vector-microbe interactions) seem somewhat exploratory, but perhaps that is the point of this type of project (unclear to this reviewer). The challenge will be in applying some of these findings to real-world (user-friendly) tools that are affordable to vector control programs. The research plan states that the proposed work focuses on "mosquitoes, sandflies, kissing bugs, ticks, and other vectors of human pathogens."...but kissing bugs were not mentioned in the resarch plan...seemed focused primarily on mosquitoes (some tick work). With the descriptive context that "This proposal has purposefully been written to define the project in broad research terms." the proposal seems appropriate and could be successful if adequate collaboration between academic, state/federal, and professional vector control programs is achieved. Multi-state collaboration between different types of "experts" to benefit public health is a plus if the leadership team has a high level of organization (reviewer cannot see composition of team, but understand this is for the northeast region of the United States).

We thank the reviewer for their feedback on the proposal. The reviewer is correct that the project aims intentionally were written broadly to encompass the scope of work of the project members' labs and to ease new members' participation in the project. Part of our proposed outreach efforts involve engaging with vector control programs to facilitate the uptake of our applied research findings. In the revised proposal, we clarified the management structure and the species addressed as part of the research plan.

Reviewer 2:

Overall, an established team with a track record of meaningful work/productivity. The framework for collaboration is well defined and they have procedures for shared ownership and responsibility. The scientific goals/objectives are important and this team is well suited to achieve them. I would have liked to have seen clear milestones. However, the objectives are clear and valuable (needed). This project should continue and in many respects they provide a role model for other multistate collaborations related to vector-borne diseases. The team may consider leveraging/coordination with the CDC's Centers of Excellence that reach into their states/region.

We thank the reviewer for their consideration of the proposal. We agree that coordination with the CDC's Centers of Excellence will improve the reach of the project, and we included language to this effect in the revised proposal.

Reviewer 3:

The rationale for a multi-state project is sound and justifiable. Each sub-objective has strengths and weaknesses.

We thank the reviewer for their detailed feedback, and have responded to comments on individual aims below.

(1.1) Predict and model the distribution and density of the vectors of public and veterinary health importance
Strengths – A robust network of sampling sites and diverse vectors will be studied.

Weaknesses – Details of the prediction / modelling methods are lacking. Are these ecological niche models? Details of trapping are not provided. If these are light traps then they will be biased toward certain species.

We omitted some details of the modeling and trapping efforts because multiple labs are engaged in this research aim, which may have slightly different protocols depending on the species of interest. We agree with the reviewer that different forms of trapping and surveillance have their own sampling biases and we will consider this in carrying out the proposed research.

(1.2) Improve the efficiency of surveillance programs vis-a-vis vector trapping and diagnostics.
Strengths – This objective applies modern techniques for surveillance.
Weaknesses - next generation sequencing will likely be prohibitively expensive for pathogen surveillance. The results will yield every potential pathogen, which may cause undue panic if used as an actual public alert surveillance system.

We agree that the next generation sequencing is expensive for pathogen surveillance, although the cost of the technology is declining and we feel this is an important tool that can be used by researchers. As noted by the reviewer, it is important to consider how the findings are communicated to the public to prevent unnecessary anxiety about pathogen exposure risk. This is encompassed in our proposed outreach plan.

(2.1) Determine the phenology, habitat, and climatic requirements of historical, extant, and emerging vectors, hosts, and pathogens using historical and contemporary datasets.
Strengths – An ambitious objective to understand how the pathosystems currently function and how key interactions may be changing.
Weaknesses – No details are provided on how the hosts or vectors will be quantified. Given that diverse wildlife (birds and mammals) are likely important as hosts for diverse pathogens (Flaviviruses, Alphaviruses, Orthobunyaviruses) the potential targets are very broad. Details of the modelling are not provided.

Again, we omitted some details of the modeling efforts and the quantification of hosts and vectors because multiple labs are engaged in this research aim, which may have slightly different approaches depending on the species of interest.

(2.2) Define physiological, ecological, and behavioral drivers of transmission of vector-borne pathogens of public health importance.
Strengths – An ambitious objective to manipulate key environmental factors and measure response in vector-borne disease ecology.
Weaknesses – A very large number of factors are proposed that will have confounding impacts on the biological traits listed. Investigating impacts of any one of these would be challenging to manage adequately. High risk.

This aim intentionally was written broadly to encompass the interests of the multiple labs involved in the proposed project. Each of these factors will be studied individually across different labs, using observational and experimental approaches to disentangle the impacts of the environmental factors on biological traits.

(2.3) Evaluate the evolutionary relationships and genetic diversity of vectors and pathogens they transmit.

Strengths – An interesting empirical assessment of genetic drivers of virulence.
Weaknesses – The application of this work is unclear. Apart from genetic modification of pathogens (which will not be permitted for release) how will this work impact vector-borne disease management?

We agree with the reviewer that pathogens with genetic modifications that enhance pathogenicity will not be permitted for release. However, the identification of genes or loci potentially associated with pathogenicity can lead to the identification of targets for attenuation of these pathogens. Likewise, the identification of differentially methylated regions (DMRs) that are potentially associated with vector capacity can be targeted with newly developed CRISPR/Cas-9DM to define their role in pathogen transmission and for the creation of vector populations with reduced vectorial capacity. We have revised the aim to add this information.

(3.1) Screening of natural products for new chemical control tools with novel modes of action (insecticides/acaricides, repellents).
Strengths – Potentially achievable goals of identifying new active compounds for control of medically important arthropods.
Weaknesses – None identified.

Thank you for these comments.

(3.2) Discovery of novel control targets by investigating vector-microbe interactions and molecular/physiological mechanisms.
Strengths – May provide novel insights in vector physiology.
Weaknesses - it is unclear how the “molecular, biochemical, and physiological techniques” will explicitly lead to a better understanding of the processes associated with pathogen transmission and vector survival, as stated. The investigators have not demonstrated how measuring electrical signals during probing (when an arthropod vector takes a blood meal), even if correlated with specific feeding behaviors, are helpful toward reducing or blocking transmission.

We revised this paragraph in the proposal to clarify the point raised by the reviewer.

(3.3) Develop countermeasures against flavivirus infection and transmission.
Strengths - Drugs that can be effective against flaviviruses are needed. The comprehensive approach, terminating in preclinical studies should determine whether the compounds have anti-flaviviral activity.
Weaknesses – May or may not lead to effective treatment.

Yes, it is a challenging task, but is essential for developing some means to combat these viral epidemics. This proposal has selected a very conserved target in the flavivirus species. Using cutting-edge technologies such as Structure-based and combined with AI based design, it will greatly increase the chances of success. We are confident that we can achieve our goals.

(3.4) Develop high throughput methods to detect insecticide resistance in Culex vectors of West Nile virus that can be combined with virus surveillance.
Strengths – Integrating insecticide resistance monitoring into ongoing vector and pathogen surveillance is a worthy goal.

Weaknesses – The methods provided are more about differentiating tow species of Culex than about high throughput assays for determining insecticide resistance.

We believe the reviewer’s comments correspond to objective 3.5 rather than objective 3.4.

Appendix G: Peer Review (Submitted)

Status: Complete

Project ID/Title: NE_TEMP2443: Biology, Ecology & Management of Emerging Disease Vectors

Rate the technical merit of the project:

1. Sound Scientific approach:
Approve/continue project
2. Achievable goals/objectives:
Good
3. Appropriate scope of activity to accomplish objectives:
Good
4. Potential for significant outputs(products) and outcomes and/or impacts:
Good
5. Overall technical merit:
Good
Comments
The objectives and reasons for research plan are laid out in a plausible manner. Some sections (e.g., flavivirus drug development, vector-microbe interactions) seem somewhat exploratory, but perhaps that is the point of this type of project (unclear to this reviewer). The challenge will be in applying some of these findings to real-world (user-friendly) tools that are affordable to vector control programs. The research plan states that the proposed work focuses on "mosquitoes, sandflies, kissing bugs, ticks, and other vectors of human pathogens."...but kissing bugs were not mentioned in the resarch plan...seemed focused primarily on mosquitoes (some tick work). With the descriptive context that "This proposal has purposefully been written to define the project in broad research terms." the proposal seems appropriate and could be successful if adequate collaboration between academic, state/federal, and professional vector control programs is achieved. Multi-state collaboration between different types of "experts" to benefit public health is a plus if the leadership team has a high level of organization (reviewer cannot see composition of team, but understand this is for the northeast region of the United States).
Your Recommendation:
Approve/continue project

Appendix G: Peer Review (Submitted)

Status: Complete

Project ID/Title: NE_TEMP2443: Biology, Ecology & Management of Emerging Disease Vectors

Rate the technical merit of the project:

1. Sound Scientific approach:
Approve/continue project
2. Achievable goals/objectives:
Good
3. Appropriate scope of activity to accomplish objectives:
Good
4. Potential for significant outputs(products) and outcomes and/or impacts:
Excellent
5. Overall technical merit:
Excellent
Comments
Overall, an established team with a track record of meaningful work/productivity. The framework for collaboration is well defined and they have procedures for shared ownership and responsibility. The scientific goals/objectives are important and this team is well suited to achieve them. I would have liked to have seen clear milestones. However, the objectives are clear and valuable (needed). This project should continue and in many respects they provide a role model for other multi-state collaborations related to vector-borne diseases. The team may consider leveraging/coordination with the CDC's Centers of Excellence that reach into their states/region.
Your Recommendation:
Approve/continue project

Appendix G: Peer Review (Submitted)

Status: Complete

Project ID/Title: NE_TEMP2443: Biology, Ecology & Management of Emerging Disease Vectors

Rate the technical merit of the project:

1. Sound Scientific approach:
Approve/continue project with revision
2. Achievable goals/objectives:
Fair
3. Appropriate scope of activity to accomplish objectives:
Good
4. Potential for significant outputs(products) and outcomes and/or impacts:
Good
5. Overall technical merit:
Good
Comments

The rationale for a multi-state project is sound and justifiable. Each sub-objective has strengths and weaknesses.
(1.1) Predict and model the distribution and density of the vectors of public and veterinary health importance
Strengths – A robust network of sampling sites and diverse vectors will be studied.
Weaknesses – Details of the prediction / modelling methods are lacking. Are these ecological niche models? Details of trapping are not provided. If these are light traps then they will be biased toward certain species.

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Strengths – This objective applies modern techniques for surveillance.
Weaknesses - next generation sequencing will likely be prohibitively expensive for pathogen surveillance. The results will yield every potential pathogen, which may cause undue panic if used as an actual public alert surveillance system.

(2.1) Determine the phenology, habitat, and climatic requirements of historical, extant, and emerging vectors, hosts, and pathogens using historical and contemporary datasets.
Strengths – An ambitious objective to understand how the pathosystems currently function and how key interactions may be changing.
Weaknesses – No details are provided on how the hosts or vectors will be quantified. Given that diverse wildlife (birds and mammals) are likely important as hosts for diverse pathogens (Flaviviruses, Alphaviruses, Orthobunyaviruses) the potential targets are very broad. Details of the modelling are not provided.

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Strengths – Integrating insecticide resistance monitoring into ongoing vector and pathogen surveillance is a worthy goal.
Weaknesses – The methods provided are more about differentiating tow species of Culex than about high throughput assays for determining insecticide resistance.

Outreach Plan
Adequate

Organization/Governance
Adequate
Your Recommendation:
Approve/continue project with revision

NE_TEMP2442: Improving Sustainable Poultry Production through Collaborative Research and Outreach

Status: Submitted As Final

Duration	10/01/2024 to 09/30/2029
Admin Advisors:	[Kumar Venkitanarayanan]
NIFA Reps:	

Non-Technical Summary

In response to the rising global population, there is an escalating demand for sustainably, efficiently, and safely produced protein, particularly in poultry. The adoption of Precision Livestock Farming (PLF) techniques in poultry production and processing systems; is identified as a strategic measure to meet this growing demand. Evolving consumer and retail preferences have led to diverse production and feeding systems, each presenting unique challenges and knowledge requirements. The collaborative research proposed here aims to further expand PLF concepts, incorporating automated continuous monitoring of animals to enable real-time recording and assessment of their health and welfare. This inclusive approach encompasses automation, robotics, equipment efficiency, facility design, as well as energy and resource allocation. Additionally, enhancements in antimicrobial intervention technologies and processing methods are, envisioned to reduce the risk of foodborne pathogens in poultry and poultry products. The target audience are those companies and personnel involved in the production of commercial poultry and eggs. In addition, poultry extension personnel, poultry researchers, allied poultry industry companies and consumers will benefit from research finding from our group. The data and technical information produced from this poultry research project will increase the knowledge of poultry and egg production in the pre-harvest and post-harvest phases.

Statement of Issues and Justification

As the demand for sustainable and efficient poultry production continues to grow worldwide, the role of advanced research and innovation in the industry becomes increasingly significant. The United States Poultry and Egg Association, recognized as the worlds' largest and most dynamic organization in the poultry industry, has acknowledged the imperative of integrating engineering and technology to optimize energy and resource efficiencies across layers, broilers, and turkeys. As a non-profit entity, it aligns its strategic goals with the evolving needs of the poultry industry, emphasizing advancements in poultry science and technology, and the assurance of safety in processed poultry and poultry products. The organization's ongoing and past research initiatives closely align with these objectives, fostering collaboration in critical areas such as nutrition, environmental control, air quality, housing systems, lighting, automation, robotics, food safety, security, health, and bird welfare.

Furthermore, the United Egg Producers (UEP), representing the laying hen industry, recognizes the necessity for enhanced technology to improve management practices and the well-being of laying hens in alternative housing systems, particularly cage-free aviaries. Additionally, industry entities like the Egg Industry Center actively support collaborative research and outreach endeavors aimed at addressing challenges faced by the poultry and egg industry.

