ESCOP Science & Technology: http://escop.info/committee/scitech/

Call Minutes: 11/25/19
4 pm ET, via Zoom (https://zoom.us/j/556081111 or 1 720 707 2699 Meeting ID: 556 081 111)

Committee Members:

Chair: Jody Jellison (NERA) **Past Chair:** Laura Lavine (WAAESD) **Delegates:** Alton Thompson (ARD) John Yang (ARD) Joe Colletti (NCRA) Bill Barker (NCRA) Indrajeet Chaubey (NERA) Mark Hutton (NERA) Susan Duncan (SAAESD) Nathan McKinney (SAAESD) Gene Kelly (WAAESD) Chris Davies (WAAESD) **Executive Vice Chair:** Bret Hess (WAAESD ED) Saige Zespy (WAAESD Recorder)	**Liaisons:** Ann Hazelrigg (NIPMCC Chair) Kristina Hains (SSCC) Parag Chitnis (NIFA) Robert Matteri (USDA ARS)

Participants:
Susan Duncan, Indrajeet Chaubey, Joe Colletti, Bret Hess (Executive Vice Chair), Mark Hutton, Jody Jellison (chair), Chris Davies, and Saige Zespy (recorder)

Call Notes:

1. Welcome and roll call – Jellison and Hess: See Participant List above
    a. Each participant introduced themselves and their affiliation.
2. Approval of meeting notes from 10/28/2019 – Jellison
    a. With no changes, Indrajeet Chaubey moved to approve the minutes. Chris Davies seconded the motion.
3. Liaison Updates, as needed
    a. No liaisons were present at the November 25, 2019 meeting.
    b. Jody suggested involving ECOP in the ESCOP STC as a liaison. She will work with Bret to have the name of a person to serve as the ECOP liaison. The purpose of the liaison would be to provide the Extension perspective on all ESCOP STC issues.
4. Report discussions:

a. National Academy Sciences Science Breakthroughs 2030: A Strategy for Food and Agricultural Research
    i. See attached file for current multistate research projects addressing the microbiome. Over the last month, Bret has worked to develop a list of all projects addressing the microbiome in some way. He delved into active projects that mentioned the microbiome in justification, related work, outline, objective, methods and so on.
    ii. Joe mentioned the document is important and helpful. He also noted the next step is to identify gaps between existing multi-state research projects and the five items in the report.
    iii. Joe also noted that it will be important to identify the pipeline from which proposals come through to help identify how funding is prioritized.
    iv. Jody mentioned it would be helpful to have a conversation about addressing these gaps on the agenda each month.
    v. Joe suggested it may be useful to also have a discussion with NIFA about funding prioritization. He specifically noted that it may be helpful to take the opportunity and have a conversation about funding allocation.
    vi. Joe further added it would be helpful to have NIFA's impressions of this report, as well as to what extent they are thinking about trans-disciplinary science and comprehensive projects.
    vii. Susan asked about analysis of projects that overlap the five summary areas. Jody asked Bret to continue working on a second topic (database analysis and data science) for the next month. Bret noted he may be able to take on two more topics prior to the next meeting.
    viii. Bret will also work to identify cross-disciplinary approach in looking at multi-state review projects.
        1. To accomplish this task, Bret noted he may be able to work with Regional EDs to engage Multi-state Review Committees and help identify projects that are multi-disciplinary in nature.

5. Other business, as needed
    a. ESS Excellence Awards in Leadership
        i. Chair Hopper distributed a call about review of the ESS Excellence Awards in Leadership. Specifically, ESCOP STC is responsible for providing recommendations on the award winner for the ESS Excellence in Multi-state Research Award. Nominations for the Regional Excellence in Leadership Award are due to the regional office on February 1, 2020. The ESS Excellence in Multistate Research Award nominations are to be reviewed by the regional offices by February 28, 2020. they are sent to. At that point, they are sent to ESCOP STC for review the first part of March. Anticipate discussing reviews on the March Zoom conference.
    b. December meeting
        i. The December meeting is scheduled for December 23, which is close to the holiday. The committee opted to cancel their December meeting and meet next on January 27, 2020.

Action Items:

- Identify an ECOP liaison for ESCOP STC (Jody and Bret)
- Add ESS Excellence Awards review schedule to March meeting agenda (Bret)
- Add agenda items to monthly call agendas on gaps in multi-state research programs as aligned to the National Academy of Sciences report *Science Breakthroughs 2030: A Strategy for Food and Agriculture Research*. (Bret)
- Extend an invitation through NIFA and ARS liaisons to invite NIFA/ARS to the conversation about research gaps related to the National Academy of Sciences report *Science Breakthroughs 2030: A Strategy for Food and Agriculture Research*. Connect with ARS and NIFA liaisons to get them more involved in ESCOP STC. (Jody)
- Assist in encouraging liaisons to join ESCOP STC monthly calls. (Bret)
- Compile initial list of multistate research projects that address data analytics and data science from NIMSS. (Bret)
- Work to keep liaisons in the loop on ESCOP STC conversations, particularly related to where NIFA is going with the National Academy of Sciences report *Science Breakthroughs 2030: A Strategy for Food and Agriculture Research*.
- Send out Zoom invite for Jan. 27 ESCOP STC Zoom meeting. (Bret)
- Send out potential agenda items for upcoming meeting to Jody and/or Bret (All)

ESCOP Science & Technology Committee (STC): http://escop.info/committee/stc/

Call Agenda for: 11/25/2019 (currently 4th Monday monthly)
4 pm ET, via Zoom (https://zoom.us/j/104447860 or 1 720 707 2699 Meeting ID: 104 447 860)

Committee Members:

Chair: Jody Jellison (NERA)	**Liaisons:**
Past Chair: Laura Lavine (WAAESD)	Ann Hazelrigg (NIPMCC)
	Kristina Hains (SSSC)
Delegates:	Parag Chitnis (NIFA)
Alton Thompson (ARD)	Robert Matteri (USDA ARS)
John Yang (ARD)	
Joe Colletti (NCRA)	
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Indrajeet Chaubey (NERA)	
Mark Hutton (NERA)	
Susan Duncan (SAAESD)	
Nathan McKinney (SAAESD)	
Gene Kelly (WAAESD)	
Chris Davies (WAAESD)	
Executive Vice-Chair	
Bret Hess (WAAESD ED)	
Saige Zespy (WAAESD Recorder)	

Call Agenda:

1. Welcome and roll call – Jellison and Hess
2. Approval of meeting notes from 10/28/2019 – Jellison
3. Liaison Updates, as needed
    a. NIFA
    b. ARS
    c. NIPMCC
    d. SSSC
4. Report discussions:
    a. National Academy Sciences Science Breakthroughs 2030: A Strategy for Food and Agricultural Research
        i. See attached file for current multistate research projects addressing the microbiome
5. Other business, as needed

Science Breakthroughs to Advance Food and Agricultural Research by 2030.

The potential of microbiomes—in the animal gut, in soil, and everywhere in between—to increase efficiency and overcome obstacles in production.

State Agricultural Experiment Station Multistate Research Projects Addressing the Microbiome.

W4002: Nutrient Bioavailability--Phytonutrients and Beyond

Duration: 10/01/2018 to 09/30/2023

Related, Current and Previous Work

1. Absorption and Metabolism Modeling

W3002 scientists have developed state of the art techniques for assessing all components of bioavailability and metabolism for several nutrients and food components. These techniques address the interactions between members of the gut microbiome, nutrient bioavailability and metabolism.

4. Factors that influence absorption, distribution, metabolism, and excretion (ADME)

B. Microbiome

NE is taking innovative approaches to assessing the interactions between dietary exosomes (and their RNA cargos) and the gut microbiome, including selection cultures, quorum sensing, delivery of microbial RNAs through bovine milk exosomes, mutational analysis of gut microbiota and interactions of microbial RNAs with Toll-like receptors. OH is also examining the reciprocal benefits of catechins and microbiota composition the generation and absorption of microbial-derived catechin metabolism that are potentially responsible, at least in part, for the anti-inflammatory activity of green tea. In OR, the impact of age on the microbiome and subsequent impact on age-related changes in nutrient utilization is an emerging area of research. PA is assessing complex interaction of dietary bioactive compounds, gut bacteria and the host in low-grade chronic inflammation models to better harness the anti-inflammatory potential of plant foods to promote gut health. PA has also established the perinatal rabbit model of chronic intestinal and liver inflammation to study the anti-inflammatory activity and gut bacterial dysbiosis ameliorating properties bioactive compounds and probiotics. PA also established the pig model to study the gut microbiome during development of low-grade inflammation and intestinal stem cell dysregulation.