In response to the rising global population, there is an escalating demand for sustainably, efficiently, and safely produced protein, particularly in poultry. The adoption of Precision Livestock Farming (PLF) techniques in poultry production and processing systems; is identified as a strategic measure to meet this growing demand. Evolving consumer and retail preferences have led to diverse production and feeding systems, each presenting unique challenges and knowledge requirements. The collaborative research proposed here aims to further expand PLF concepts, incorporating automated continuous monitoring of animals to enable real-time recording and assessment of their health and welfare. This inclusive approach encompasses automation, robotics, equipment efficiency, facility design, as well as energy and resource allocation. Additionally, enhancements in antimicrobial intervention technologies and processing methods are, envisioned to reduce the risk of foodborne pathogens in poultry and poultry products.

Optimizing poultry production systems for energy/resource efficiency, minimizing carbon footprint, and ensuring sustainability across the production chain (breeder, hatchery, producer, processor, and consumer) is paramount. Collaborative research serves as the foundation that connects different components of the system and their intricate relationships. The failure to renew this project would jeopardize the poultry industry's standing in the global marketplace, hindering its ability to deliver safe and nutritious poultry products to consumers worldwide.

The multi-state poultry research team, composed of diverse experts including environmental physiologists, behaviorists, animal welfare scientists, nutritionists, engineers, extension scientists, microbiologists, and economists, operates with access to commercial-type, pilot-scale, and laboratory-scale facilities. Their collective expertise and collaborative efforts have yielded documented success in the past. Moreover, the team includes several leading industry experts actively engaged in the collaborative process, providing invaluable insights and establishing crucial links between researchers and commercial operations, birds, and equipment. This dynamic collaboration enhances the relevance and feasibility of research endeavors, ensuring their practical applicability in the field.

Objectives:

Collaborators at the experimental stations in AL, AR, CA,CT, DE, GA, USDA-ARS (GA), HI, IL, IA, IN, KY, MD, MI, MN, MS, NE, NC, PA, SC, TN, TX, and VA will work on research related to the following objectives:

1.) Advancing Sustainable Poultry Systems through Precision Management:

a. ***Pre-harvest:*** This section will cover environmental control and management, housing, litter management, ventilation, lighting, pre-harvest food safety, nutrition, feed processing, behavior, and welfare.

b. ***Post-harvest:*** Post-harvest considerations will encompass food safety, processing methods, waste-water-management, offal, and rendering.

c. ***Environmental footprint:*** This section will explore avenues related to carbon footprint, nutrient utilization, production systems, and life cycle analysis.

2.) Fostering Innovative Production Practices through Research and Extension:

1. ***Pre-harvest:*** This section will cover incubation and hatchery, reproductive physiology, nutrient excretion reduction, precision nutrition, alternative feed ingredients, NAE/ABF practices, alternative feeding strategies, gut health, gut microbiome, poultry health and disease management, alternative sustainable production systems, production systems under regulatory exemption, economic analysis, and bird welfare. Various research initiatives are underway.

2. ***Post-harvest:*** This section will cover cutting-edge processing methodologies, production systems under regulatory exemption, and the quality of meat and eggs.

3. ***Outreach and training:*** This section will cover various aspects of outreach and training that several of the universities will and are pursuing.

4. ***Poultry economics:*** This section will cover the aspect of how poultry economics plays a role in conducting the studies to answer some of the questions outlined in the objectives.

We have state of the art commercial poultry production facilities, which will allow us, to conduct research (AL, IA, IN, IL, MN, MS, NC, PA, and VA). Facilities with fully functioning processing plants for converting birds to food (GA and AL) are available for use. The specialized equipment located at these stations can enhance collaborative research efforts and could bring synergistic outcomes. Therefore, the collaborative use of these facilities by participating universities and USDA stations will maximize research productivity.

Due to the complexity of the poultry industry needs, research objectives addressed by this multi-state project could not, be accomplished at any single station/university. Each of the researchers has expertise and facilities to address some component(s) of poultry production efficiency, well-being, nutrition, environmental concerns, facility management, and/or technical monitoring of poultry environments and poultry processing. To best, comprehensively address the challenges facing the poultry industry identified in this project, a collaborative effort is necessary and will eliminate duplication of effort and conserve resources.

Data generated from these collaborative research efforts will enhance the resiliency of the poultry industry through real-time monitoring of facilities, leading to improved poultry welfare, performance, food safety and security throughout poultry production.

The successful completion of the objectives outlined in this proposal will lead to:

1. Incorporation of advanced science, engineering and technology into poultry production facilities to enhance system efficiency, and improve production efficiency and poultry well-being.

2. Identification of relationships between environmental, nutritional, and disease factors that affect poultry well-being, food safety, and economics.

3. Establishment and adoption of current and future poultry husbandry practices and development of a trained scientific workforce to address a changing industry landscape.

4. Development of new antimicrobial intervention technologies or processing methods to control foodborne pathogens in poultry and poultry products.

Related, Current and Previous Work

A CRIS search on poultry environment or production systems did not identify any active multi-state projects, other than the existing NE1942 state projects, other than the existing NE1942.

Contributions NE1942 investigators.

NE1942 was, founded in 1978 to facilitate research in the area of poultry production recognizing collaboration and communication amongst the research institutions would advance poultry management strategies. NE1942 has grown from 11 universities in 1999 to 18 universities and 2 USDA-ARS units for the 2019 renewal. The multistate group has expanded to 22 universities and 1 USDA-ARS unit, for this 2024 rewrite. An annual, meeting is, held each year to discuss research results and plans collaborative projects for the coming year. The NE1942 philosophy is to help implement sustainable practices in poultry production for the industry from a multi-state collaborative approach. Recognizing that more advances can be, made as a collaborative group rather than working as a single investigator-initiated grant. The complexities of animal-environment interactions overlaid with animal welfare and consumer demands require a multi-discipline, collaborative approach to identifying ways to best achieve the producer and societal goals.

Examples of sustained endeavors by NE1942 participants include:

1. History of publication in the scientific literature. Over the last 4 project years of this 5-year project, this regional poultry research group has published 284 peer-reviewed journal articles, 380 abstracts, 45 popular-press articles, 35 peer-reviewed extension reports, and 80 proceedings at national and international meetings.

2. Success with joint proposals demonstrates the capacity of NE1442 to coordinate ideas, resources, and execute multi-state research projects.

3. The multistate research group has secured over $20 million in grants to conduct research with the NE1442 project during the last project cycle (5-year) period.

Collaborative research previously conducted and currently being, worked on by these research stations is, listed, below.

Collaborative research between stations in CA and TN is underway to develop and validate systems tracking hens' resource utilization in cage-free housing systems. Noteworthy multistate collaborators include Dr. Richard Blatchford from UC Davis and Dr. Yang Zhao from the University of Tennessee.

Collaborative research between the GA and NC stations involve the study of hen-housing interactions on welfare parameters. UGA and NC researchers are also collaborating to understand the gut-brain-behavior axis in chickens. Multistate members include Dr. Prafulla Regmi from UGA, Dr. Kenneth Anderson, and Dr. Allison Pullin from NC.

Researchers at the University of Minnesota (UM) will persistently investigate sustainable food safety solutions applicable to poultry production systems. Collaborations with industry and other university partners will inform multiple research projects. Ongoing efforts include investigating targeted microbial interference strategies against pathogens relevant to the turkey industry, with a focus on producing gut friendly and environmentally friendly solutions, to strengthen a sustainable poultry food supply.

The CA station collaborates on the investigation of various causes of woody breast and its measurement methods in broilers. The CA and TN stations are jointly exploring housing and genetic factors contributing to keel bone fractures in laying hens, with collaboration from multistate members Dr. Richard Blatchford (UC Davis) and Dr. Yang Zhao (U. Tennessee).

Current Work: Literature Review

According to the United Nations, the global population will reach 9.8 billion in 2050; this rapid growth necessitates a dramatic increase in the world's food supply, requiring 60 to 110% more agricultural products produced. However, the yield projected based on the current increasing rates are insufficient to meet the 2050 demand. Therefore, farmers worldwide will have to boost agricultural production, either by increasing the amount of agricultural land/facilities or by enhancing productivity with existing resources. The objectives of this multistate project aim to help in achieving this growth in agricultural production.

OBJECTIVE 1

1. **Advancing Sustainable Poultry Systems through Precision Management.**

1a. Pre-harvest:

Engineering and Technology. Poultry producers will inevitably rely on advanced tools and technologies to handle challenges in the process of production expansion. Compared to other industrial sectors, agriculture has been a sector tardy in applying high-tech engineering systems. In the early '80s, scientists promoted the concept of 'precision agriculture,' which aims to optimize agricultural returns on inputs and preserve resources by observing, measuring and responding to inter and intra-field variabilities with advanced technologies. Since then, precision agriculture has been exhibiting significant impacts to sustainable agricultural production. While most efforts in precision agriculture have been, dedicated to crop production, its application and research on livestock and poultry are scarce. Real-time monitoring of metrics (i.e., ventilation, air quality, lighting, acoustics, microbial, and others) within the production system needs to be at both a flock and individual bird basis; this allows for welfare elements and distinctions to be, balanced for the flock and individuals, which is a critical component of precision agriculture.

Technology, Monitoring Systems, and Environmental Control Management. Currently, poultry producers have a limited ability to identify and address problems within in a production system rapidly. This is because more real-time monitoring systems have been, directed at larger animals, such as swine or dairy. The discrepancy in innovation efforts directed at poultry likely reflects monitoring difficulties caused by the small size of the birds, and related to tracking similarly looking individuals within very large flocks (up to 100,000 plus per poultry house). While there has been some development of sensor technology to track hen movement, the existing methods are not without problems. Many rely on video to track patterns of the flock (therefore, do not provide information at the individual level) or electronic technologies that are incompatible with poultry housing systems (e.g., other birds and objects within the environment interfere with signals). The development of hen-mounted acoustic sensors and acoustic monitoring systems offers a novel approach with the potential for mitigating these problems. For instance, sensors can be placed under the feathers, reducing damage from pecking, and allow continuous recording of hen location compared to sensors that require readers in fixed locations. These devices can be, quite small, easily automated, and, do not need external readers, compared to devices used now.

Poultry Welfare and Housing Environment. A need for objective determination of poultry welfare is of utmost importance as guidelines transition from resource-based allocations to outcome-based inspections of the birds in their housing environment (Hester, 2005). Many factors in the housing environment have been, shown to effect poultry welfare (Kang et al., 2023; Jacobs et al., 2023). Currently, outcome-based welfare assessments of poultry (Abdelfattah et al., 2020; Johnsonon et al., 2019), are performed by subjective evaluation of trained individuals. This can still lead to different interpretations of the numerous indicators of welfare, such as, overall plumage score, cleanliness, keel deformation, comb pecking, footpad dermatitis, claw length, skin lesions, beak trimming, and toe damage. Furthermore, the scoring of these indicators is discrete without any discretion for intermediate scores. Advances in technology have led to machine vision systems capable of advanced pattern recognition needed to identify and classify different indicators of bird welfare accurately. The use of technology to aid in welfare assessments could substantially decrease observer-observer differences, and allow, for a rapid and widespread application of welfare assessments to advance the industry at the rate needed to match demand. This is especially relevant in the layer industry, where legislation in states (California) has mandated for certain alternative housing standards. Due to consumer demand and this legislation, producers have had to change to these alternative hen-housing systems, without knowing the impact on bird health and welfare.

Environmental Control Management. As novel technologies, new housing, and system designs emerge, there is a need for evaluation and optimization of these systems to establish best management practices for producers. Bist et al. (2023) published a review of the effects of ammonia emissions, impacts, and mitigation strategies for poultry. Particular interest is, focused on perches, lighting, system layout, and ventilation (including equipment, thermal and air quality) depending on poultry production system (Zheng et al., 2020; Liang et al., 2022). Considerable research has been, conducted on ventilation requirements of broiler houses as well (Li et al., 2023b). The provision of perches in hen housing systems could still lead to many detrimental effects (e.g., keel bone deformities, foot disorders, and bone fractures) that would negatively, impact production and welfare of the birds (Ali et al., 2019). Considerable efforts are still needed towards optimizing perch design (e.g., shape, size, texture, material, and temperature), spatial arrangement (e.g., height, angle, and relative position), and management (e.g., timing of bird’s introduction to perches). Furthermore, lighting is of critical importance and a crucial environmental factor that affects behavior, development, production performance, health, and well-being of poultry (Lewis and Morris, 2000; Parvin et al., 2014). The effects of blue and red light can influence egg production in laying hens (Poudel et al., 2022). Recently, LED lights have become more readily available and affordable to poultry producers; these lights, are promoted as being more energy-efficient, readily dimmable, and long lasting. However, the existing lighting guidelines/recommendations were mainly, established based on the traditional incandescent or CFL lights and may not accurately reflect the operational characteristics and impact of the LED lights on birds.

Aviary and Floor Rearing Systems. Although layer aviary systems have existed in the US for the last decade, the ventilation and environmental control requirements are vastly different from the conventional housing (Hayes, 2012; Zhao et al., 2015). This situation is in part due to the lower stocking density and access to litter on the floor. Mainly during the winter, ventilation for indoor air quality at the lower stocking density requires supplemental heat, which must be, properly distributed throughout the house. Further challenges in aviary systems include design ventilation rates, indoor air quality, heat, and moisture production of the birds and the surroundings, fuel usage, and the birds’ preference for winter temperature-ammonia combinations (Zhao et al., 2013). Another concern with these housing systems is that a portion of manure from the birds is, held in the house on the floor as litter. Litter on the floor impacts indoor air quality and can lead to elevated ammonia and dust concentrations (Shepherd et al., 2015).

There have been, resent research published, that has been, directed at the effects of aviary design on laying hen behavior and pullet rearing (Yang et al., 2023). Some research has looked at providing ramps in a cage-free aviary to provide for better movement of birds (Stratmann et al., 2022a; 2022b), while more recent research has used machine learning to track floor eggs produced by cage-free hens and monitor and record their pecking behavior (Subedi et al., 2023a; 2023b). Ali et al. (2020) researched the effects of aviaries on the general health of laying hens, and Yang et al. (2022) developed a model for detecting cage-free hens on the litter floor. These studies have indicated that there is still much to learn about, how to, properly manage cage-free aviaries for the optimum health and welfare of laying hens.

Nutrition, Alternative Ingredients and Feedstuffs. The use of alternative feed ingredients in poultry diets has many implications. Many of these ingredients and feedstuffs, impact, broiler and laying hen welfare, health, growth, and performance. Feeding ingredients such as cassava root tips (Yadav et al., 2019), high-oleic peanuts (Toomer et al., 2019), orange corn (Abraham et al., 2021), insects (Koutsos et al., 2021), and bigheaded carp meal (Upadhyaya et al., 2022) have sown to have an enhanced effect on broiler growth and performance. In addition, numerous studies have focused on feeding varying levels of microbial phytase to broilers in hopes of determining the optimum levels of phytase to include in the diet (Babatunde et al., 2020; Broch et al., 2021; Gulizia et al., 2023). Other studies have concentrated on feeding varying levels of organic minerals (Macalintal et al., 2020), total sulfur amino acids (Adhikari et al., 2022), and zinc methionine and manganese methionine (Pacheco et al., 2021), and dietary antibiotic alternatives (Wang et al., 2019). Considerable work has also been, reported on the effect of varying particle size of diets for starting broilers (Brown et al., 2023). Finally, research conducted within our group has tested the impact of pellet quality on broiler performance (Ovi et al., 2020a; 2020b; Lynch et al., 2023; Poholsky et al., 2023).

Feed Processing and Feed Manufacturing. Feed and feed manufacture represent the largest production cost (60-70%) for a commercial integrator. All commercial broilers and most turkeys are, fed, diets that are, pelleted due, to improvements in body weight gain, feed efficiency, and feed intake, with these benefits being more pronounced as feed form improves (Sellers et al., 2017). Therefore, research relating to feed manufacture and feed quality has the potential to have a dramatic impact on commercial integrator production costs and ultimately reduce the cost for the consumers. During the pelleting process, mash feed is, subjected to conditions of high moisture, pressure, and temperature; thus, adding additional costs and variables to the feeding process. In addition, it has been, shown that steam conditioning can have an impact on the quality of processed feed (Boltz et al., 2019; Homan et al., 2019). Commercial poultry feed mills (in general) utilize the same equipment to accomplish the pelleting process. However, due to variations in equipment manufacturers, the age of the mill, ingredients in the diet, feed throughput demands, ambient temperature, and others, the quality of the feed produced at a given mill and at a given time may vary (Buchanan et al., 2010). Feed quality, also referred to as durability or pellet quality, is, defined as how durable a pellet responds to intense handling and transportation from the time the feed is, manufactured to the point of consumption. This is important to consider because finished feed at the feed mill will have to be loaded onto a truck transported, to a farm, augured into a feed bin, and finally augured throughout the feed system at a house before reaching the point of consumption. The delivery process adds severe stress on the feed and pellets often dramatically deteriorate before being consumed, placing more importance on creating high-quality feed at the feed mill.

The starter growth phase represents a critical stage in a broiler's lifecycle. During this time, gastrointestinal tract formation is rapid and extremely critical for improvements in digestion to maximize performance (Lilburn and Loeffler, 2015). While ample research on feed quality improvement on broiler performance exists, research pertaining to feed quality presented in the early growth phases is lacking. Determining the optimal starter feed particle size may optimize starter performance, and impact, the overall performance of the bird (Lemons et al., 2019).

Role of Space on Behavior. The role of 3-D space available for broilers on a litter floor pen is difficult to determine; however, recently Li et al. (2023a) provided evidence as to how to track broilers use of floor pen space. Chai et al. (2022a) developed an automated approach for monitoring poultry distribution on floors. In addition, Li et al. (2022) used technology to design, and develop a broiler, mortality removal robot. For laying hens, aviaries, offer laying hens more space per bird in comparison to conventional (Cooper and Albentosa, 2003). However, merely providing more space does not ensure hens will be able to perform essential behaviors. Therefore, it is important to understand factors that may interfere with hens' abilities to use resources and perform key behaviors, which would reduce potential welfare benefits of transitioning hens to cage-free housing.

In a production setting, hen housing guidelines and codes of practice frequently include recommendations on the amount of space or resource allocation per hen with the intent of allowing expression of behavioral needs such as standing, lying, perching, wing flapping, and dust bathing. If hens cannot perform these behaviors, possible results are frustration, injury, or deprivation. For example, hens will work to gain perch access, particularly at night (Olsson and Keeling, 2000), and if sufficient perch space is provided for each hen, hens may spend 100% of their night perching (Olsson and Keeling, 2000). Dust bathing, is also well documented as having a positive benefit for hens. This "high priority behavior" (EFSA, 2015) is a maintenance behavior that can improve feather condition and dislodge skin parasites (Weeks and Nicol, 2006).

However, relatively little research has directly examined the amount of space required for laying hens to perform these key behaviors (Mench and Blatchford, 2014; Spindler et al., 2016) or postures, such as standing and lying, in which hens spend much of their day (Channing et al., 2001). Recently, we developed a unique approach to determine the physical space required by hens to perform key behaviors in commercial style aviaries and reported differences in space requirements between four different strains to perform certain behaviors (Riddle et al., 2018). We found that popular commercial hen strains such as Hy-Line W36, Bovan Brown, Hy-Line Brown, and DeKalb White required more physical space to perform key behaviors such as standing, lying, dust bathing, wing flapping and perching than proposed by housing guidelines and codes of practice or reported by previous research (Mench and Blatchford, 2014; Spindler et al., 2016).

However, there is more to a hen being able to perform a behavior than just the amount of space physically taken up by her body during the behavior. For example, we must also consider how many hens will engage in a given behavior simultaneously, a phenomenon called flock synchrony (Mench and Blatchford, 2014) as well as how much space a hen will place between herself and others in her group when performing a behavior (Collins et al., 2011). Aviary systems are, designed to promote behaviors shown by hens, and to provide more space per enclosure and per hen than conventional cages. However, to understand if the amount and type of space provided by aviaries allow hens to meet behavioral needs, space needs, to be, considered in the context of the presence of other birds. To understand how laying hens may use 3-D space, Rentsch et al. (2023) has provided some insight into a hen's use of space in an aviary.

Broiler Enrichment. *A*n area of animal welfare with poultry even less explored is broiler enrichment. Although several animal welfare certification programs have encouraged the scattering of feeding as a form of broiler enrichment, there is little evidence to support that broilers would benefit from this practice. The idea that the scattering of whole grains or other food items can be used, as a form of environmental enrichment is grounded in the assumption that foraging behavior is important for broilers and will be readily expressed if the scattered substrate is provided. However, what constitutes a normal behavior (i.e., a behavior that is important to the animal) can be altered by selective breeding and environmental conditions. It has been, suggested that along with selection for fast growth the broiler behavioral repertoire has shifted towards the performance of behaviors that allow the birds to conserve their energy. For example, previous research has shown that broiler chickens are less willing to work for food when a freely available food source is available as compared to laying hens or red jungle fowl, their wild counterparts (Linqvist et al., 2006). Additionally, provision of foraging or pecking materials as an enrichment in the presence of freely available food has not been associated with benefits, such as increased locomotion or improvements in leg condition (Pichova et al., 2016). Together, the results of these previous studies put into question whether scatter feeding has the potential to be an effective form of broiler enrichment. If scattering of feed is, to be, recommended, as a form of environmental enrichment for broilers, its implications for broiler welfare must first be, examined.

Heat Stress. Climate change-associated hot weather is an increasing threat to the animal production industries (Hajat et al., 2010). When ambient temperature increases toward the critical upper thermal limits in animals, heat gain exceeds heat loss, resulting in hyperthermia. As one of the critical reasons causing pathophysiological damage, hyperthermia leads to oxidative stress, i.e., disturbance in the pro-oxidant/antioxidant balance in favor of producing reactive oxygen species (ROS; Droge, 2002). Increased ROS contributes to cytotoxicity, causing cell death, tissue injury and organ damage resulting in increased morbidity and mortality (Solymosi et al., 2010). Estimated heat stress (HS) related losses for livestock was estimated at $2.4 billion in the U.S. (St-Pierre et al., 2003).

Heat stress (HS) is of great concern for poultry operations, as chickens, have been continuously selected for fast growth with much heavier harvest weight (broilers) or increased egg production (layers), which may increase susceptibility to HS by shifting energy from maintenance in response to stressors to productivity (Berong and Washburn, 1998). When the temperature exceeds the comfort level of a bird, behavioral and physiological changes occur, that are accompanied by changing physiological homeostasis (Jiang et al., 2019; Greene et al., 2022) and oxidative balance (Lin et al., 2006). These changes reduce feed intake and nutrient metabolism and immune response (Mohammed et al., 2019) while increasing morbidity and mortality. Thus, it is critical to increasing their heat tolerance. Gut microbiota reacts to various internal and external stimuli, influencing brain function in regulating the host's health via the gut-brain axis (Yarandi et al., 2016). Some probiotics have demonstrated beneficial effects on alleviating oxidative stress and related tissue damage in rodents (Lei et al., 2015). These data suggest that supplementing probiotics may provide a new strategy to inhibit HS-associated damage to health and welfare in poultry.

1b. Post-harvest:

Food Safety. During the post-harvest phase of poultry production, food quality and safety is very important. Since the poultry industry is all about producing a safe affordable product, it is extremely important that a high level of food security be, maintained. Previous research conducted by our group has published numerous studies examining ways to reduce the *Salmonella* and Campylobacter contamination of poultry meat products as an example (Nair et al., 2019; Kumar et al., 2020; Bourassa et al., 2021; Shijinaraj et al., 2021; Grace Dewi et al., 2021). These studies have shown some of the various ways in which poultry meat products can be, kept safe. In addition, some studies have, investigated several egg-processing techniques, to enhance, egg safety (Cassar et al., 2021; Beining et al., 2020; Chai et al., 2022b). Further research conducted by our group will provide more answers to discovering potential methods to keep poultry meat safe for human consumption.

OBJECTIVE 2

2. **Fostering Innovative Production Practices through Research and Extension.**

2a. Pre-harvest and Post-harvest:

Understanding the way in which chickens and turkeys partition their nutrient intake in different production systems is mostly unknown. Therefore, research is, needed on the breadth of poultry feeding programs by examining feed form, dietary ingredients, feed additives, and individual nutrients and how these influence not only the bird performance but also other elements of the production system such as well-being, environment, health, and food safety. This research will create replicated data to investigate the relationship between physical activity and production uses of nutrients in the current egg and meat-type bird genotypes and bird performance and health. Identification of bird strain and feed form effects on bird performance and nutrient utilization will be determined. Further research conducted by our group on the effects of the poultry house environment will help determine optimum raising environments. Future research conducted by our group during the post-harvest phase of poultry production will help ensure the production of safe and affordable poultry meat and eggs.

Objectives

1. 1. Advancing Sustainable Poultry Systems through Precision Management.
   Comments: This will include collaborative research, which covers environmental control and management, housing, litter management, ventilation, lighting, pre-harvest food safety, nutrition, feed processing, behavior, and welfare during the pre-harvest stage of production. Post-harvest considerations will encompass food safety, processing methods, waste-water-management, offal, and rendering. Another consideration in this section will explore avenues related to carbon footprint, nutrient utilization, production systems, and life cycle analysis.
2. 2. Fostering Innovative Production Practices through Research and Extension.
   Comments: This collaborative research will cover incubation and hatchery, reproductive physiology, nutrient excretion reduction, precision nutrition, alternative feed ingredients, NAE/ABF practices, alternative feeding strategies, gut health, gut microbiome, poultry health and disease management, alternative sustainable production systems, production systems under regulatory exemption, economic analysis, and bird welfare, during the pre-harvest stage of production. Cutting-edge processing methodologies, production systems under regulatory exemption, and the quality of meat and eggs, will be, examined in the post-harvest phase. Outreach and training will be a part that several of the universities will pursue. Poultry economics plays a role in conducting the studies to answer some of the questions outlined in the objectives.

Methods

Objective 1

1. **Advancing Sustainable Poultry Systems through Precision Management.**

1a. Pre-harvest: This section will, cover, environmental control and management, housing, litter management, ventilation, lighting, pre-harvest food safety, nutrition, feed processing, behavior, and welfare.

Engineering and Technology/Aviary and Floor Rearing Systems:

Collaborative research between stations in CA and TN is underway to develop and validate systems tracking hens' resource utilization in cage-free aviary housing systems, including their use of perches an movement within the system. Noteworthy multistate collaborators include Dr. Richard Blatchford and Maja Makagon from UC Davis and Dr. Yang Zhao from the University of Tennessee.

Researchers at Auburn will engage in assessing artificial intelligence (AI) and deep learning techniques to identify and map broiler and broiler breeder facilities to support responses to animal health outbreaks. Geo-tagged remote sensing, imagery will, be acquired from the USDA National Aerial Imagery Program (NAIP) for the years 2021 – 2023. The ability of two deep-learning-based paradigms to, correctly identify broiler and broiler breeder facilities from aerial imagery will be explored and compared by computing average precision, mean average precision, sensitivity, specificity, and intersection over union metrics. In other experiments, video analysis techniques will be used to evaluate lighting programs (natural light versus traditional) and thermal management strategies on bird performance (body weight, body weight gain, FCR, mortality), welfare (footpad scores, gait scores) and behavior (response to observe test, novel object test).

Research at Michigan State University (MSU) will focus on computer vision strategies to mitigate problematic behaviors in laying hens occurring in the litter area. This will involve automating detection of problematic behaviors and developing targeted intervention strategies to disrupt the behavior without causing unintended consequences to hen welfare, such as increased fear, injury, or reduction in positive behavior.

Nutrition, Alternative Ingredients and Feedstuffs/ Environmental Control Management/ Food Safety:

Research efforts in CT will be, dedicated to devising alternatives to antibiotic growth promoters, aiming to support optimal broiler/layer performance while effectively controlling the transmission of pathogens like *Salmonella* in poultry and their environment.