Bioavailability of nutrients and bioactive food components

C. Nitrates/isothiocyanates/indole-3-carbinol

Isothiocyanates and indoles derived from cruciferous vegetables such as sulforaphane and indole-3-carbinol have been studied extensively for their potential health benefits including cancer prevention, anti-inflammatory activity and immune dysfunction (Ho et al., 2011). However, the bioavailability of these compounds and their metabolites in humans, especially to target tissues, is relatively unknown. Researchers in OR developed sensitive mass spectrometry methods to better understand the distribution of sulforaphane and indole-3-carbinol and their respective metabolites using pre-clinical models and human subjects. Researchers have also studied effects of whole foods versus supplement sources and have recently found that supplements that lack myrosinase, a key enzyme the helps release sulforaphane, markedly decreases bioavailability of the compounds. Several cancer clinical trials are also underway to test the effects of sulforaphane supplementation. Factors such as polymorphisms in metabolizing enzymes such as glutathione-S-transferases and the gut microbiota may also alter bioavailability and determine individual responses to these phytochemicals (Clarke et al., 2011). As fruit and vegetable consumption are associated with enhanced indices of bone health across the lifespan, W3002 scientists have evaluated the efficacy of dietary nitrate to contribute to bone growth or slow bone loss after surgical ovariectomy in a female rat model system (OR). While ovariectomy in these rats altered the plasma metabolome and fecal microbiome, it was demonstrated that dietary nitrate has no effect on indices of bone health or the community structure of the microbiome in young or ovariectomized rats (Conley et al. 2017). Research at MA found that 3,3'-diindolylmethane, a major metabolite of indole-3-carbinol, inhibits adipogenesis and reduces overall fat accumulation in C. elegans. Research in this area is important for determining optimal dietary recommendations for disease prevention.

Factors that modulate absorption and metabolism

C. Nutrient-Gene interactions

Research in NE has shown that dietary depletion of exosomes and their RNA cargos elicits phenotypes such as impaired spatial learning and memory, loss of fecundity, changes in the gut microbiome, aberrant purine metabolism, changes in immune function, and changes in the hepatic and muscle transcriptome. These phenotypes are consistent with effects of dietary exosomes in Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways in human peripheral blood mononuclear cells and murine liver, brain, skeletal muscle and placenta and milk/egg (exosome) feeding studies.

Methods

Objective 1: Determine the bioavailability (absorption, distribution, metabolism, elimination) of nutrients and bioactive food components.

1. Absorption and Metabolism Modeling

W3002 scientists have developed state of the art techniques for assessing all components of bioavailability and metabolism for several nutrients and food components. These techniques address the interactions between the gut microbiome, and nutrient bioavailability and metabolism. Novel

methodologies include use of rare and stable isotopes for calcium, and 15N-labelled nitrate and nitrite and vitamins such as folate (UC-D) and vitamin E (OH). The use of these isotopes will allow W3002 scientists to precisely monitor each nutrient, its uptake, distribution into plasma and elimination in urine & feces across different life stage and physiological conditions, including physical activity. W3002 scientists can examine alterations in nutrient metabolism during different physiological conditions, including malnutrition, inflammation, and physical activity. Researchers in UC-B together with UC-D investigated the influence of folate and vitamin B12 status in our experimental animals including some novel mouse models that recapitulate some of the symptoms and metabolic disturbances observed in human B12 deficiency. Some of the proposed studies in zebrafish will allow for the quantification of effect of gut microbial metabolism on the physiological effects of dietary polyphenols. NE will study the absorption and distribution of bovine milk exosomes and their RNA and protein cargos in mice and humans with a particular focus on the blood/brain barrier and placental transfer.

3.Factors that influence ADME

Microbiome

The influence of age-related alterations in the microbiome on response to bioactive supplementation and susceptibility to stress will be examined in OR using mouse models (OR). We propose using the zebrafish model to determine if dietary elagitannins, rich in pomegranate and walnuts, improve the efficiency of muscle contraction (as evidenced by lower oxygen cost of physical activity) via the production of specific water-soluble metabolites by the gut microbiome. These studies will involve interrogating the effects of a dietary pomegranate extract on the brain and plasma metabolome, as well as the fecal microbiome, and their association with performance and behavioral phenotypes. The influence of dietary blueberries, dietary patterns, and pre-biotic fibers on the gut microbiome in rodent models and humans is being evaluated in IN.

NE will study the effects of exosomes in bovine milk on the microbiome at four levels: 1) changes in microbial communities, 2) selection of microbes in murine and human fecal samples in exosome-defined minimal media, 3) selection of mutations in microorganisms in exosome-defined minimal media, and 4) the roles of microbial RNA cargos in bovine milk exosomes. MA will also conduct the study determining the role of microbiome in metabolisms of bioactive compounds and their bioactivities using a rodent model.

Nutrient/gene interactions

Gene polymorphisms that affect nutrient flux through metabolic pathways may well be important determinants of nutrient requirements for optimal health. Initially, folate and vitamin B12 will be further studied (UC-D and UC-B). UC-B scientists are studying the influence of common genetic polymorphisms as confounders of biomarkers of vitamin status. Effects of nutrients (i.e. amino acids) and dietary supplementation of other bioactive food components on the expression of genes related to gut and brain health will be evaluated at UC-D. The influence of polymorphisms in glutathione-s-transferases and cytochrome P450, and non-coding RNA expression on bioavailability of phytochemicals found in cruciferous vegetables will also be examined in OR. NE will focus on exosome/RNA-dependent gene pathways that play roles in spatial learning and memory and purine metabolism and pathways that link the gut microbiome with human metabolism. Work in MT has

progressed along the line of selection of high free lysine cultivars of spring wheat and potatoes, and these are now in field testing.

Objective 2. Determine the efficacy and mechanisms of action of nutrients and dietary bioactive compounds towards improved health.

Obesity and metabolic diseases

CVD

Specific dietary patterns are associated with either improved or diminished cardiovascular function. In adults, sodium reduction and the Dietary Approaches to Stop Hypertension (DASH) style diet that is rich in fruits, vegetables, dairy, fish, nuts, legumes and low in added fats and sugars are associated with lower blood pressure and blood lipids. These dietary interventions will be tested in adolescents when antecedents for CVD are being established (IN). Human consumption of dietary nitrate primarily comes from vegetables in clinical studies, with reduced blood pressure and decreased the oxygen cost of exercise in humans. W3002 scientists will use the zebrafish model system to elucidate the physiological determinants of improved efficiency of muscle contraction during exercise caused by dietary nitrate (OR). Additional work in zebrafish is proposed to determine of dietary elagitannins, rich in pomegranate and walnuts, improve efficiency of muscle contraction during exercise via the production of metabolites, such as urolithins, by the gut microbiome. MT has released a new cultivar of Camelina sativa selected for low glucosinolate and high omega-3 (ALA) content, and it is being produced by an Illinois based food processing company for the value-added aspect of its extracted oil. Given omega-3 consumption is associated with decreased CVD mortality.

Gut Health

CA-D and MA will focus on determine the impact of dietary bioactive components, such as, plant extracts, on gut health and physiology with in vitro cell culture models and pig as well as rodent models. Dual benefits will be generated from this work for utilizing the bioactive food components to improve both animal and human gut health. At OR, scientists are investigating whether members of the gut microbiome in zebrafish hydrolyze ellagitannins from pomegranates to urolithins. Urolithins are associated with anti-inflammatory, improved vascular function, lowered blood pressure, and increased efficiency of muscle contraction. NE will study the effects of exosomes in bovine milk on the microbiome at five levels: 1) changes in microbial communities, 2) selection of microbes in murine and human fecal samples in exosome-defined minimal media, 3) selection of mutations in microorganisms in exosome-defined minimal media, 4) quorum sensing, and 5) the roles of microbial RNA cargos in bovine milk exosomes.