Mississippi State University is set to conduct research identifying best management practices for poultry producers. The focus will be on readily employable practices that can reduce the incidence of *Salmonella*, *Campylobacter*, and other pathogens across commercial poultry operations, spanning farms, hatcheries, feed mills, and transportation. Management practices, to be, explored are improvements in biosecurity measures, such as novel pest and insect control measures that would reduce these vectors and in turn reduce these pathogens across all poultry operations. In addition, developing best management practices in, regard to litter and water lines in the poultry house are also, being, explored for improvements that can reduce these pathogens.

Auburn will be conducting evaluations on the gut microbiome and transcriptome, exploring their relationship to the reduction of foodborne pathogens such as *Salmonella* and *Campylobacter* in live poultry. This will involve the use of feed and water additives like probiotics and organic acids.

In IA, research will be concentrated on examining the impacts of feed alternatives, technologies, ventilation, and the thermal environment on turkey and laying hen performance, intestinal health, and behavior.

NC researchers will be actively developing and validating innovative interventions applicable in the poultry environment. This will include the assessment of feed additives and water treatment to reduce the prevalence, population, and virulent serotypes of *Salmonella* and *Campylobacter* in poultry. Dr. Lin Walker from NC will be conducting this work.

In HI, ongoing research includes the evaluation of novel feedstuffs and feed additives. This will aim to develop sustainable feeding programs and nutrition strategies to modulate gut health and manage stressors, including those induced by climate change in broilers. Strategies to enhance reproductive efficiency in layers and broiler breeders will also be, developed.

Researchers at the University of Minnesota (UM) will persistently investigate sustainable food safety solutions applicable to poultry production systems. Collaborations with industry and other university partners will inform multiple research projects. Ongoing efforts include investigating targeted microbial interference strategies against pathogens relevant to the turkey industry, with a focus on producing gut-friendly and environmentally friendly solutions to strengthen a sustainable poultry food supply. We are investigating probiotics (for e.g., Propionibacterium and Ligilactobacillus), phytobiotics (essential oils and their ingredients) and vaccination strategies against the colonization and dissemination of foodborne pathogens, including Salmonella, relevant in turkey production. Parameters, including the populations of pathogens in dependent tissues and organs, will be assessed using microbiological approaches.

Poultry Welfare and Housing Environment/ Role of Space on Behavior/Heat Stress:

Collaborative research between GA and NC stations will involve the study of hen-housing interactions on welfare parameters. UGA and NC researchers are also collaborating to understand the gut-brain-behavior axis in chickens. Multistate members include Dr. Prafulla Regmi from UGA and Dr. Kenneth Anderson, and Dr. Allison Pullin from NC.

Researchers at, the University of Arkansas (UA) are evaluating environmental management, genetic selection, and welfare strategies for broiler chickens. A common focus among researchers will be the exploration of multidisciplinary strategies to mitigate heat stress, involving Drs.' Liang, Orlowski, and Weimer. Experiments will evaluate the effects genetic selection for high and low water efficiency, temperature, relative humidity, lighting, and commercial sprinklers on broiler performance, stress physiology, behavior, and welfare.

Researchers at Purdue University are persisting in efforts to improve housing, welfare, and production in pullets/layers, turkeys, and ducks. Their multidisciplinary approach combines behavior, environmental factors, egg quality, general physiology, nutrition, and neuroendocrine disciplines. Ongoing investigations include understanding the physiological stress response, evaluating the epigenetic impacts of heat stress, and determining if feed additives can ameliorate these effects. The team is also exploring benefits of environmental enrichment on production values and welfare variables, such as body condition, behavior, glucocorticoids and organ morphometrics. We are also evaluating visual perception in poultry to determine how different lighting environments impact birds' ability to visualize resources, and to develop a holistic view of layer chicken behavior, performance, physiology (glucocorticoid and immune factors), and neuroendocrine status (stress and reproductive neurohormones) to determine the best housing design form the birds' perspective.

Feed Processing and Feed Manufacturing:

University of Kentucky (UK) researchers continue to generate valuable data on feed ingredient evaluations. Their focus is on increasing the efficiency of energy and nutrient utilization while concurrently reducing nutrient excretion into the environment. The team is particularly interested in evaluating field contamination of grains, especially mycotoxin-contaminated corn, and its impact on performance, nutrient utilization, and gut health. Selected feed additives are also being, assessed for their effectiveness in ameliorating the effects of mycotoxin contamination and cyclic heat stress on performance, nutrient and energy digestibility, and utilization in broiler and laying hens, as well as egg quality. Response variables to be measure include performance, nutrient and energy digestibility and utilization, intestinal morphology, blood gas (heat stress study only), organ (liver, spleen, etc) relative weight, cecal microbial composition, changes in intestinal and serum cytokines levels, skeletal integrity, gene expression of intestinal tight junction genes, liver heat shock proteins, etc.

1b. Post-harvest: Post-harvest considerations will encompass food safety, processing methods, waste-water-management, offal, and rendering.

Food safety:

CT's research focus will center on the development of natural antimicrobials, nano emulsions, phytochemicals, including probiotics. The objective is to control *Salmonella* in meat and eggs without compromising their quality and shelf life.

Auburn's investigation will extend to evaluating the microbiome of poultry products and its correlation with the presence of foodborne pathogens like *Salmonella* and *Campylobacter*. Alternative interventions, such as high-intensity light and the use of bacteriophages will be, assessed for potential implementation in commercial facilities.

NC's research initiatives aim to develop and validate innovative technologies, including high-intensity pulsed light, to mitigate the food safety risks associated with poultry and egg products. Additionally, predictive models will be, devised to estimate the growth and survival of foodborne pathogens in these products, with LW from NC leading these efforts.

Researchers at the University of Minnesota are exploring plant-based solutions (for e.g., lemongrass and Pimenta essential oils) to enhance the post-harvest safety of turkey products. Collaborations with industry partners and other universities will be, established to facilitate multiple projects addressing this aspect. We are collaborating with the leading turkey processors for fine-tuning the use of essential oils in turkey products. Parameters, including their pathogen reduction potential, color parameters, pH, antioxidant status, and spoilage organisms will be determined.

1c. Environmental footprint: This section will explore avenues related to carbon footprint, nutrient utilization, production systems, and life cycle analysis.

The University of Minnesota researchers are actively engaged in seeking environmentally sustainable solutions. Their focus will be on developing industry-friendly approaches (based on the preharvest and postharvest approaches) that effectively address challenges faced by poultry producers and turkey growers while simultaneously minimizing the environmental impact of these solutions. Emphasis will be on collaborative and translational research incorporating science, engineering, and technology to enhance system efficiency and sustainability through infrastructure development of block-chain production.

Objective 2

2. **Fostering Innovative Production Practices through Research and Extension.**

2a. Pre-harvest: This section will cover incubation and hatchery, reproductive physiology, nutrient excretion reduction, precision nutrition, alternative feed ingredients, NAE/ABF practices, alternative feeding strategies, gut health, gut microbiome, poultry health and disease management, alternative sustainable production systems, production systems under regulatory exemption, economic analysis, and bird welfare. Various research initiatives are underway.

The CA station collaborates on the investigation of various causes of woody breast and its measurement methods in broilers, with CY from UC Davis. The CA and TN stations are jointly exploring housing and genetic factors contributing to keel bone fractures in laying hens, with collaboration from multistate members Drs. Richard Blatchford and Maja Makagon (UC Davis) and Dr. Yang Zhao (U. Tennessee). The project will focus on provision of resources (e.g. ramp, perch type) that facilitate the hens’ movements within multi-tier aviaries.

In CT, the focus will be on devising alternative strategies supporting growth, health, and performance in layer and broilers throughout the production pipeline, spanning incubation, hatching, grow-out, and laying periods. This will include studying the chicken microbiome's acquisition, temporal evolution, and impact on intestine development, immune health, and function.

Mississippi State University will be addressing ways to enhance hatchability of fertile eggs and increase the production of fertile eggs in broiler breeder hens. The multifaceted approach will involve examining changes in the hens' diet, storage conditions, temperatures, and incubation methods. Additionally, culturomics will be, employed to explore and harness the benefits of poultry microbiota, identifying beneficial microbial species and their functions for improved gut health and growth performance. Investigations will also cover new feed additives, precision nutrition, and bone health assessments in laying hens, with economic analyses conducted in both layers and broilers. Research includes both short term and long laying hen and pullet feeding study that will include- alternative feed ingredients evaluation, formulating into low crude protein and additive enzymes like protease and combination of enzymes, gut health additives such as pre-pro and symbiotics. The concept of precision feeding will be, applied where hens will be, only fed feed formulated into their dietary intake level where all the nutrients, are matched and met according to the age and status of them. In addition, bone health and welfare will be a component of these studies as there is a regular exchange and trade-off with calcium and egg production that affects the egg and bone system in laying hens. The necessity to evaluate the low oil residue of soybean meal and compare that with the similar and lower protein level ingredients such as DDGS has been the primary focus of the lab for the next 2 years. In all the research, the egg and egg economics will be a part of the nutrition studies in layers.

The University of Arkansas team will evaluate the economic, environmental, and bird performance and welfare implications of sprinkler systems in commercial houses. Research projects will also include experiments to determine the effects of environmental and genetic impacts on broiler performance, behavior, and welfare. Research results will be, presented at stakeholder engagement activities, including university workshops and symposia.

UGA's Chen Lab will be evaluating the impact of diet formulation on the economics and carbon footprints of broiler production. The focus will be on the role of synthetic amino acids, the metabolic energy level of diets on body composition, and their impacts on carbon footprints. Additionally, other factors such as parasite challenges, mycotoxins, and heat stress will be, integrated into different research models and study their contributions to the economic and sustainability of broiler production. While UGA's Regmi Lab will be delving into the genetic basis of hen activity in aviary houses.

Clemson University will focus on evaluating the effectiveness of alternative feedstuffs in preventing coccidiosis and necrotic enteritis in broilers, along with studying changes in the transcriptome and intestinal microbiome. The effect of sorghum on the intestinal tract will be evaluated for male commercial broilers from 1 to 42 days of age. A 2 x 2 factorial design will be used with 2 challenge treatments (unchallenged or challenged with E. maxima + Clostridium perfringens), and 2 dietary treatments (corn-based diet as the standard or sorghum-based diet). Performance parameters will be evaluated weekly and intestinal lesion scores will be, evaluated 21 days of age. Digesta and jejunal mucosa will be, collected for microbiome and transcriptomic analysis, respectively.

The Purdue Team is actively engaged in translational research addressing production challenges in ducks, turkeys, broilers, and layers. Their projects will cover environmental enrichment, parasite control, prevention of keel bone issues, and understanding behavioral aspects in ducks. Stakeholder engagement will include extension activities, industry meetings, and educational outreach.

Michigan State's research continues to explore non-cage aviary design and management impacts on laying hen behavior and welfare, with a specific focus on floor laying rates and spatially intense behaviors in the litter area such as wing flapping, dust bathing, and play behavior.

Virginia Tech will be investigating dietary energy in response to challenges posed by renewable fuels' standards, exploring alternative energy sources and strategies to enhance energy utilization in broiler chickens and laying hens.

The University of Minnesota researchers will be testing strategies to identify and implement alternative protein sources (e.g., insects, algae, plant proteins), targeted probiotics, and immune-enhancement strategies (vaccination, probiotics) in the breeder, hatchery, and production segments, collaborating with partner universities and industry stakeholders.

2b. Post-harvest: This section will cover cutting-edge processing methodologies, production systems under regulatory exemption, and the quality of meat and eggs.

Mississippi State University is, dedicated, to conducting assessments, of eggshell quality, offering producers insights into the structural integrity of their eggs and shedding light on the condition of the hens. The research will include strategies to enhance eggshell quality, primarily through dietary manipulations aimed at producing eggs with improved quality, ultimately reducing the likelihood of cracked or contaminated eggs reaching consumers.

CT will research use of novel antibacterial nano emulsions and ultra-fine bubble technology to reduce foodborne pathogen contamination on poultry carcass, chicken skin, breast, thigh and other cuts. The application of this would be, further tested in processing environments.

2c. Outreach and Training: This section will cover various aspects of outreach and training that several of the universities will and are pursuing.

The Purdue Team has successfully mentored a total, of five graduate students who have subsequently pursued advanced academic degrees, entered veterinary school, or embarked on careers within the poultry industry. In addition, team members have actively involved a combined total, of 25 undergraduate students in their research initiatives. The team will continue its efforts in training the next generation of researchers. Serving as co-advisors for the Purdue Poultry Club, team members will continue to play an integral role in facilitating presentations at various meetings and exhibits during campus-wide events such as Spring Fest, the Boiler Barnyard, and the Annual Alumni Fish Fry. Furthermore, Poultry Club undergraduates will actively participate in notable events, including the PEAK conference and internship program, with plans to engage in IPSF/IPPE in 2024. The Purdue Team takes pride in teaching several poultry-related courses, such as "Cracking the Poultry Industry" and upper-level courses in Welfare and Poultry Management. The latter includes a Course-Based Undergraduate Research Experience (CURE), providing teams of undergraduates with opportunities to investigate real-life challenges in poultry management. The team will continue its commitment to diversity and inclusion as team members continue to recruit, educate, and train underrepresented individuals for the poultry industry, contributing to a more diverse workforce.

The Purdue team will actively engage with stakeholders through extension activities, including monthly article publications and biannual symposia through outlets like the Poultry Extension Collaborative. Participation in industry meetings, such as PEAK, will ensure the effective dissemination of valuable information. Moreover, the Purdue team will extend its impact to the public through outreach and educational initiatives at events such as the Indiana State Fair, collaborations with community partners like The Farm at Prophetstown, and workshops for 4-H youth. Purdue Extension Specialists maintain a strong presence, engaging with poultry companies in Indiana and nationally, addressing key industry concerns, and providing essential training programs for poultry workers.

The University of Connecticut will focus on various challenges associated with ensuring food safety in poultry production that are underscored by the prevalent presence of bacterial pathogens like *Salmonella* and *Campylobacter,* in both layer hens and broilers. Studies will emphasize the potential health risks to consumers, if these pathogens, are not, effectively managed throughout the poultry production chain. To address these concerns, a comprehensive series of poultry extension activities will be, carried out in CT. These initiatives, developed in collaboration with the CT agricultural agencies, farm bureau and other local poultry groups, will encompass multifaceted training programs. Workshops and seminars will not only disseminate the latest research findings but also focus on industry best practices related to biosecurity measures, sanitation protocols, and effective management strategies. Additionally, specific extension activities will target poultry health, emphasizing disease prevention, vaccination protocols, and application of alternatives to antibiotics in poultry production. These extension activities, led by the UConn extension group, aim to empower poultry producers with practical tools for risk mitigation and the improvement of overall poultry health. By fostering a culture of continuous improvement, these initiatives aspire to enhance the safety, sustainability, and productivity of poultry production in CT. Through collaborative efforts and evidence-based practices, the extension activities seek to contribute to the resilience of the poultry industry in the face of evolving climate changes.

The University of Arkansas team actively collaborates and interacts with a diverse range of stakeholders. The team organizes and hosts quarterly Tyson Short Course workshops with Tyson employees and their customers on topics spanning the U.S. poultry industry, hatchery management, broiler and breeder production and management, animal wellbeing, biosecurity, nutrition and feed milling, processing and meat quality, food safety, and industry challenges. The course includes tours of hatchery, breeder, broiler, as well as hands-on necropsy and processing activities. The Center for Food Animal Wellbeing hosts an annual symposium and engages with poultry industry organizations. Symposium speakers include scientists, veterinarians, and industry leaders that share their insights into research findings, best practices, responsibilities in their respective roles, and legal perspectives on relevant challenges and advancements in agricultural animal wellbeing. The Center also works with poultry industry stakeholders, including the Center for Food Integrity, Professional Animal Auditor Certification Organization, and American Humane Association on scientific committees to review and improve poultry wellbeing standards.

Extension specialists from the University of Arkansas, University of California Davis, and Purdue University are members of the Poultry Extension Collaborative (PEC), a cohesive group of poultry welfare experts focusing on developing and disseminating science-based information to guide poultry welfare decisions in practice. The PEC’s mission encompasses creating and disseminating educational and outreach materials, including the monthly Poultry Press, offers scientific expertise to poultry industry stakeholders, and hosts online symposia on topics spanning poultry welfare assurance and research from international perspectives. The PEC disseminates this information online through their website, YouTube channel, and Facebook page.

2d. Poultry Economics: This section will cover the aspect of how poultry economics plays a role in conducting the studies to answer some of the questions outlined in the objectives.

North Carolina State University will be conducting many studies on the economic analysis for the poultry industry. (i) Analysis of Industry Organization, Structure, and Competitiveness: Monitor industry trends and conduct research to assess the impacts of industry concentration, market power, mergers, and acquisitions on consumer welfare and cost synergies. (ii) Evaluating Economics of Poultry Contracts and Integrator-Grower Relations: Investigate the relative performance-based settlement schemes, such as tournaments, in broiler production contracts. Explore potential alternatives with fewer incentives and analyze their effects on firm-level profitability and growers' income. (iii) Economic Assessment of Animal Welfare: Perform cost-benefit and/or enterprise budgeting analyses for innovative animal welfare production technologies or regulatory proposals. This analysis will be, conducted at the individual project level or within the context of partial market equilibrium settings.

Mississippi State University will also cover economic analyses in both broilers and in layers with respect to new feed additives, precision nutrition, and bone health assessments. Moreover, UGA's Chen Lab will be evaluating the impact of diet formulation on the economics and sustainability of broiler production.

Measurement of Progress and Results

Outputs

- Enhanced collaborative research and development of research proposals for submission to federal agencies
- Successful extramural funding from industry and government programs that target specific collaborative research goals outlined in the project
- Support and training for undergraduate, graduate and post-doctoral students
- Data published in peer-reviewed high impact journals and presented at major scientific venues including association conferences such as Poultry Science, Worlds Poultry Science, International Poultry Scientific Forum, Institute of Food Technologists, International Association for Food Protection, and American Society of Agricultural and Biological Engineers
- Results are translated and available for various stakeholders including government agencies, poultry associations, poultry, and food industry and consumers. Different formats used include various media (print, web-based, webinar and other media specific to the target audience
- Optimize poultry house environments, monitoring and management will improve producer economic situations
- Performance and well-being data generated from alternative housing and pasture-raised studies will improve decision-making in a rapidly changing industry
- Data generated from nutrition trials utilized by producers for feed formulations, feed milling and feed suppliers
- Producers will have access to data related to poultry well-being to supplement decisions related to management practices
- Economic analyses of poultry production systems will improve industry profitability and consumer demand for poultry products
- Industry adoption of recommendations generated from research findings

Outcomes or Projected Impacts

- Improved productivity in broiler, turkey and layer chickens (feed conversion, weight gain, dozens of eggs produced)
- Improved disease management and food safety as a result of feed supplements or management practices
- Adoption of new engineering strategies and technology for reducing energy consumption and improve poultry house environments and food safety
- Improved poultry well-being through advanced monitoring systems and precision livestock farming
- Management recommendations on alternative housing systems for laying hens and other poultry that will assist producers in making sound business decisions on which systems are most suitable to their operations; and identify best practices to be adopted to allow these systems to function to full potential
- Strategized feeding programs with the use of alternative feed additives or feed ingredients or under constrained ingredient use while maintaining or improving broiler, turkey, and/or hen performance, well-being, and environment parameters

Milestones

(**2025**):• Studies conducted at the stations in CA and TN will validate various systems used to track hens' resource utilization in a cage-free housing environment • Research studies in CT will be, dedicated to finding alternatives to antibiotic growth promoters to support broiler/layer performance and control transmission of Salmonella in poultry and their environment • Artificial intelligence (AI) and deep learning techniques will be conducted at Auburn to identify and map poultry facilities to support responses to animal health outbreaks • Research studies in IA will examine the impacts of feed alternatives, ventilation, and the thermal environment on turkey and laying hen performance • Studies conducted in AR will evaluate environmental management, genetic selection, and welfare strategies for broilers • Feed ingredient evaluation studies will be conducted at KY that focuses of increasing the efficiency and nutrient utilization of poultry while reducing nutrient excretion in the environment • Translational research conducted by Purdue (IN) will address production challenges in ducks, turkeys, broilers, and layers. Environmental enrichment, parasite control, prevention of keel issues and behavioral aspects in ducks will be the focus • Experiment conducted at MS will address various ways to enhance hatchability of fertile eggs and increase the production of fertile eggs in broiler breeder hens

(**2026**):• The incidence of Salmonella, Campylobacter and other pathogens will be assessed at commercial poultry operations, including various farms, hatcheries, feed mills, and transportation (MS) • Auburn will be conducting gut microbiome and transcriptome relationships to reduce foodborne pathogens such as Salmonella and Campylobacter • NC researchers will develop innovative interventions applicable to the poultry environment. Feed additives and water treatment will be the focus • Experiments will be conducted by the CA and TN stations to investigate woody breast causes, and evaluate contributing factors leading to keep bone fractures in laying hens kept in cage-free environments

(**2027**):• NC will continue developing technologies, including high-intensity pulsed light to mitigate food safety risks associated with poultry and egg products • CT will continue to develop natural antimicrobials, including probiotics to control Salmonella contamination in poultry meat and eggs • MN will explore plant-based solutions to enhance post-harvest safety of turkey products • Alternative feedstuffs will be the concentration of the station at Clemson (SC) and their effect on preventing coccidiosis and necrotic enteritis in broilers

(**2028**):• The MN station will be testing strategies to identify alternative protein sources, targeted probiotics, and immune-enhancement strategies in breeder, hatchery, and production segments • VA will investigate dietary energy responses to challenges posed by renewable fuels' standards which will enhance the energy utilization in broilers and layers • MI will continue to explore non-cage aviary design and management's impact on laying hen behavior and welfare. Solutions to floor laying rates and related aspects will be analyzed • Outreach and training of students, at all university experiment stations will be the focus throughout the project period. In IN (Purdue), graduate and undergraduate students will be trained to understand the various aspects of the poultry industry • CT will summarize various challenges associated with ensuring food safety in poultry production systems

(**2029**):• Numerous studies at NC will be summarized which center on assessing the economic condition of the poultry industry. The analysis of industry organization, structure, and competitiveness will be included • MS will also summarize the economic condition of the broiler and layer industries with respect to the effect of new feed additives, precision nutrition, and bone health of poultry

Outreach Plan

Study findings will be disseminated promptly to the academic communities, industry stakeholders, and the general public through press releases, web publications, extension reports, presentations at professional conferences and industry educational workshop, such as the Annual Industry Issues Forum organized by the Egg Industry Center (www.eggindustrycenter.org). The results will also be disseminated via extension publications (e.g., Animal Industry Report produced at ISU, NCLP and MT Reports at http://poultry.ces.ncsu.edu/layer-performance/), graduate student theses and dissertations, and peer-reviewed journal articles. While some of the outreach will be passive (user identifies information through web searching or other means), other outreach will be active (planned "events"). The "events" will be, planned by the Extension specialists participating in the project including, seminars, field days, and workshops at which results of the research technologies will be discussed/disseminated to poultry producers. On-farm demonstrations, will be conducted when feasible to allow farmers to see firsthand the results of the research in conjunction with traditional producer education programs. Members of the group will identify relevant information to share with interested clientele groups within their states or regions. Engagement of the poultry industry stakeholders (UEP, National Chicken Council, National Turkey Federation, USPEA, and others) will allow for input on the relevance of research being conducted and provide feedback on strategic ways to actively share information as well as provide ideas on future research topics. We believe a partnership between industry, government, and academia must exist to improve poultry production systems and well-being.

Organization/Governance

The Technical Committee is responsible for the planning and supervision of the Multi-State Research Project. The membership of this committee shall consist of an Administrative Advisor, a technical representative of each participating agency or experiment station, and representative of the USDA Cooperative States Research Service. Each participating agency or experiment station is entitled to one vote. The Technical Committee shall be responsible for review and acceptance of contributing projects, preparation of reviews, modification of the regional project proposal, and preparation of an annual report. Each technical committee member will prepare annual written reports and distributed at the annual meeting. Annual reports will be, compiled and distributed to Technical Committee members, and Agricultural Experiment Station Directors. The Technical Committee will meet yearly and conduct an election for the office of Junior Executive. The position should alternate between Poultry Scientists and Agricultural Engineers. The person elected to serve as Junior Executive will rotate through the remaining offices of Senior Executive and Secretary and will serve as Chair in the fourth year. All voting members of the Technical Committee are eligible for office. The Chair prepares the meeting agenda and presides at meetings. The Chair is responsible for the preparation of the annual report. The Secretary records minutes and assists the Chair. The Senior and Junior executives help with policy decisions and nominations. The Technical Committee functions as a unit with sub-committees formed as necessary, which is, preparing nominations for elections.

Literature Cited

Abdelfattah, E., G. Vezzoli, and M.M. Makagon. 2020. On-farm welfare assessment of commercial Pekin duck: A comparison of methods. Poult. Sci.99:689-697.

Abraham, M. E., S. L. Weimer, K. Scoles, J. I. Vargas, T. A. Johnson, C. Robison, L. Hoverman, E. Rocheford, T. Rocheford, D. Ortiz, and D. M. Karcher. 2021. Orange corn diets associated with lower severity of footpad dermatitis in broilers. Poult. Sci. 100:101054. DOI: 10.1016/j.psj.2021.101054.

Adhikari, P., F. L. Castro, G. Liu, and W. K. Kim. 2022. Effects of total sulfur amino acids on growth performance, immunity, and meat yield in broilers fed diets with and without antibiotics. Front. Vet. Sci. 9:903901.

Ali A. B. A., D. L M. Campbell, J. M. Siegford. 2020. A risk assessment of health, production, and resource occupancy for 4 laying hen strains across the lay cycle in a commercial-style aviary system. Poult. Sci. DOI: 10.1016/j.psj.2020.05.057.

Ali A.B. A., M.J. Toscano, and J. M. Siegford. 2019. Later exposure to perches and nests reduces individual hens’ occupancy of vertical space in an aviary and increases force of falls at night. Poult. Sci. 98:6251-6262. DOI: 10.3382/ps/pez506.

Babatunde, O. O., J. A. Jendza, P. Ader, P. Xue, S. A. Adedokun, and O. Adeola. 2020. Response of broiler chickens in the starter and finisher phases to three sources of microbial phytase. Poult. Sci. https://doi.org/10.1016/j.psj.2020.05.008.

Beining Ouyang, B., A. Demirci, and P. H. Patterson. 2020. Inactivation of escherichia coli K12 in liquid egg white by a flow-through pulsed UV light treatment system. J. Food Protect. 83:418-425.

Berong, S. L., and K. W. Washburn. 1998. Effects of genetic variation on total plasma protein, body weight gains, and body temperature responses to heat stress. Poult. Sci. 77:379-85.

Bist, R.B., S. Subedi, L. Chai, and X. Yang. 2023. Ammonia emissions, impacts, and mitigation strategies for poultry production: A Critical Review. J. Environ. Manage. 328:116919.

Boltz, J. W., C. Boney, J. Shen, J. Jaczynski, and J. S. Moritz. 2019. The effect of standard pelleting and more thermally aggressive pelleting utilizing a hygieniser of feed manufacture and reduction of *Enterococcus faecium*, a *Salmonella* surrogate. J. Appl. Poult. Res. 28:1226-1233.

Bourassa, D. V., R. J. Buhr, C. E. Harris, and L. N. Bartenfeld Josselson. 2021. Assessment of stabilized hydrogen peroxide for use in reducing *Campylobacter* levels and prevalence on broiler chicken wings. J. Food Prot. 84:449-455.

Broch, J., V. D. L. Savaris, L. Wachholz, E. H. Cirilo, G. L. S. Tesser, W. J. Pacheco, C. Eyng, G. M. Pesti, and R. V. Nunes. 2021. Influence of phytate and phytase on performance, bone, and blood parameters of broilers at 42 days old. S. Afr. J. Anim. Sci. 51:160-171.