MT has developed a cultivar of barley (from an Ethiopian landrace population of barley) that shows an unusually high level of resistance to plant viruses, and it appears to be active in curing bacterial plasmids as evidenced by the mitigation of scours syndrome in lambs and calves. As plasmids are a source of toxin, intestinal binding and antibiotic resistance genes, this “remove the messenger” approach is a novel way to tackle the problem of constantly emerging antibiotic resistance.

Inflammation

Chronic inflammation is a common precursor to many chronic disease states. W3002 researchers are examining the impact of bioactive food components on mitigating inflammatory processes. OR is examining the interaction among the microbiome, inflammation and zinc status. A particular focus is on identifying bioactive food components to mitigate age-related chronic inflammation in mouse models and in humans. More research will be conducted at UC-D and MA to examine the influences of bioactive components on gut and systemic inflammation caused by infectious diseases. Research will focus on the regulation of both gut microbiome and immunity. NE will study the activation of toll-like receptors (TLRs) receptors by RNAs, encapsulated in bovine milk exosomes, in murine TLR reporter cells and TLR reporter mice; the latter will be challenged with influenza A virus to elicit a strong TLR response.

Neurological Health

Aging, specific morbidities, dietary patterns and specific bioactive food components have been associated with improved or decreased brain function, as evidenced by changes in cognition, memory and learning. W3002 scientists propose to study dietary compounds, including zinc, iron, nitrate and polyphenols, in C. elegans model (MA), the zebrafish model (OR), rodent model (AZ), and pig model (UC-D) to determine the effect on behavior, learning, memory and various other measures of cognitive function. These studies will investigate the effects of these dietary bioactive compounds on the brain and plasma metabolome, as well as the fecal microbiome, and their association with these behavioral phenotypes and physical performance. NE will study the transfer of bovine milk exosomes and their RNA cargos across the blood-brain barrier and their roles in spatial learning and memory, and prevention of seizures.

NE1602: Explorations in the Turfgrass Phytobiome: Understanding Microbial Associations and Developing Tools for Management

Duration: 10/01/2016 to 09/30/2021

Statement of Issues and Justification

Anticipated Impacts:

Advances in understanding the microbiome of the human gut has had far reaching impacts in gastrointestinal health and transformed the field of personalized medicine. We anticipate that impacts from our study of the turfgrass phytobiome will be similarly transformative for the turfgrass industry, ultimately enabling a more predictive and systems-based approach in the management of turfgrass. Our research approach to studying the turfgrass phytobiome involves a series of interdependent projects across multiple environments and will provide a basis for turfgrass research in this growing area. The microbial composition of the turfgrass phytobiome will be classified at a higher level, allowing researchers to pinpoint candidates for plant health promotion and subsequent future research. Moreover, the effects of cultural and chemical inputs on the turfgrass phytobiome will be elucidated, and stewardship of this microbial resource will be communicated to turfgrass managers. The project will bring together researchers from diverse backgrounds and result in improved exchange of information between turfgrass agronomists, plant pathologists, computer scientists,

bioinformaticians, and breeders. In our attempts to improve turfgrass management through applications in the phytobiome, it is imperative that outreach projects are effectively constructed to educate practitioners. New management concepts can be met with apprehension and confusion, but the strong extension experience offered by project participants will ensure dissemination of research results in a manner that practitioners can use, be it on golf courses, home lawns, or athletic fields. Social media and web-based tools are popular within all sectors of the turfgrass industry as a means to communicate ideas, local trends, and even management tools. With this in mind, a website will be developed in an effort to provide the most up to date information on the proposed project, along with research descriptions and a forum for questions related to our research. In addition to web-based tools, collaborations and independent work amongst the group will be broadcast through peer-reviewed publications, seminars at national meetings, research field days, and trade journal publications. Opportunities to share findings with the general public through mass media will also be sought. Continued updates will help to spread new research concepts, promote our research goals, and ultimately provide new tools for practitioners that are effective and environmentally friendly. Applications developed through our collaborative effort will result in improved sustainable management practices for major biotic and abiotic stressors of turfgrasses managed in our respective regions and throughout the U.S. Turfgrass growers will be surveyed throughout our region in year 1 and 4 of this multistate project in an effort to evaluate project impacts.

Related, Current and Previous Work

Advances in next generation sequencing have significantly improved our ability to study individual microorganisms and entire microbial communities. Although, previous research has shown that analyses of microbial communities using next generation sequencing technologies have many limitations with respect to microenvironment, plant host, sampling methods and experimental reagents (Bent and Forney, 2008; Prakash and Taylor, 2012; Yang et al., 2001). Peiffer et al. (2013) showed that recovered maize rhizosphere microbial diversity was significantly impacted by 16S primer selection as the relative abundance of phyla recovered varied according to primers associated with different variable regions of the 16S subunit. Therefore, previous and ongoing research of microbiomes reflects a growing concern for the development of standardized research practices. Standardized research methods will allow for a better comparison of data across different environments and subsequently allow for more robust recommendations in the future.

Methods

1. Developing standardized research methods for studying microbial community dynamics within the turfgrass phytobiome. Ongoing research of microbiomes reflects a growing concern for the development of standardized research practices. Previous research has shown that analyses of microbial communities using next generation sequencing technologies are significantly impacted by microenvironment, plant host, sampling methods and experimental reagents. Data generated from different research studies may not be comparable if substantitively different methods are applied, as methodological differences is a potential source of variation. Variation based on methodology reduces our ability to generate experimentally valid, systems-based conclusions about the turfgrass microbiome. Furthermore, the unique characteristics of the turfgrass ecosystem may present unique

experimental challenges, particularly in cases where the environment is intensively managed and chemical residues are abundant. Our collaborative research group will partner to provide a more thorough understanding of experimental methodology and its impact on the turfgrass phytobiome, and develop a set of core recommendations for microbiome research in this area. 1.1 Plant and soil sampling, environmental DNA/RNA extraction, and sample processing Identify and develop standardized methods for plant and soil sampling for microbial community analysis, environmental DNA processing, and sample processing for next generation sequencing platforms. Due the strong environmental impact on microbial communities, special care is needed when sampling both plant and soil for microbial communities. Laboratory technical controls are vital, due to the sensitivity of next generation sequencing technology. There is increasing data that shows even experimental reagents such as DNA extraction kits can contribute contaminant sequences to microbiome analyses. Environmental conditions can have significant impacts on the ability to extract nucleic acids suitable for next generation sequencing, especially where chemical inputs are abundant and soil organic matter is reduced. 1.2 Data analysis and metadata storage As turfgrass scientists develop research to further understand turfgrass phytobiomes, there is a growing need for a common database to store and share sequence data. A significant challenge in the application of next generation sequencing tools for studies of the microbiome is the enormous amount of data produced. Millions of sequencing reads are generated from each project. Infrastructure is needed for data storage, and specialized training and expertise in bioinformatics is required to effectively manage and analyze these data. Custom computer tools and scripts are often needed for analyses and general processing. We will partner to develop a cloud-based computer platform for maintenance and storage of turfgrass data generated on the metagenomic and genomic scale. An open-source database will allow for more efficient analysis within turfgrass microbiomes and facilitate application development within the phytobiome. A primary limiting factor for turfgrass microbial community analyses is the paucity of available genome-scale resources for common pathogens, endophytes and free-living inhabitants of the turfgrass ecosystem. True metagenomic analyses, where the gene expression of all resident microorganisms is evaluated, are stymied by our inability to provide sequence identification from reference databases, as no such reference data yet exists. Individual research groups are beginning to generate genome-scale resources for some of the key turfgrass pathogens, however these resources represent only a fraction of the organisms shown to inhabit these environments. Research from the Crouch lab shows that soil within a single golf course putting green can host 103,000 unique bacteria and 46,600 unique fungi in a single growing season (unpublished data), yet the vast majority of these organisms cannot even be identified to the genus or species level using standard ribosomal DNA signatures. To understand how these organisms function at the systems level to impact turfgrass health, simple identification is an essential first step. As a group, we will partner to further develop genome resources for turfgrass-inhabiting microorganisms, and make these resources readily available through a common platform. 2. Characterizing geographic and temporal norms of turfgrass phytobiomes. Initial research projects exploring phytobiomes in turfgrass systems are expanding our knowledge of microbes that develop with plants and how management practices are impacting community development. Further research will improve our knowledge of these plant microbe interactions and result in downstream applications to reduce the need for fresh irrigation water, synthetic fertilizers, and pesticides necessary to maintain turfgrass. The diversity among constituents of turfgrass phytobiomes will be characterized in mature and immature cool-season turfgrass stands in the Northeast, Mid-Atlantic, and North Central United