Brown, A. T., M. J. Alvarenga, M. E. Lemons, C. D. McDaniel, J. S. Moritz, and K. G. S. Wamsley. 2023. Determining the average particle size consumed (APSC) between two genetic strains (GS) receiving starter diets varying in feed form (FF) and feed quality (FQ). J. Appl. Poult. Res. 100336.

Buchanan, N. P., K. G. S. Lilly, C. K. Gehring, and J. S. Moritz. 2010. The effects of altering diet formulation and manufacturing technique on pellet quality. J. Appl. Poult. Res. 19:112-120.

Cassar J. R., L. M. Bright, P. H. Patterson, E. W. Mills, and A. Demirci. 2021. The efficacy of pulsed ultraviolet light processing for table and hatching eggs. Poult. Sci. 100(3) March 2021, 100923. https://doi.org/10.1016/j.psj.2020.12.021

Chai, L., S. Aggrey, A. Oladeinde, C. Ritz, and T. Applegate 2022a. An Automated Approach to Monitoring Poultry Floor Distribution. *UGA Extension Bulletin*.

Chai, L., Y. Zhao, H. Xin, and B. Richardson 2022b. Heat treatment for disinfecting egg transport tools. Appl. Eng. Agric. 38;:343-350.

Channing, C. B. Hughes, and A. Walker. 2001. Spatial distribution and behaviour of laying hens housed in an alternative system. Appl. Anim. Behav. Sci. 72:335-345.

Collins, L. M., L. Asher, D. U. Pfeiffer, W. J. Browne, and C. J. Nicol. 2011. Clustering and synchrony in laying hens: The effect of environmental resources on social dynamics. Appl. Anim. Behav. Sci. 129:43-53.

Cooper, J. J., and M. J. Albentosa. 2003. Behavioural priorities of laying hens. Avian Poult. Biol. Rev. 14:127-149.

Droge, W. 2002. Free radicals in the physiological control of cell function. Physiol. Rev. 82:47-95.

EFSA, E. A. 2015. Scientific Opinion on welfare aspects of the use of perches for laying hens. EFSA J. 197:1-23.

Grace Dewi, Shijinaraj Manjankattil, Claire Peichel, Shiliang Jia, Zata Vickers, Timothy Johnson, Carol Cardona, Sally Noll, Anup Kollanoor Johny. 2021. Effect of plant-derived antimicrobials against multidrug-resistant *Salmonella* Heidelberg in ground Turkey. Poult. Sci. 101:101581. https://www.sciencedirect.com/science/article/pii/S0032579121006027

Greene, E. S., E. Adeogun, S. K. Orlowski, K. Nayani, and S. Dridi. 2022. Effects of heat stress on cyto (chemo) kine and inflammasome gene expression and mechanical properties in isolate red and white blood cells from 4 commercial broiler lines and their ancestor jungle fowl. Poult. Sci. 101:101827. https://doi.org/10.1016/j.psj.2022.101827

Gulizia, J. P., S. M. Bonilla, J. I. Vargas, S. J. Sasia, S. Llamas-Moya, T. Doung, and W. J. Pacheco. 2023. The effects of phytase and multicarbohydrase complex containing ahpha-galactosidase on performance, processing yield, and nutrient digestibility in the broiler chicken. J. Appl. Anim. Res. 1:308-322.

Hajat, S., M. O'Connor, and T. Kosatsky. 2010. Health effects of hot weather: From awareness of risk factors to effective health protection. Lancet. 375:856-863.

Hayes, Morgan Davis. 2012. "Environmental and energy assessment of an aviary laying-hen housing system in the Midwestern United States". Graduate Theses and Dissertations. 12601. https://lib.dr.iastate.edu/etd/12601.

Hester, P. Y. 2005. Impact of science and management on the welfare of egg laying strains of hens. Poult. Sci. 84:687-696.

Homan, V. B., J. W. Boney, and J. S. Moritz. 2019. The effects of steam conditioning temperatures on commercial phytases and subsequent broiler performance and tibia mineralization. Appl. Anim. Sci. 35:298-303.

Jacobs, L., R. A. Blatchford, I. C. de Jong, M. A. Erasmus, M. Levengood, R. C. Newberry, P. Regmi, A. B. Riber, and S. L. Weimer. 2023. Enhancing their quality of life: environmental enrichment for poultry. Poult. Sci. 102:102233. DOI: 10.1016/j.psj.2022.102233.

Jiang, S., A. A. Mohammed, J. A. Jacobs, T. A. Cramer, and H. W. Cheng 2019. Effect of synbiotics on thyroid hormones, intestinal histomorphology, and heat shock protein 70 expression in broiler chickens reared under cyclic heat stress. Poult. Sci. 99:142-150.

Johnsonon, A. K., J. D. Colpoys, A. Garcia, C. Jass, S. T. Millman, M. D. Pairis-Garcia, C. J. Rademacher, S. Weimer, and S. Azarpajouh. 2019. A proactive blueprint to demonstrate on-farm animal welfare. CAB Reviews. 14:1-8. DOI: 10.1079/PAVSNNR201914037.

Kang, S. W., K. D. Christnesen, M. T. Kidd Jr., S. K. Orlowski, and J. Clark. 2023. Effects of a variable light intensity program on the welfare and performance of commercial broiler chicken. Front. Physiol. 14. https://doi.org/10.3389/fphys.2023.1059055

Koutsos, E. A., P. H. Patterson, K. Livingston, and T. Freel: Book Chapter: 2021. The Role of Insects for Poultry Feed: Present and Future Perspective. *In* Mass Production of Beneficial Organisms: Invertebrates and Entomopathogens, 2nd Edition, Dr. Morales-Ramos, Editor. Elsevier, Cambridge, MA.

Kumar, S., M. Singh, D. E. Cosby, N. A. Cox, and H. Thippareddi. 2020. Efficacy of peroxy acetic acid in reducing *Salmonella* and *Campylobacter* spp. populations on chicken breast fillets. Poult. Sci. 99:2655-2661.

Lei, K., Y. L. Li, Y. Wang, J. Wen, H. Z. Wu, D. Y. Yu, and W. F. Li. 2015. Effect of dietary supplementation of Bacillus subtilis B10 on biochemical and molecular parameters in the serum and liver of high-fat diet-induced obese mice. J. Zhejiang Univ. Sci. B. 16:487-95.

Lemons, M., C. D. McDaniel, J. Moritz, and K. G. S. Wamsley. 2019. Increasing average feed particle size during the starter period maximizes Ross × Ross 708 male broiler performance. J. Appl. Poul. Res. 28:420-434.

Lewis, P. D., and Morris, T. R. 2000. Poultry and coloured light. World’s Poult. Sci. J. 56:189-207.

Liang, Y., M. Janorschke, and C. E. Hayes. 2022. Low-cost solar collectors to pre-heat ventilation air in broiler houses. *Energies* 2022, 15, 1468. https://doi.org/10.3390/en15041468

Li, G., G. D. Chesser, J. L. Purswell, C. Magee, R. S. Gates, and Y. Xiong. 2022. Design and development of a broiler mortality removal robot. Appl. Engin. Agric. 38: 853-863.

Li, G., Gates, R. S., Meyer, M. M., and E. A. Bobeck. 2023a. Tracking and characterizing spatiotemporal and three-dimensional locomotive behaviors of individual broilers in the three point gait-scoring system. Animals. 13(4):717.

Li, G., Gates, R. S., Xiong, Y., Ramirez, B. C., and R. T. Burns. 2023b. Evaluating draft EPA emissions models for broiler operations. J. Appl. Poult. Res. doi: 10.1016/j.japr.2023.100365

Lilburn, M. S., and S. Loeffler. 2015. Early intestinal growth and development in poultry. Poult. Sci. 94:1569-1576.

Lin H., E. Decuypere, and J. Buyse. 2006. Acute heat stress induces oxidative stress in broiler chickens. Comp. Biochem. Physiol. A Mol. Integr. Physiol. 144:11-17.

Lindqvist C. E., P. Zimmerman, and P. Jensen. 2006. A note on contrafreeloading in broilers compared to layer chicks. Appl. Anim. Behav. Sci. 101:161-166.

Lynch, E., K. Bowen, V. Ayers, T. Boltz, K. G. S. Wamsley, J. W. Boney, and J. S. Moritz. 2023. Hygenic pelleting can decrease Hubbard x Ross 708 parent ileal amino acid digestibility, broiler performance, and increase digestible amino acid requirement. J. Appl. Poult. Res. 32:100355. DOI: https://doi.org/10.1016/j.japr.2023.100355

Macalintal, L. M., A. J. Pescatore, T. Ao, M. J. Ford, and K. A. Dawson. 2020. Organic minerals restore the acid-base and electrolyte balance in broiler chicks with nutritionally induced metabolic acidosis. J. Appl. Anim. Nutr. 8:41-48.

Mench, J. A., and R. A. Blatchford. 2014. Determination of space use by laying hens using kinematic analysis. Poult. Sci. 93:794-798.

Mohammed, A. A., S. Jiang, J. A. Jacobs, and H. W. Cheng. 2019. Effect of a synbiotic supplement on cecal microbial ecology, antioxidant status, and immune response of broiler chickens reared under heat stress. Poult. Sci. 98:4408-4415.

Nair, V. T., and A. Kollanoor Johny. 2019. *Salmonella* in poultry meat production. *In* Food Safety in Poultry Meat Production, Steven Ricke, Siddhartha Thakur, and Kumar Venkitanarayanan, Editors. Springer. Pp. 1-24.

Olsson, I. A. S., and L. J. Keeling. 2000. Night-time roosting in laying hens and the effect of thwarting access to perches. Appl. Anim. Behav. Sci. 68:243-256.

Ovi, F. K. C. Bortoluzzi, T. J. Applegate, C. R. Starkey, K. S. Macklin, A. Morey, W. J. Pacheco. 2020a. Effects of pre-pelleting whole corn inclusion on broiler performance, intestinal microbiota, and carcass characteristics. J. Appl. Poult. Res. https://doi.org/10.1016/j.japr.2020.100114

Ovi, F. K., R. Hauck, J. Grueber, F. Mussini, and W. J. Pacheco. 2020b. Effects of pre-pelleting whole corn inclusion on feed particle size, pellet quality, growth performance, carcass yield, and digestive organ development and intestinal microbiome of broilers between 14 to 42 day of age. J. Appl. Poult. Res. https://doi.org/10.1016/j.japr.2020.10.012

Pacheco, W. J., D. B. Patiño, J. I. Vargas, J. P. Gulizia, K. S. Macklin, and T. J. Biggs. 2021. Effect of partial replacement of inorganic zinc and manganese with zinc methionine and manganese methionine on live performance and breast myopathies of broilers. J. Appl. Poult. Res. 30: https://doi.org/10.1016/j.japr.2021.100204

Parvin, R., M. M. Mushtaq, M. J. Kim, and H. C. Choi. 2014. Light emitting diode (LED) as a source of monochromatic light: a novel lighting approach for behaviour, physiology and welfare of poultry. World's Poult. Sci. J. 70:543-556.

Pichova, K, J. Nordgreen, C. Leterrier, L. Kostal, and R. O. Moe. 2016. The effects of food-related environmental complexity on litter directed behaviour, fear and exploration of novel stimuli in young broiler chickens. Appl. Anim. Behav. Sci. 174:83-89.

Poholsky, C. M., L. S. Erb, A. M. Lyons, P. Rohlf, and J. Boney. 2023. Improving pellet quality enhances Nicholas Select turkey performance in targeted phases of production. J. Appl. Poult. Res. 32:100340. https://doi.org/10.1016/j.japr.2023.100340

Poudel, I., M. M. Beck, A. S. Kiess, and P. Adhikari. 2022. The effect of blue and red LED light on the growth, egg production, egg quality, behavior, and hormone concentration of Hy-Line W-36 laying hens. J. Appl. Poult. Res. 31:100248. https://doi.org/10.1016/j.japr.2022.100248

Rentsch, A. K, E. Ross, A. Harlander, L. Niel, J. Siegford, T. M. Widowski. 2023. The development of laying hen locomotion in 3D space is affected by early environmental complexity and genetic strain. Scientific Reports. 13:10084. doi: 10.1038/s41598-023-35956-1.

Riddle, E. R., A. B. Ali, D. L. Campbell, and J. M. Siegford. 2018. Space use by 4 strains of laying hens to perch, wing flap, dust bathe, stand and lie down. PloS ONE 13: e0190532.

Sellers, R. B., P. B. Tillman, J. S. Moritz, and K. G. S. Wamsley. 2017. The effects of strain and incremental improvements in feed form on d 28 to 42 male broiler performance. J. Appl. Poult. Res. 26:192-199.

Shepherd, T. A., Y. Zhao, H. Li, J. P. Stinn, M. D. Hayes, and H. Xin. 2015. Environmental assessment of three laying-hen housing systems. Part II: Ammonia, greenhouse gas, and particulate matter emissions. Poult. Sci. 94:534-543.

Solymosi, N., C. Torma, A. Kern, A, Maroti-Agots, Z. Barcza, L. Konyves, O. Berke and J. Reiczigel. 2010. Changing climate in Hungary and trends in the annual number of heat stress days. Int. J. Biomet. 54:423-431.

Spindler, B., M. Giersberg, A. Briese, N. Kemper, and J. Hartung. 2016. Spatial requirements of poultry assessed by using a colour-contrast method (KobaPlan). Br. Poult. Sci. 57:23-33.

St-Pierre, N. R., B. Cobanov, and G. Schnitkey. 2003. Economic losses from heat stress by US livestock industries. J. Dairy Sci. 86:E52-E77.

Stratmann, A., D. Guggisberg, C. Benavides-Reyes, J. Siegford, and M. J. Toscano. 2022a. Providing ramps in rearing aviaries affects laying pullet distribution, behavior and bone properties. J. Appl. Poult. Res. 31:100283. doi:10.1016/j.japr.2022.100283.

Stratmann A, J. Siegford, M. Toscano. 2022b. Laying hen chicks make earlier use of elevated areas and perform more intertier transitions when provided with ramps in the rearing aviary. Proc. 55th Congr. Intl. Soc. Appl. Ethol. 55:92.

Shijinaraj Manjankattil Divek V. T. Nair, Claire Peichel, Sally Noll, Timothy J. Johnson, Ryan B. Cox, Annie M. Donoghue, Anup Kollanoor Johny. 2021. Effect of caprylic acid alone or in combination with peracetic acid against multidrug-resistant *Salmonella* Heidelberg on chicken drumsticks in a soft scalding temperature-time setup. Poult. Sci. 100:101421. https://doi.org/10.1016/j.psj.2021.101421

Subedi, S., R. B. Bist, X. Yang, and L. Chai. 2023a. Tracking floor eggs with machine vision in cage-free hen houses. Poult. Sci. 102637.

Subedi, S., R. B. Bist, X. Yang, and L. Chai. 2023b. Tracking pecking behaviors and damages of cage-free laying hens with machine vision technologies. Comput. and Elect. Agric. 204: 107545.

Toomer, O. T., A. M. Hulse-Kemp, L. L. Dean, D. L. Boykin, R. Malheiros, and K. E. Anderson. 2019. Feeding high-oleic peanuts to layer hens enhances egg yolk color and oleic fatty acid content in shell eggs. Poult. Sci. 98:1732-1748. https://doi.org/10.3382/ps/pey531

Upadhyaya, I., K. Arsi, A. Fanatico, B. Wagle, S. Shrestha, A. Upadhyay, C. N. Coon, C. Owens-Hanning, B. Mallman, J. Caldas-Cueva, J. Trushenski, M. N. Riaz, M. B. Farnell, D. J. Donoghue, A. M. Donoghue. 2022. Impact of feeding bigheaded carp fish meal on meat quality and sensory attributes in organic meat chickens. J. Appl. Poult Res. https://doi.org/10.1016/j.japr.2021.100224.

Wang, X., E. D. Peebles, K. G. S. Wamsley, A. S. Kiess, and W. Zhai. 2019. Effects of coccidial vaccination and dietary antibiotic alternatives on the growth performance, internal organ development, and intestinal morphology of Eimeria-challenged male broilers. Poult. Sci. 98:2054-2065. https://doi.org/10.3382/ps/pey552.

Weeks, C., and C. Nicol. 2006. Behavioural needs, priorities and preferences of laying hens. Worlds Poult. Sci. J. 62:296-307.

Yadav, B., R. Mishra, and R. Jha. 2019. Cassava (*Manihot esculenta*) root chips inclusion in the diets of broiler chickens: effects on growth performance, ileal histomorphology, and cecal volatile fatty acid production. Poult. Sci. 98:4008-4015.

Yang, X., R. Bist, S. Subedi, Z. Wu, T. Liu, and L. Chai. 2023. An automatic classifier for monitoring applied behaviors of cage–free laying hens with deep learning. Eng. Appl. Artif. Intell. 123:106377.

Yang, X., L. Chai, R. B. Bist, S. Subedi, and Z. Wu. 2022. A deep learning model for detecting cage-free hens on the litter floor. *Animals* 12:1983.

Yarandi, S. S., D. A. Peterson, G. J. Treisman, T. H. Moran, and P. J. Pasricha. 2016. Modulatory effects of gut microbiota on the central nervous system: How gut could play a role in neuropsychiatric health and diseases. J. Neurogastroenterol. Motil. 22:201-212.

Zhao, Y. T. A. Shepherd, J. Swanson, J. A. Mench, D. M. Karcher, and H. Xin. 2015. Comparative evaluation of three laying-hen housing systems: Description of the production systems and management practices. Poult. Sci. 943:475-484.

Zhao, Y., H. Xin, T. A. Shepherd, M. D. Hayes, J. P. Stinn, and H. Li. 2013. Thermal environment, ammonia concentrations, and ammonia emissions of aviary houses with white laying hens. Transactions of the ASABE. 56:1145-1156.

Zheng, W., Y. Xiong, R. S. Gates, Y. Wang, and K. W. Koelkebeck. 2020. Air temperature, carbon dioxide and ammonia assessment inside a commercial cage layer barn with manure-drying tunnels. Poult. Sci. 99:3885-3896. DOI: 10.1016/j.psj.2020.05.009.

Land Grant Participating States/Institutions

CT,AR,MS,MD,NC,AL,MN,VA,CA,IN

Non Land Grant Participating States/Institutions

Participation

Participant	Is Head	Station	Objective	Research						Extension	
				KA	SOI	FOS	SY	PY	TY	FTE	KA

Combined Participation

Combination of KA, SOI and FOS	Total SY	Total PY	Total TY
Grand Total:	**2.10**	**3.00**	**2.05**
712	0.1	0	0
307	0.05	0	0
315	0.05	0	0
307	0.03	0	0
308	0.03	0	0
311	0.03	0	0
712	0.03	0	0
311	0.1	3	2
307	0.2	0	0
311	0.05	0	0
712	0.05	0	0
307	0.03	0	0.05
315	0.03	0	0.05
503	0.03	0	0.05
712	0.1	0	0
311	0.02	0	0
311	0.02	0	0
712	0.02	0	0
712	0.02	0	0
722	0.02	0	0
302	0.03	0	0
302	0.03	0	0

302	0.03	0	0
307	0.25	0	0
315	0.05	0	0
307	0.08	0	0
307	0.08	0	0
307	0.08	0	0
315	0.08	0	0
315	0.08	0	0
315	0.08	0	0
307	0.1	0	0
301	0.03	0	0
305	0.03	0	0
306	0.03	0	0

Program/KA	**Total FTE**
Grand FTE Total:	**2.6**
712	0.27
311	0.03
307	0.03
308	0.03
712	0.03
307	0.23
712	0.03
311	0.03
307	0.03
712	0.02
302	0.03
307	0.05
307	0.08
307	0.08

Response to the Reviewers Comments

NETEMP_2442: Improving Sustainable Poultry Production through Collaborative Research and Outreach

Duration: 10/01/2024 to 09/30/2029

Thank you for the reviews. We appreciate the time and effort that the reviewers have invested in critically perusing the project. We have carefully prepared responses for their comments below.

Reviewer 1:

1. This is the largest poultry specific multistate regional project currently, and the members should be proud for the breadth of issues they are and are planning on addressing. While the proposal was strong, reorganization would aid in clarifying and leveraging collective strengths across institutions. For example, topics/sub-topics outlined (for the most part) were good in the current work/literature review, but when describing planned work, these sub-topics were difficult to follow. Objective 2b (post-harvest) also indicates work in both meat and eggs, yet only eggs are listed as part of this objective. Reorganization to clarify efforts in those sub-topics may be warranted, with special attention paid towards leveraging projects beyond the capabilities of singular scientists and singular experiment stations.

Response: The section has been revised to reflect the topics/sub-topics discussed in current work. Considering that the rewrite slightly varies and adds to the ongoing projects, we have tried to incorporate the changes in a reasonable manner. The changes have been highlighted in the methodology section.

2. For the outreach and training, the group should consider ways to elevate the work of collective group (or larger subgroups therein). While only briefly mentioned, the Poultry Extension Collaborative is an example of a collective effort. The group should also be considering ways to actively engage stakeholders (beyond just presentations at meetings, and this was not directly communicated. Some stakeholder groups to engage with and become involved in meetings could include the Egg Industry Center, the International Poultry Welfare Alliance, and the US Roundtable for a Sustainable Poultry & Egg Supply. No specific plan was relayed for recruitment of other scientists to this group. Very much appreciate involvement of any economist and value they would bring to efforts, and strategies to bring in other scientists beyond animal/poultry faculty would be rewarding to broaden efforts (e.g. systems modeling - LCA).

Response: This has been, addressed, in the outreach and training section of the project objectives.

Reviewer 2:

1. The participants need to include additional details including the specific parameters to be evaluated in proposed experimental trials. The document contains mainly general nonspecific

terms such as: feed ingredients, additives, feedstuffs, alternatives, supplements, formulations, management, programs, strategized feeding programs, disease management, and food safety.

Response: More details have been provided on methodology regarding specific compounds and parameters used in the Methods section.

2. This project has identified several broad areas and the timely issues important to today's poultry production industry in the Unites States for improving sustainable poultry production.

The proposal is multi-disciplinary/multi-state and represents research approaches that should improve productivity, well- being, and food safety.

Hopefully, the results will be significant improvements that the poultry industry stakeholders can utilize to address both pre-harvest and post-harvest challenges.

I am concerned about the absence of details provided and the over broad general goals presented in the proposal.

The proposal identifies both institutions and researchers that have been displayed solid expertise in their specific fields of study and past productivity.

Response: Thank you for the review. As mentioned in the previous response, we have revised the method section accordingly.

Reviewer 3:

1. This project has identified critical and timely issues to the poultry industry. The proposed multi-disciplinary/multi-state proposal represents a logical and well-outlined approach to the topic of improving sustainable poultry production. I am positive that the industry stakeholders await the successes and contributions that this collaborative effort will generate. The contributions will be significant to the poultry industry stakeholders, addressing a variety of both pre- and post-harvest challenges. I was originally concerned about the extremely diverse and complex objectives and goals set forth in this proposal, but after thoroughly reviewing the research institutions and researchers partnering in this project, I am fully comfortable in their likelihood of success. The proposal identifies both institutions and researchers that have been displayed solid expertise in their specific fields of study.

The only statement that I feel needs clarification is toward the bottom of the Section "Examples of sustained endeavors by NE1942 participants include:" and in then repeated in the "2a. Pre-harvest" section. The statement "The CA station collaborates on the investigation of woody breast causes a measurement method in broilers". It appears that this statement is incomplete and needs additional clarification.

Response: We appreciate the feedback from the reviewer. The statement has been revised for clarity in both places as "The CA station collaborates on the investigation of various causes of woody breast and its measurement methods in broilers".

<u>Reviewer 4:</u>

1. This is an impressive and expansive project. Very impressive outputs from the last 5-year cycle. Good cooperation across groups and some important research areas proposed. Appreciate the inclusion of AI technology. Strong team that should generate impactful data. May have been the uploading system but some editing issues throughout, lots of commas randomly through document.

Response: Thank you for your comments. The uploading system did not allow for certain changes and hence the final document is differently formatted. We will try to sort the issue with the website.

Thank you!

Appendix G: Peer Review (Submitted)

Status: Complete

Project ID/Title: NE_TEMP2442: Improving Sustainable Poultry Production through Collaborative Research and Outreach

Rate the technical merit of the project:

1. Sound Scientific approach:
Approve/continue project with revision
2. Achievable goals/objectives:
Good
3. Appropriate scope of activity to accomplish objectives:
Good
4. Potential for significant outputs(products) and outcomes and/or impacts:
Good
5. Overall technical merit:
Good
Comments
This is the largest poultry specific multistate regional project currently, and the members should be proud for the breadth of issues they are and are planning on addressing. While the proposal was strong, reorganization would aid in clarifying and leveraging collective strengths across institutions. For example, topics/sub-topics outlined (for the most part) were good in the current work/literature review, but when describing planned work, these sub-topics were difficult to follow. Objective 2b (post-harvest) also indicates work in both meat and eggs, yet only eggs are listed as part of this objective. Reorganization to clarify efforts in those sub-topics may be warranted, with special attention paid towards leveraging projects beyond the capabilities of singular scientists and singular experiment stations.
For the outreach and training, the group should consider ways to elevate the work of collective group (or larger subgroups therein). While only briefly mentioned, the Poultry Extension Collaborative is an example of a collective effort. The group should also be considering ways to actively engage stakeholders (beyond just presentations at meetings, and this was not directly communicated. Some stakeholder groups to engage with and become involved in meetings could include the Egg Industry Center, the International Poultry Welfare Alliance, and the US Roundtable for a Sustainable Poultry & Egg Supply. No specific plan was relayed for recruitment of other scientists to this group. Very much appreciate involvement of any economist and value they would bring to efforts, and strategies to bring in other scientists beyond animal/poultry faculty would be rewarding to broaden efforts (e.g. systems modeling - LCA).
Your Recommendation:
Approve/continue project with revision

Appendix G: Peer Review (Submitted)

Status: Complete

Project ID/Title: NE_TEMP2442: Improving Sustainable Poultry Production through Collaborative Research and Outreach

Rate the technical merit of the project:

1. Sound Scientific approach:
Approve/continue project with revision
2. Achievable goals/objectives:
Fair
3. Appropriate scope of activity to accomplish objectives:
Good
4. Potential for significant outputs(products) and outcomes and/or impacts:
Excellent
5. Overall technical merit:
Good
Comments
General comment:
The participants need to include additional details including the specific parameters to be evaluated in proposed experimental trials. The document contains mainly general nonspecific terms such as: feed ingredients, additives, feedstuffs, alternatives, supplements, formulations, management, programs, strategized feeding programs, disease management, and food safety.
Specific comments:
This project has identified several broad areas and the timely issues important to today’s poultry production industry in the Unites States for improving sustainable poultry production.
The proposal is multi-disciplinary/multi-state and represents research approaches that should improve productivity, well-being, and food safety.
Hopefully, the results will be significant improvements that the poultry industry stakeholders can utilize to address both pre-harvest and post-harvest challenges.
I am concerned about the absence of details provided and the over broad general goals presented in the proposal.
The proposal identifies both institutions and researchers that have been displayed solid expertise in their specific fields of study and past productivity.
Your Recommendation:
Approve/continue project with revision

Appendix G: Peer Review (Submitted)

Status: Complete

Project ID/Title: NE_TEMP2442: Improving Sustainable Poultry Production through Collaborative Research and Outreach

Rate the technical merit of the project:

1. Sound Scientific approach:
Approve/continue project
2. Achievable goals/objectives:
Excellent
3. Appropriate scope of activity to accomplish objectives:
Excellent
4. Potential for significant outputs(products) and outcomes and/or impacts:
Excellent
5. Overall technical merit:
Excellent
Comments
This project has identified critical and timely issues to the poultry industry. The proposed multi-disciplinary/multi-state proposal represents a logical and well-outlined approach to the topic of improving sustainable poultry production. I am positive that the industry stakeholders await the successes and contributions that this collaborative effort will generate. The contributions will be significant to the poultry industry stakeholders, addressing a variety of both pre- and post-harvest challenges. I was originally concerned about the extremely diverse and complex objectives and goals set forth in this proposal, but after thoroughly reviewing the research institutions and researchers partnering in this project, I am fully comfortable in their likelihood of success. The proposal identifies both institutions and researchers that have been displayed solid expertise in their specific fields of study.