States (CT, MA, MD, MO, WI). Emphasis will be placed on identifying operational taxonomic units (OTUs) conserved across all regions which may serve as keystone representatives of the turfgrass phytobiome. Temporal changes in microbiome diversity will be assessed coincident with peak spring and fall root growth and summer root decline of perennial cool-season turfgrasses in all regions (CT, MA, MD, MO, WI). Selection of turfgrass sites will be standardized among collaborators, and specific details about each site will be recorded. Methodologies for sample collection and metagenomic analysis developed by MD-Beltsville in Objective 1.1 will be employed by collaborators. MD-Beltsville will examine the diversity of epiphytic and endophytic microbes in/on seed of various turfgrass species and cultivars. Plants will be grown under sterile conditions to compare the contribution of seed-borne organisms versus environmental cohorts in shaping the turfgrass phytobiome. Data will be shared among all collaborators in an open-source database (objective 1.2). 3. Assess the impact of turfgrass management systems on phytobiome preservation and development. Cultural management practices are integral to reducing disease and maintaining turf function on golf courses, athletic fields, and home lawns. Initial research has shown how fertilization practices can impact soil microbial communities in annual bluegrass with specific links to reductions in anthracnose disease. Moreover, research has alluded to chemical management impacts on culturable microbes in the creeping bentgrass phyllosphere. Future projects will evaluate additional cultural and chemical management practices, including organic methods for their impact on the turfgrass phytobiome. The intense management and perennial growth nature of turfgrass can also provide a model system for understanding the impact of management on the phytobiome. Moreover, identification of beneficial relationships associated with changes in management will be employed in future applications. CT, MA, MD, and NC will study the impact of turfgrass management on diversity and composition of the phytobiome. Collaborators will focus investigations on various turfgrass uses from intensively managed golf course turf to no-input residential lawn turf. CT will compare the influence of management systems including calendar-based fertilizer and pesticide lawn care, integrated pest management based, organic based, and no-input residential lawn maintenance programs on the phytobiome composition. All management systems were established at the same site in 2014; which provides a unique opportunity to study phytobiome evolution under various management inputs in the same environment. Metagenomic and bioinformatic analyses of CT samples, conducted by MD-Beltsville, will seek to identify unique OTUs among management systems; with an emphasis on those which proliferate in high performing turf stands with minimal inputs. MD, NC, and WI will evaluate the effect of various commonly used fungicide active ingredients on the phyllosphere and soil microbiome of high maintenance golf course turf. Studies will seek to address the short- and longer-term impact of fungicide use on various partitions of the turfgrass phytobiome. MA will continue to assess organic and conventionally managed golf courses for correlations between the soil microbiome and resident pathogenic and non-pathogenic nematode populations. MD will assess the role of the turfgrass phytobiome on the utility of effluent water sources as an irrigation source. The ability of various organisms to degrade, sequester, or transform common anthropogenic contaminants (e.g., personal care products, pharmaceuticals, etc.) of treated waste water may be important to expanding the use of this underutilized resource. Results will be shared and discussed among all collaborators to identify common OTUs that are promoted or suppressed by various management systems or practices. 4. Identifying constituents of the turfgrass phytobiome which confer improved abiotic and biotic stress tolerance. Candidate OTUs associated with improved turfgrass performance from phytobiome characterization studies will be identified. CT,

MA, MD, MO, MD-Beltsville, NC, and WI will use bioinformational approaches developed by MD-Beltsville to screen candidates for putative plant health functions. Microbiome constituents associated with phytohormone production or regulation, siderophore production and nutrient availability, enhanced water use, or allopathic compounds will be targeted. Microbes involved in disease suppression through induced systemic resistance, antimicrobial compound production or other mechanisms will also be identified. These attributes could contribute to greater sustainability of turfgrass systems through reductions in water, nutrient, and pesticide use. In vivo and/or in situ studies will be developed to characterize plant health effects of candidate OTUs as well as mechanisms for potential application development.

W4147: Managing Plant Microbe Interactions in Soil to Promote Sustainable Agriculture

Duration: 10/01/2018 to 09/30/2023

Statement of Issues and Justification

The other more recent development is next-generation sequencing technologies, also known as high-throughput sequencing. This technology is presently being utilized by our members. Companies are engaging in microbiome research, and using this as a tool to discover new products. These include startup companies such as Agbiome (supported by Syngenta and Genective), Bioconsortia, and Indigo. Monsanto has also invested millions in conducting microbiome studies on the hundreds of test plots for variety development.

Continued Interest in Biological Control.

Interest and enthusiasm about biocontrol continues within the science of plant pathology. Since 2012, over 3,804 peer-reviewed articles have been published on biological control of plant pathogens (Web of Science, October 2017). During this same time, 10,360 papers were published on the subject of soil health. Much of this research is based on understanding how soil physical and chemical properties influence plant performance, but soil health must be studied in the context of microbiomes and how these affect plant diseases. This will be focus of the new project. Combined with the increasing resistance in parts of the world to transgenic plants, it appears that the W-3147 regional project is both very timely and successful. Commercial interest has also increased substantially, as outlined above. A new biocontrol agent, developed by one of our members, Barry Jacobsen (MT), was just registered in 2017 by EPA as a biological plant activator and is now marketed as LifeGard by Certis. The active ingredient is a species of Bacillus mycoides that has been shown to induce resistance. This is just one example of the products that have been developed by this project over the last 40 years.

Related, Current and Previous Work

The Phytobiome

A new journal was launched in 2017 called Phytobiomes, and one of our members (Borneman) is a senior editor. The Phytobiome Initiative was launched about 3 years ago, and several of our members are a part of that effort, as members of the American Phytopathological Society. This initiative is

stimulating research support and collaborations among the scientific community. We believe that the Phytobiome and more specificaly the rhizobiome is the key to understanding how soilborne pathogens can be managed. There has also been a proliferation of new companies funded by millions of dollars of venture capital, to capitalize on using the microbiome in agriculture to find new biological agents, much like the efforts of the 1980s. These include companies such as AgBiome, Indigo Agriculture, and Bioconsortia.

Over the past several years, a number of members of the W-3147 project have been involved in microbiome research. Researchers at ARS-WA and WA have used these techniques to identify suppression to Rhizoctonia bare-patch (75) and to compare communities in the soil and rhizosphere of long-term no-till and conventional tilled wheat plots, both fungi and bacteria (74, 53). Recently, they have shown the minimal effects of glyphosate on bacterial and fungal communities (64), a paper with a large impact because of the recent interest in this herbicide, the most widely used in the world. They have shown how biosolids, processed sewage sludge, can shift fungal communities in the soil and dust. (63) They compared the communities of arbuscular-mycorrhizal in organic vs conventionally grown irrigated onions (24). Researchers in MN have pioneered the studies of the ecology of Streptomyces and their role in suppression of plant diseases (61, 66, 15).

Researchers at WA are also working on the rhizosphere microbiome of the bioenergy crop switchgrass to understand the interplay between plant physiology and microbial nitrogen fixation / nitrogen cycling (9). They are also working on understanding the ecology, evolution, and genomics of nitrogen-fixing bacteria, both free-living (27, 42, 43) and symbiotic (50, 51).

Researchers in CA have examined the bacteria and fungi in roots of citrus trees that exhibit tolerance to Huanglongbing disease in Florida. These trees have been termed survivor trees, and they are found in groves where most of the other trees are very unhealthy. Since the trees in such groves are clonal, the cause of this tolerance is likely not genetic. They posit that the cause of this phenotype is the citrus microbiome (16). In their research, they have identified numerous microorganisms that negatively correlate with disease ratings, which may prove to be useful biological control agents. One example is the discovery of several phylotypes of mycorrhizal fungi, which are known to provide phosphorus to plants, and citrus trees with Huanglongbing have been shown to be deficient in phosphorus.

Disease Suppressive Soils and Plant Protecting Microorganisms

Over the last 25 years there have been surprising and exciting discoveries for natural methods to suppress or eliminate pathogens, and/or protect plants. Intensive studies of disease suppressive soils have led to the development of new methods of analysis (17, 8, 7, 3) and new insights into the nature of soilborne disease suppression (69,20). The most interesting direction has been the use of microbiome research to describe these complex communities. Members of W-3147 are recognized as leaders in this area, as evidenced by an invited review article titled “Disease Suppressive Soils: New Insights from the Soil Microbiome” which was published in Phytopathology in 2017 (65). In this paper, Schlatter, Weller, Thomashow and Kinkel, all members of W-3147, speculate on the future of research in this area, show three case studies (take-all, Rhizoctonia, and Streptomycetes) and construct a series of testable hypotheses. Many of the advances in the study of suppressive soils have been made by members of W-3147. This includes the first identification of new bacterial genera associated with

Rhizoctonia decline in North America (75), the role of complex communities of phenazine producing Pseudomonas spp. (47), and the role of actinomycetes in suppression by glucosinolate biofumigation (13, 14, 31, 32). Such advances indicate that active management of soil microbial communities can be an effective approach to develop natural suppression of soilborne diseases and improve crop productivity (31).

Generally speaking, there are two approaches to actively managing crop-associated microbial communities. The first approach is to develop disease suppressive soils through manipulation of carbon inputs. This involves adjusting the types and timing of organic inputs, such as cover crops, animal manures, composts, compost teas, and crop sequencing. The second approach involves inoculation with disease suppressive microorganisms. These disease suppressive organisms may be identified using a microbiome approach, and then developed as effective and low-cost inocula. Members of W-3147 have done research with both approaches.

Objectives

Objective 1. To discover, identify, and characterize microbes, biological control agents, biorational compounds, pathogen-suppressive microbiomes, as well as cultural practices and organic amendments that reduce plant diseases and damage caused by soilborne plant pathogens and improve plant health.

Methods

Objective 1. To discover, identify, and characterize microbes, biological control agents, biorational compounds, pathogen-suppressive microbiomes, as well as cultural practices and organic amendments that reduce plant diseases and damage caused by soilborne plant pathogens and improve plant health.

2. Biocontrol Agents.

In the past, extensive efforts were made to isolate microorganisms at random from soil and plant material and then identify, through in vitro, greenhouse and field tests, those with potential as biological control agents or plant growth promoters. This strategy tended to yield candidate species that occur in high populations or those that grow quickly in culture. Past members of this project have produced Bacillus (41) and Trichoderma products (19). With the development of high-throughput sequencing and microbiome studies, we can now implicate and identify new fungi and bacteria. But much of the community work is still correlative- certain OTUs are associated with a phenomenon, such as disease suppression. Few studies have isolated, identified and tested candidate organisms, i.e. performing Koch's postulates. Project researchers in WA and CA were among the first to do this (75, 8). The other limitation is that many of the bacteria and fungi that are implicated in a function have been isolated or cultured. This will require new methods of directed isolation. For example, genomic understanding of the organism may provide clues to specific catabolic processes and unique carbon and nitrogen sources that can only be used by the organism. Other novel techniques include the use of isolation chips (5), co-culturing, or manipulation of the environment eg. acid conditions, high CO2.

Members will continue to search for novel biocontrol agents using more directed methods based on high-throughput sequencing. Members will share protocols, data pipelines, and develop common projects.

B. Pathogen suppressive microbiomes.

However, little is known about how larger groups or consortia function in disease suppressiveness. One line of research has focused on making synthetic microbial consortia and testing them in model systems such as Arabidopsis. Core microbiomes are identified, and combinations are tested. However, because our group is more oriented to practical applications, we will focus on describing and characterizing entire microbiomes. To this end, some of our members (WA-ARS, KS-KSU) have begun to describe the core rhizosphere microbiome of wheat in disease suppressive soils, under long-term no-till (which can promote suppression). Network analysis has provided a powerful tool to see interactions that are not evident by just analyzing the abundance of OTUs. Members of WA-ARS have begun to use these tools in describing how herbicides such as glyphosate and fertilization may have subtle effects on microbial communities (52, 62, 63, 64). W-3147 members are on the cutting edge of soil and root microbiome research, which will provide a powerful tool for understanding how natural disease suppression occurs and give clues to cultural methods that can be used to enhance this under real grower conditions. One major advantage of investigating microorganisms associated with suppressive soils is that these organisms have demonstrated the ability to function in production agricultural systems. We can study both fungal and bacterial communities, and even nematode communities. This can lead to the development of more sustainable and effective strategies to manage soilborne pathogens and enhance soil health.

C. Examining cultural practices and organic amendments that influence soilborne pathogens.

-how does Anaerobic Soil Disinfestion reduce pathogen inoculum and alter the microbiome of soil, which could reduce the pathogen recolonization? What are the best organic substrates to use in controlling strawberry diseases (ARS-WA)?

-how do Brassica seed meal amendments alter the microbiome and pathogen spectrum involved in apple replant and nematodes on vegetables (ARS-WA, CA)?

- how is the soil microbiome and soil community (fungi, bacteria, Oomycetes) changed by soil solarization and biosolarization (OR)?

Objective 2. To determine how microbial populations function to suppress disease and how plants and the environment relate to this function.

Secondary metabolite production, lytic enzymes and novel compounds.-Researchers at ARS-WA have been conducting a long-term experiment, under irrigated and non-irrigated conditions. Under irrigated conditions, the Pseudomonas population shifts to phloroglucinol-producers, but under dryland conditions, the population reverts to phenazine producers. How do these two antifungal compounds influence the soil microbiome? Researchers from NJ are looking at novel lipopeptides produced by endophytic Bacillus in grasses, and their role in protecting them from soilborne

pathogens. They are also looking at how endophytes enhance the ability of roots to take up organic forms of N and enhance plant growth. Researchers at NE working with Lysobacter discovered a novel antibiotic (HSAF) and lytic enzymes to be biocontrol mechanisms against fungi and bacteria.

Objective 3. Develop, assess, and promote sustainable strategies and practices to manage soilborne plant pathogens that are IPM-based and are compatible with organic and soil health management practices.

This objective covers the applied aspect of the project. How do we demonstrate, evaluate and promote a workable, realistic tool that growers can use for long- sustainable disease management? This first involves field testing under real conditions. Almost all of our members have been involved in this (ARS-WA, CA-R, MD, MI, MN, MS, MT, NE, NJ, NM, NY, OR, and WA). This may involve soil management practices like incorporation of cover crops and organic amendments, applying microbials to the seed, greenhouse mix or directly to the soil; or enhancing suppression by crop rotation. Plant genetics are also part of the equation. Recent research has focused on how different cultivars may support a different root microbiome and thus enhance suppression of pathogens. But it also involves assessment of the impacts on soil health through microbiome research using high-throughput sequencing and also the impacts on the pathogen. This is done with recent advances in identification and quantification of pathogens and beneficials with real-time quantitative PCR, loop-mediated isothermal amplification, and smart chip based real time PCR (Wafergen). In other words, many of the findings in Objective 1 and 2 will be applied to this objective.

Outreach Plan

4. Training the next generation of biocontrol scientists and practitioners. Almost all of our members have teaching responsibilities, both at the graduate and undergraduate levels. They teach courses in biological control and plant pathology, train students in the lab and supervise graduate students. Many undergraduate students do special or honors projects in our labs. For example, over the last four years, one member (NE) has reached 240 undergrad students in intro plant pathology and over 80 grad students in his distance education grad level course on biocontrol of pests. We train and mentor postdoctoral research associates in our labs and research stations. Finally, we have extensive international collaborations, and travel to other countries to give seminars, and host international scientists in our labs. An example is collaborations between ARS-WA and China, Turkey, Tunisia, and Morocco. Some of our members (NE) also teach in long-distance web-based courses. Over the past 4 years, project members have supervised 61 undergraduate projects, 40 graduate students and 11 postdocs. This is only a partial count, based on a survey of the members. They also teach formal courses, such as a course at CA-UCR, MCBL 126. This is an undergraduate course on microbiomes, and includes a section on biological control on plant pathogens.