The only statement that I feel needs clarification is toward the bottom of the Section "Examples of sustained endeavors by NE1942 participants include:" and in then repeated in the "2a. Pre-harvest" section. The statement "The CA station collaborates on the investigation of woody breast causes a measurement method in broilers". It appears that this statement is incomplete and needs additional clarification.
Your Recommendation:
Approve/continue project

Appendix G: Peer Review (Submitted)

Status: Complete

Project ID/Title: NE_TEMP2442: Improving Sustainable Poultry Production through Collaborative Research and Outreach

Rate the technical merit of the project:

1. Sound Scientific approach:
Approve/continue project
2. Achievable goals/objectives:
Excellent
3. Appropriate scope of activity to accomplish objectives:
Excellent
4. Potential for significant outputs(products) and outcomes and/or impacts:
Excellent
5. Overall technical merit:
Excellent
Comments
This is an impressive and expansive project. Very impressive outputs from the last 5-year cycle. Good cooperation across groups and some important research areas proposed. Appreciate the inclusion of AI technology. Strong team that should generate impactful data. May have been the uploading system but some editing issues throughout, lots of commas randomly through document.
Your Recommendation:
Approve/continue project

Nominating Region: Northeast (NE)
Nominator: Robert L. Taylor, Jr. (AA, bob.taylor@mail.wvu.edu)
Project or Committee Number and Title: NE2334 *Genetic Bases for Resistance and Immunity to Avian Diseases*
Technical Committee Chair: Theros Ng (therosng@westernu.edu)
Administrative Advisor: Robert L. Taylor Jr. (bob.taylor@mail.wvu.edu)

Issue, problem or situation in context of Grand Challenge(s) addressed: Worldwide poultry consumption has increased steadily since 1960. United States per capita poultry consumption equals the combined per capita consumption of beef and pork. Disease prevention and control strategies strengthen a sustainable poultry industry that can increase production to feed a growing world population, which is forecast to be 9.7 billion people by 2050. Multistate project NE2334 *Genetic Bases for Resistance and Immunity to Avian Diseases* meets this crucial need by researching, developing methods, and enhancing stakeholders' knowledge of the genetics of resistance and immunity to poultry diseases. Three Grand Challenges (**GC**) are addressed: **GC1 - Sustainability, Competitiveness, and Profitability of Food and Agriculture** by reducing disease losses; **GC2 - Adapting to and Mitigating the Impacts of Climate Change** by improving immune responses in suboptimal environments; and **GC4 - Ensuring a Safe, Secure and Abundant Food Supply** by enhancing productivity through genetic resistance to disease.

Objectives: The NE2334 project *Genetic Bases for Resistance and Immunity to Avian Diseases* began on October 1, 2024. However, its lineage traces to 1968, at a minimum, a total of 56 years. Members have continued to address changing disease issues over the project's long tenure by integrating new technologies and communicating information to stakeholders. Multiple, impactful discoveries (genes, vaccine components, immune system modulators) have improved disease responses. Work has focused on basic immunology and disease resistance to increase poultry production. The project objectives are: 1) **To determine how genetics, epigenetics and gene regulation influence innate and acquired immune functions; 2) To identify factors and agents affecting poultry immune development, function, dysfunction, and pathology; and 3) To develop and employ genetic stocks, methods, reagents and other tools to assess basic immune function, characterize immune evolutionary processes, guide genetic selection, and increase resistance to or protection against avian diseases**.

Accomplishments: The cooperative, multidisciplinary nature of project NE2334 embodies frequent collaboration, with at least 25% of the total publications having authors from multiple project institutions. Between 2013 and 2023, members published 380 peer-reviewed publications, 32 book chapters, 397 abstracts, and 18 technical reports. An additional 696 peer-reviewed papers were published between 1998 and 2013, for a total of 1,076 papers. These works appeared in high-impact journals, including *Poultry Science*, *Avian Diseases*, *Proceedings of the National Academy of Sciences USA*, *Nature*, *Journal of Immunology*, *Immunogenetics*, and *Virology*. Project members have written or edited books on avian immunology and poultry diseases. International collaborators from Canada, Denmark, Ghana, Kenya, Netherlands, Scotland, and Tanzania have also contributed to the project research. Participating scientists conduct research to reduce disease impacts **to increase profitability (GC1)**, enhance immune responses **to mitigate climate change effects (GC2)**, and increasing resistance to pathogens **to promote a safe, sustainable food supply (GC4)**. Through all these efforts, the following selected outputs were realized:

- Participants have developed and maintained genetic resources such as inbred lines, congenic lines differing by a single gene or gene family, advanced intercross lines, and highly selected lines. Some lines have been inbred for 50 to 100 generations. **(GC1, GC4)**
- Collaborating with industry, members created a line of immunoglobulin (Ig) knockout chickens that do not produce antibodies, facilitating basic investigation of immunity. **(GC2)**
- Project members discovered, developed, and patented the nonpathogenic Marek's disease (MD) virus, SB-1. **(GC1, GC4)**
- Genomic tools were created to identify specific disease-resistance genes. **(GC2, GC4)**
- Project members discovered major histocompatibility (B) complex (MHC) gene effects on Marek's disease (MD) outcome. First demonstration of genetic resistance to cancer. These two papers were cited 451 times and 241 times, respectively. **(GC1, GC2)**
- Major histocompatibility (B) complex (MHC) genes also affected the outcome of tumors caused by Rous sarcoma virus (RSV) or cloned RSV oncogene DNA. **(GC1, GC2)**
- A second group of MHC-like genes, MHC-Y, was discovered. These genes are independent of the MHC but impact disease responses. **(GC2, GC4)**
- Project scientists collaborating with industry mapped specific alloantigen genes' chromosomal location and identified their protein products. **(GC1)**
- Research characterized feed amendments that augment chicken immune responses. **(GC1, GC2)**
- Identified genetic markers associated with increased immunity and enhanced survival time in indigenous chicken ecotypes in Africa challenged with NDV. **(GC2, GC4)**

Selected Short-term outcomes
- Specific alloantigens genes impacted responses against the parasite, *Eimeria tenella*.
- Used genomic tools to track advantageous genes in commercial and research populations.

Selected Medium-term outcomes
- Discovery of MHC-like genes, MHC-Y, allowed poultry breeders to select for increased resistance to pathogens and food-borne bacteria such as Campylobacter.
- Poultry breeders use techniques developed in the project to identify chicken alloantigens to enhance frequencies of favorable alleles that affect production traits and disease resistance.
- Studies of the MHC association with diseases have augmented the identification of beneficial alleles for resistance to Marek's disease virus, Rous sarcoma virus, Salmonella enteriditis, and Eimeria species. Other disease association studies with the MHC are ongoing.

Selected Long-term outcomes
- The SB-1 Marek's disease virus is a principal component of bivalent and trivalent MD vaccines used worldwide.
- Genetic stocks have been used by multiple project stations and collaborators, as well as other scientists, to study the genetics of disease resistance. Line UCD 001 was used in the first chicken genome sequence in 2004. This associated publication has been cited 2,067 times. **(GC1)**
- Poultry breeders conduct ongoing assessment of major histocompatibility (B) complex (MHC) and other gene alleles to select for disease resistance and enhanced vaccine responses.

Impacts: Improved disease resistance and prevention strategies enhance production efficiency, animal health, welfare, and producer acceptance, while reducing antibiotic use and improving food safety of poultry products. Identifying crucial immune response genes aids vaccine design. Understanding heat stress and inflammatory response interactions allows improved poultry health and production as climates change. Studies on the host response to food-safety bacteria may reduce microbiological contamination of poultry products. Multistate project NE2334 research has generated **significant economic impact**. All poultry companies use Marek's disease vaccine with SB-1. Companies now evaluate their breeders for specific genes that impact disease resistance. The 2022 total U. S. poultry production value was $151.6 billion. If project work had a 1% impact on this figure, the value would be **$1.5 billion**. The worldwide value is likely more substantial.

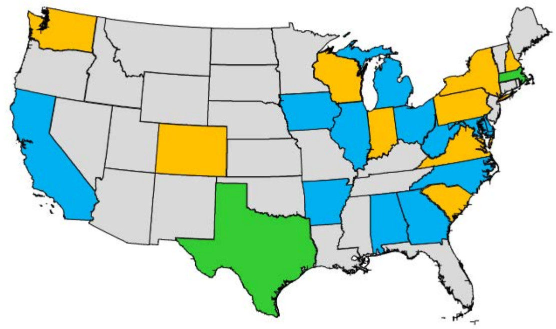
Added Value and Synergistic Activities: ***Multi-disciplinary activities:*** Continued identification of genes that enhance immunity and disease resistance facilitates response against continuously evolving pathogens. This work ensures an adequate, safe, food supply that is sustainable and adaptive to climate changes in accordance with Grand Challenges (**GC1, GC2, GC4).**

Multi-functional integrated activities: Project members regularly present at national and international meetings. Some presentations are invited as participants are experts in poultry genetics and disease resistance. Joint meetings have been held with other multistate project committees (i.e., NC168). Interactions with industry participants and Extension personnel within and outside the annual technical committee meeting communicates the latest research data, allowing the industry to adapt their breeding and production strategies.

Additional partnerships, associations or collaborations: Industry and international collaborators regularly participate with the committee (**participating institutions, page 4**). Their perspective assists research efforts through sponsored research funding and graduate fellowships. The collaborative nature of the work enables NE2334 to **leverage accomplishment beyond what would have been possible through individual investigation**. Multiple accomplishments were cited, and at least 25% of the publications were collaborative products.

Multi-institutional and Leveraged Funding: Committee members have leveraged funds from multiple sources. The US Agency for International Development granted $6 million, followed by a $5 million renewal, to improve African food security by enhancing resistance to Newcastle disease virus and heat stress in chickens through Feed the Future Innovation Lab to Improve Poultry. Another $1 million USDA-AFRI project studied climate change effects on heat stress and immune responses. A joint USDA and the UK's Biotechnology and Biological Sciences Research Council grant examined genomics and immunology of responses to avian pathogenic E. coli (APEC). Over the project's lifespan, other funds were provided by the National Cancer Institute for genetic effects on Rous sarcoma virus-induced tumors and the National Science Foundation for studies on ecological and life-history factors influencing the evolution of maternal antibody allocation. Industry scientists collaborated with project members to 1) create immunoglobulin gene knockout chickens that produce no antibody, a valuable resource for examining immune responses; 2) identify and locate five chicken alloantigen genes that impact disease resistance and production traits; 3) fund a competitive graduate student fellowship. Multi-institutional funding is demonstrated by the collaborative refereed publications among project members.

Summary of Participating Institutions and Units:
State (Region) current FTE, function, additional prior FTE - *Institution/Institutional Unit*
Alabama (S), 1 R, (3) - *Auburn University*
Arkansas (S), 1 R, (2) – *University of Arkansas*
California (W), 2 R, (4) - *University of California-Davis*
California (W), 2 R, (0) – *Western University*
Georgia (S) – 2 R, (3) - *University of Georgia*
Illinois (NC), 1 R, (0) - *University of Illinois*
Iowa (NC), 1 R, (1) - *Iowa State University*
Maryland (NE), 2 R, (0) - *University of Maryland*
Michigan (NC), 1 R, (3) - *USDA-ARS, East Lansing*
North Carolina (S), 2 R, (3) – *N. C. State University*
Ohio (NC), 1 R (2) - *The Ohio State University*
West Virginia (NE), 2 R, (0) – *West Virginia University*



NE2334 has 18 participants from 12 states (blue) across all four U. S. regions. Two states are current collaborators (green). Prior participating stations and collaborating institutions are shown in orange. Seventy-three individuals from 49 institutions have participated across all categories.

Current Collaborators
Texas – 1 - *USDA-ARS, College Station*
North Carolina - 1 - *USDA-ARS, Raleigh*

Industry and Stakeholder Collaborators
Industry – *Cobb-Vantress, Inc. (primary breeder)*
Industry – *Hy-Line International (primary breeder)*
Industry – *Perdue Farms, Inc. (producer, primary breeder)*
Industry – *Chrystal Bioscience, Inc. (biotechnology company)*
Industry – *ImmunBio, Inc. (biotechnology company)*

International Collaborators (10 institutions, 18 individuals)
Canada = *Agriculture, Canada; McGill University; University of Prince Edward Island: Ontario Veterinary College;* Denmark = *University of Aarhus;* Ghana = *University of Ghana;* Kenya = *International Livestock Research Institute (ILRI);* Netherlands = *Wageningen University;* Scotland = *Roslin Institute, University of Edinburgh;* Tanzania = *Sokoine University of Agriculture*

Former Participating Institutions and collaborators (20 institutions, 30 individuals)
Arkansas = *USDA-ARS, Fayetteville;* California = *Beckman Research Institute;* Connecticut = *University of Connecticut;* Illinois = *Northern Illinois University;* Indiana = *Purdue University ;* Maryland = *USDA-ARS, Beltsville;* Massachusetts = *Cotter Laboratory, Framingham State College, University of Massachusetts;* New Hampshire = *University of New Hampshire;* Colorado = *Colorado State University*; New York = *Cornell University;* Pennsylvania = *Pennsylvania State University, University of Pennsylvania, Wistar Institute;* South Carolina = *Clemson University;* Virginia = *Virginia Tech;* Wisconsin = *University of Wisconsin-Madison;* Texas = *Texas A&M University;* Washington = *Washington State University;*