W4122: Beneficial and Adverse Effects of Natural Chemicals on Human Health and Food Safety

Statement of Issues and Justification

Overview. This application is a renewal of a productive regional project that was started in 1971. The overall goal of W-3122 researchers is to examine the effects that bioactive components of the diet such as phytochemicals, foodborne toxicants, microbial metabolites, and specific macro- and micronutrients exert on human health and in the safety of the food supply. W-3122 participants collectively utilize mechanistic, preclinical, and clinical research methods to provide a comprehensive translational approach towards understanding the role of natural chemicals in human health and food safety. These efforts include use of cutting-edge research methodologies to address a broad range of research questions. Topics addressed include examination of the effects of whole foods and specific dietary components on gut ecology, understanding the molecular basis of both carcinogenesis from food-borne toxicants and chemo-protection by beneficial dietary chemicals, effects of food processing on bioactivity and bioavailability of food-borne chemicals, and trans-generational health effects of dietary and environmental exposures. The objectives of this renewal application represent our continued commitment to understanding the relationship between dietary components and human health while emphasizing emerging areas of scientific inquiry, such as the interplay between dietary chemicals and the gut microbiome and dietary regulation of the host epigenome. W3122 was selected for the Western Region Award of Excellence in 2015 and 2016, and has been highly successful as measured by numerous collaborative efforts, extensive publications and other outreach initiatives such as presentation of lectures and development of websites and curriculum modules. We anticipate that this renewal project will be equally successful and will continue to have an impact on issues related to food safety and human health.

Dietary interactions with the gut microbiome. W-122 researchers have an established record of exploring mechanisms of action of beneficial and harmful dietary chemicals and for exploring ways to mitigate or enhance their effects through agricultural practices or food processing. However, the advent of new sequencing technologies has allowed us to identify and examine how the trillions of microorganisms in our intestines contribute to host health and physiology. It has been established that these organisms are critical to digestion, pathogen protection, and immune modulation (Sekirov et al. 2010). An imbalance, or dysbiosis, of the microbiota has been associated with inflammatory diseases of the intestines but also with cardiometabolic dysfunction like Type 2 diabetes and heart disease (Festi et al., 2014) and with autoimmune conditions like rheumatoid arthritis (Wu et al., 2016) and Parkinson's disease (Sampson et al., 2016). Several mechanisms have linking microbiota, diet, and disease development or prevention are being established. One prevalent and well-supported hypothesis suggests that high fat diet induced microbial dysbiosis is associated with loss of integrity of the intestinal epithelial barrier and translocation of bacterial components such as lipopolysaccharides (ie. bacterial endotoxin), which results in a condition referred to as metabolic endotoxemia (Cani et al. 2007). Metabolic endotoxemia is associated with chronic low-grade inflammatory processes that contribute to various components of cardio-metabolic disease.

Specific microbial metabolites of dietary components are also key modulators of host disease processes. Dietary fiber serves as food for the colonic bacteria and is fermented to short chain fatty acids such as butyrate, proprionate, and acetate. These products can interact with free fatty acid receptors in the gut, liver, and adipose tissue to regulate intestinal transit time and glucose and lipid storage (Kasubuchi et al. 2015). Butyrate serves as the primary food source for colonic epithelial cells

and is though to have anti-tumorogenic effects by acting as an HDAC inhibitor (Davie, 2002). It has also been shown that butyrate is critical in maintaining hypoxic conditions at the epithelium-lumen interface and stabilizing the expression of Hypoxic Inducible Factor (HIF-1a), which regulates tight junctions between epithelial cells (Kelly et al. 2015). Conversely, other metabolites produced by microbial processes can have detrimental effects to the host. Protein degradation by colonic bacteria is associated with production of pro-carcinogenic metabolites such as N-nitroso compounds and hydrogen sulfides (Hughes et al. 2000). Choline and carnitine consumption are associated with microbial production of trimethylamine oxide (TMAO) which can interfere with reverse cholesterol transport processes and result in development of atherosclerotic plaques (Koeth et al. 2013). Therefore, understanding the influence of diet on the microbiota and microbial processes is emerging as an important aspect of understanding how dietary chemicals can influence or prevent certain diseases. W-122 researchers are making important contributions to this area, particularly with respect to understanding how dietary microbiota manipulation can be used to prevent colorectal cancer and metabolic syndrome.

Impacts of Studying Dietary Bioactive Chemicals. There are a number of positive impacts that will result from this work. First, this research will continue to improve our understanding of the mechanisms responsible for the beneficial and detrimental effects of dietary bioactive chemicals. This knowledge is the foundation for determining recommendations of dietary intakes for optimal health and disease prevention, and advancing the field of personalized nutrition which strives to provide individualized dietary recommendations based on a person's genetics, microbiome, and other factors. Second, this research will improve the safety of the food supply by determining toxic exposure levels of adverse dietary bioactive compounds as well as identifying ways that food can be grown or processed to mitigate safety risks. Third, the discovery of novel bioactive compounds, beneficial human-associated bacteria, or development of new crop varieties as a result of this research could provide new opportunities for disease prevention or treatment. Finally, research tools developed by W-122 researchers, such as reporter cell lines, new animal models, and biomarker identification can be widely implemented to improve the quality future research in this and related fields.

Related, Current and Previous Work

Interactions between diet and host microbiome are emerging as important factors in the development of many diseases. As such, understanding how dietary components are metabolized by gut microbiota, how diet changes the composition and production of important microbial short chain fatty acids, and what the downstream effects are on mucin production, immune responses, gut barrier function, and intestinal inflammation are becoming increasingly important in preventive medicine. W-122 researchers have already made important contributions and are continuing to make headway in this area. As a result, we have made investigation of effects of phytochemicals and other dietary components on gut microbiota and intestinal function a separate objective for this renewal application.

Methods

Objective 1: Examine the effects of phytochemicals and other dietary components on gut microbiota and intestinal function.

c) HI plans to discover and characterize novel bioactive compounds or chemical fingerprints that have beneficial or adverse effects on human health by modulating gut microbiome. In particular, we will collaborate with CO to explore how noni juice, a traditional Polynesian medicinal preparation, bitter melon, and coffee affect gut microbial composition and parameters related to insulin sensitivity and metabolic syndrome in mice. In addition, we will look at how coffee consumption affects gut microbiota composition and health-related parameters in a human population.

d) OSU will determine the impact of the microbiome on the synthesis of endogenous ligands for the aryl hydrocarbon receptor (AhR) and subsequent impact on the response to dietary carcinogens, such as polycyclic aromatic hydrocarbons (PAHs) that are thought to act via AhR signaling and metabolized by AhR regulated genes. In addition, we will explore the impact of the microbiome on levels of trimethylamine-N-oxide (TMANO) and interaction with genetic variants of the human liver enzyme, flavin-containing monooxygenase 3, responsible for conversion of trimethylamine (from the breakdown of dietary choline and from consumption of fish) to TMANO. The levels of TMANO have been shown to have a marked influence on cardio-vascular disease. OSU is further interested to understand the role of diet and inflammation on the lung mucosal microbiome, which similar to the gut microbiome may play an important role in host response to inflammatory-related diseases. We propose to evaluate the importance of diet on the lung microbiome in mice linked to specific markers of inflammation.

f) UT plans to determine the impact of dietary supplementation with functional foods rich in bioactive polyphenols, including BRB, on colon tumorigenesis using the azoxymethane/dextran sodium sulfate mouse model of colitis-associated colorectal cancer (CAC) and a rodent basal diet that reflects typical US nutrient intakes (the total Western diet). Changes in the gut bacteria associated with inflammation and tumorigenesis directly contribute to colon tumorigenesis, and dietary interventions with bioactives known to modulate the gut microbiome and reduce gut inflammation may contribute to reduced incidence or severity of CAC. Specifically, we will 1) Determine the efficacy of black raspberries for suppression of colon tumorigenesis using a pre-clinical mouse model of CAC and 2) assess the effects of dietary interventions on composition of the gut bacteria community and capacity for BRB-conditioned bacteria to protect the host against CAC following fecal transfer. Our experimental approach takes into account the dynamic response of the gut microbiome, as well as the dynamic response of the gut epithelium, to intervention with bioactive food components during active colitis, a period of recovery and (if failed recovery) progression to colitis-associated carcinogenesis.

Objective 4. Determine how food processing influences chemical composition to affect human health.

d) UT-AB, in a collaboration with IL, proposes to investigate the impact of thermal abuse of cooking oil on gut inflammation, composition of the gut microbiome and development of inflammation-associated colorectal cancer in mice fed a standard basal diet (AIN93G) or the total Western diet. Diets will be prepared using soybean oil that has not been abused or oil that was abused by repeated high temperature thermal cycling while cooking fish. The azoxymethane/dextran sodium sulfate model of inflammation-associated colorectal carcinogenesis will be employed using male C57BL/6J mice. Endpoints assessed will include food/energy intake, body weight gain, body composition, colitis in

response to dextran sodium sulfate, colon tumorigenesis, biomarkers of inflammation (Fluidigm PCR as described above in 2), and assessment of the gut microbiome (taxonomy, alpha and beta diversity and metagenome).

Measurement of Progress and Results

Outcomes or Projected Impacts

Improved dietary recommendations for optimizing health throughout the lifespan. W-122 work in areas such as diet-microbiome and epigenetic effects of dietary components will help inform how dietary recommendations can be tailored for improved outcomes related to maternal-child health, healthy aging, and other milestone throughout the lifespan.

NC1202: Enteric Diseases of Food Animals: Enhanced Prevention, Control and Food Safety

Duration: 10/01/2017 to 09/30/2022

Statement of Issues and Justification

Unique dynamic interactions between the enteric pathogens, animals and humans, their gut microbiota (microbiome), sharing the same environment, is considered within the “One Health” approach. In the upcoming project, we will attempt to more fully understand the role of the gut microbiome in contributing to or preventing enteric diseases and will perform studies to determine how the productive functions of the gut microbiome can be manipulated without the need for antibiotics. This new NC1202 project will develop and employ inter-disciplinary systems approaches to address critical areas that will enhance animal health, food safety and food security by maintaining efficient pork, beef and chicken production and reducing reliance on antibiotic use through development of alternative approaches for sustainable food animal agriculture. The list that follows are the major and significant enteric animal, zoonotic and AMR pathogens on which the NC1202 group will continue to work to seek novel prevention and control measures.

Antimicrobial resistance (AMR). The widespread use of antimicrobials in both food animals and humans has heightened concerns about the emergence of AMR, which impacts animal health, public health, food safety and environmental exposure. The role of the microbiome in transmission of AMR is a new avenue for understanding the extent and emergence of this problem. The NC1202 group has expertise and extensive research experience in AMR with focus on epidemiology, emergence, transmission, molecular mechanisms as well as development of innovative and sustainable approaches to mitigate AMR. The AMR mitigation strategies include but are not limited to development of non-antibiotic alternatives to antibiotics, manipulation of the gut microbiota to improve gut health, boostering innate defense to enhance disease resistance, and vaccine development. Historically, the AMR has been one of important research areas of NC1202 multistate project and will continue to be an significant topic of enteric diseases of food animals. Notably, the AMR component of this new NC1202 project does not duplicate another new project with focus on AMR (NC_temp1206).

IMPACTS, INNOVATION, OUTCOMES. 1) Emerging diseases. We expect to identify, characterize and develop improved detection and prevention methods related to newly recognized, novel or emerging causes of zoonotic enteric disease and enteric pathogens of food animals. 2) Developing preventions and interventions. We expect to develop and improve preventative measures and interventions to reduce the incidence and prevalence of infections of food animals with enteric and foodborne pathogens. We also expect to develop effective and sustainable approaches to mitigate AMR. 3) Disseminating knowledge. We will provide training or continuing education to disseminate new information to students, producers, veterinarians, diagnostic labs and others to implement interventions and preventative measures. A major expected outcome will be increased understanding of the mechanisms of initiation of acute and chronic enteric infections for known and emerging enteric pathogens. This will provide science-based best practices and implementation strategies for preventive measures and interventions for the major enteric diseases of food animals. The new NC1202 project addresses critical, timely, cross-cutting research areas and objectives (e.g. antimicrobial resistance, intestinal microbiome) that will enhance food safety while maintaining efficient pork, beef and poultry production.

Related, Current and Previous Work

Lawsonia intracellularis enhances shedding of Salmonella enterica (Isaacson Lab, Minnesota).

Our data has shown that pigs co-infected with S. enterica serovar Typhimurium and L. intracellularis shed higher levels of S. enterica and for a longer period of time (Borewicz, et al, 2015 and Patterson, et al, 2016). Our hypothesis is that an L. intracellularis infection of pigs increases the risk of salmonellosis in humans. The goals of this project are to determine the duration and quantity of S. enterica shed by pigs co-infected with L. intracellularis and to determine if vaccination with an L. intracellularis specific vaccine mitigates S. enterica shedding. A third goal is to quantify microbiome changes in the intestinal tract in response to infections with these two pathogens to identify new targets that could be exploited to reduce shedding of S. enterica.

Alternatives to antibiotic growth promoters (AGPs) (Lin Lab, Tennessee).

Developing effective alternatives to AGPs is urgently required in order to maintain current animal production levels without threatening public health. Our recent functional microbiota research in chicken strongly suggested that intestinal bile salt hydrolase (BSH) is a key mechanistic microbiome target for developing novel alternatives to AGPs; we have identified a unique BSH enzyme from a chicken Lactobacillus salivarius strain, developed an efficient high-throughput screening system to discover BSH inhibitors, and performed a series of functional, structural, and broiler studies to develop innovative alternatives to AGPs (Geng & Lin, 2016; Lin et al., 2013, 2014; Lin 2014; Smith et al., 2014; Wang et al., 2012; Xu et al., 2016).

Methods

OBJECTIVE 2. FOCUS ON PREVENTION AND INTERVENTION

Microbiomes and enteric pathogens (Hardwidge, Nagaraja, Renter Labs, Kansas).

We are developing and using techniques to study the role of the intestinal microbiota in the control of enteric

pathogens in both livestock and in the mouse pathogen Citrobacter rodentium, which is used as a model organism for the study of attaching/effacing pathogens.

Manipulating the pathogenesis Campylobacter jejuni and enterohemorrhagic E. coli (EHEC) with the microbiome (Mansfield Lab, Michigan). We are studying the role of the microbiome in acute enteritis due to Campylobacter jejuni (invasive) and enterohemorrhagic E. coli (EHEC; adherent). We expect to determine which cellular signals and pathways are differentially regulated upon adherence, invasion or translocation of humanized microbiota mice by C. jejuni or EHEC in absence/ presence of innate cells or particular microbiota.

Campylobacter and Salmonella studies (Rajashekara Lab, Ohio). We are deploying traditional and molecular microbiological techniques in our research on Campylobacter and Salmonella. These include: 1) high throughput screening of small molecules, 2) metagenomics (16S rRNA gene sequencing) to characterize gut microbiome, 3) mutagenesis; 4) physiological assays for respiration, 5) quantitative real time PCR to quantify gene expression and gene copy numbers in matrices, 6) standard isolation and enumeration culture techniques, 7) broth microdilution assay to test antimicrobial resistance, 8) PCR arrays to test innate and adaptive immunity in response to probiotic.

Lawsonia intracellularis enhances shedding of Salmonella enterica (Isaacson Lab, Minnesota). To determine the duration and quantity of S. enterica shed by pigs infected with S. enterica or S. enterica and L. intracellularis groups of pigs will be orally challenged with one or both microbes and S. enteric shedding monitored using a most probable number analysis tool. These data also will be compared to non-challenged pigs. A comparison of singly or co-challenged pigs that were vaccinated with a commercial vaccine against L. intracellularis will be performed and shedding S. enterica will be determined. Finally, DNA will be extracted from the collected fecal samples, subjected to amplification of the 16S rRNA gene, and sequenced to determine the composition of the microbiomes in these pigs.

Salmonella and Campylobacter studies (Shah/Sischo lab, WSU). We are employing traditional and molecular biological tools in our research on Salmonella and Campylobacter. These include (i) high throughput screening of mutant libraries of these pathogens for pathogenicity, and persistence in the environment (ii) characterization of individual genes of these pathogens for their contribution to infectivity in food animals such as poultry to identify suitable vaccine candidates (iii) identification of non-antibiotic alternatives (e.g., CpG islands) as immunopotentiating agents to enhance innate immunity against Salmonella and (i) characterize microbiomes of dairy animals to identify suitable probiotic agents to reduce calf mortality and prevent infection with food borne pathogens.

Campylobacter studies (Rajashekara Lab, Ohio). We are deploying traditional and molecular microbiological techniques in our research on Campylobacter and Salmonella. These include: 1) high throughput screening of small molecules, 2) metagenomics (16S rRNA gene sequencing) to characterize gut microbiome, 3) mutagenesis; 4) physiological assays for respiration, 5) quantitative real time PCR to quantify gene expression and gene copy numbers in matrices, 6) standard isolation

and enumeration culture techniques, 7) broth microdilution assay to test antimicrobial resistance, 8) PCR arrays to test innate and adaptive immunity in response to probiotic.

Prevention using probiotics (Saif/Vlasova/Rajashekara Lab, Ohio). Ongoing research focuses on new oral adjuvants (vitamins, probiotics) and vaccine approaches to improve RV vaccines using the neonatal gnotobiotic (Gn) piglet model transplanted with human infant fecal microbiota (HIFM). This model provides controlled conditions to constrain confounding variables in ways not possible in infants or conventional pigs naturally infected with diverse RV field strains. We will use omics approaches (metagenomics, metabolomics and metatranscriptomics) to understand the impact of malnutrition on host metabolome/transcriptome, microbiome composition, gut immunity, HRV pathogenesis and HRV vaccine efficacy.

Measurement of Progress and Results

Milestones

(2019): Identify genes and genetic markers utilizing high throughput mutagenesis, transcriptomics and proteomic approaches. Significant new knowledge will be gained on the emergence, development and persistence of antibiotic-resistant Campylobacter Development of an ETEC vaccine candidate Metagenomics to characterize gut microbiome Determine the pathogenicity of PDCoV to develop and assess vaccine candidates Perform both in vivo pig and in vitro cell culture experiments to study the phenotype (growth kinetics, fidelity, etc.) of PEDV

NC1206: Antimicrobial Resistance

Related, Current and Previous Work

Emergence, persistence, accumulation and propagation of antibiotic resistance (AMR) is occurring at an alarming rate in animal and human populations1-4. The Centers for Disease Control and prevention (CDC), the World Health Organization, and President's Council of Advisors on Science and Technology (PCAST) have identified AMR as one of the greatest threats to human health worldwide, and it is a threat to economic growth, public health, agriculture, economic security, and national security5. The CDC estimates that more than 23,000 Americans die annually because of infections caused by antimicrobial resistant bacteria1. Furthermore, AMR also threatens many modern medical procedures like cancer chemotherapy, complex surgeries, dialysis for renal disease and organ transplantation5. The annual domestic impact of antibiotic-resistant infections to the U.S. economy is estimated to be to be in excess of $20-30 billion with an additional $35 billion due to direct health care costs and lost productivity, respectively6,7. Prophylactic and metaphylatic use of antibiotics in livestock are major concerns considering their possible impact on selection for antibiotic resistance8. Excessive use of antimicrobials stresses the naturally occurring microbiome and allows for resistant bacteria to become dominant9. Therefore, unrestricted use of antibiotics in the livestock industry is likely contributing to the increase of resistant bacteria and emergence of antibiotic resistant strains. Such selection has a direct impact on human health as many of the antibiotics used in animal agriculture are also prescribed for the treatment of diseases in humans (e.g. tetracycline, penicillin, sulfonamides, and 3rd generation cephalosporins)10. Indeed, research has suggested that AMR might spread to humans11 through food

products of animal origin12, the environment13, and by direct contact in the case of agricultural workers14. Therefore, the development and evaluation of strategies that can reduce the prophylactic and metaphylatic antimicrobial use in agriculture animals can be pivotal to improving antimicrobial stewardship and containing the AMR threat to global health.

Because of the established links between antibiotic use and selection of plasmids described earlier, alternative approaches have been sought as a means to reduce or eliminate the use of antibiotics in poultry. One approach at UMN has involved first defining the baseline microbiome of commercial turkeys44, then understanding how antibiotics such as penicillin, virginiamycin, tylosin, and monensin modulate the turkey microbiome45 and/or broiler microbiome46. These baseline studies pave the way for development and assessment of alternative products. In a recent study at MSU, probiotics were investigated as a means to reduce AMR in pre-weaned calves. However, the probiotic significantly increased cephalosporin resistance in coliform bacteria as compared to calves not fed the probiotic47. This study stresses the need to determine the impacts on AMR for any intervention to reduce AMR and for investigation of alternatives to antibiotics.

The CU groups are investigating the mechanistic pathways by which antibiotic contamination of freshwater ecosystems may enrich resistant bacteria and impact on pathogen transmission among farmed fish via modulation of their microbiome and other mechanisms; and assess the likelihood of each pathway, severity of the consequences and associated uncertainty.

The UI group is investigating whether selective dry cow therapy can be used strategically to reduce antimicrobial use and what is its impact on the dissemination of AMR. A series of studies from Europe and Canada have suggested that combined use of teat sealant and on-farm culture might allow targeting the use of antimicrobial on selective dry cow therapy only for the high-risk group of cows. However, the impact of these strategies might vary considerably from different regions, and its impact on the milk microbiome has not been evaluated yet.

Methods

Objective 1: The goal of this objective is to continue and enhance our current surveillance methods for detection of antimicrobial resistance in a range of pathogens and commensals associated with food animals. Researchers focus on methods to enhance detection of resistance as well as design of new methods to detect new resistance traits identified through phenotypical and genotypic analysis and to address the use of newer technologies (e.g. MALDI-TOF) that can be investigated and implemented to identify new and novel resistance traits.

Investigators at ISU will study the antimicrobial resistance in pathogens of poultry (APEC), and those of commensals associated with poultry including avian fecal E. coli, Campylobacter and Salmonella. A new project will focus on understanding the role of the poultry environment in the selection of pathogens, and they will be assessing the entire microbiome as well as the genomic traits (resistance genes) of the environment using a metagenomics based approach. They will use agar, broth and disc assays to phenotypically screen for resistance as well as novel designed multiplex PCR panels to rapidly screen

for a range of resistance associated genes for use in standard PCR and real time assays52. Additionally, collaborators at ISU will investigate AMR in S. aureus and MRSA of swine and explore the emergence of novel resistance genes linked with methicillin resistance, and the resistances associated with Campylobacter15. Other researchers at ISU will work on a method to enrich metagenomic samples for targets of interest, in this case the targets would be AMR genes. These will then be combined with long read sequencing and will be used to look at horizontal gene transfer (HGT) events in the resistome between different species.

Objective 3: Investigators from UI and NCSU will test several phytochemicals for antimicrobial activity. Phytochemicals showing positive activity will be further investigated to determine the cellular, biochemical and molecular mechanisms underlying the inhibitory effect. CU investigators will attempt to develop recombinant subunit vaccines to control Salmonella Dublin and Leptospira sp. Researchers at ISU will explore the development of peptide nucleic acids as treatment for infections caused by Campylobacter jejuni. Researchers at UMN will investigate the use of probiotics and prebiotics to modulate mucosal immunity with the goal of improving overall gut health. Culturomics39 methods will be used at SDSU to identify beneficial bacteria from healthy animals. Researchers at SDSU have setup facilities for high-throughput screening of gut bacteria. Culturomics will be directed towards isolating bacteria from healthy microbiota that provide colonization resistance to pathogens. Main criteria for selecting such species will be the production of beneficial metabolites such as butyrate and propionate. Strains will be isolated using strict anaerobic culture methods and will be identified using MALDI-TOF. Inhibition of these strains against drug resistant bacteria will be then identified using co-culture assays.

At UMN, investigators will assess turkey-specific and broiler-specific probiotic blends for their ability to enhance performance and modulate the microbiome of the bird.

Objective 5: University of Illinois researchers will investigate the effect of selective dry cow therapy in US farms and its effects on milk microbiome and milk resistome to determine downstream impacts on dissemination of AMR. The study will compare decision-making strategies based on on-farm culture, somatic count cells, and history to determine which approach translates into a better outcome for milk quality and containment of mastitis. Furthermore, an economic analysis will be conducted to evaluate the feasibility of implementing these strategies for the food supply chain. Finally, an evaluation of the impact of these strategies on AMR based on the characterization of milk microbiome and resistome will be performed.

NC1180: Control of Endemic, Emerging and Re-emerging Poultry Respiratory Diseases in the United States

Related, Current and Previous Work

POULTRY RESPIRATORY DISEASE CAP PROJECT. As mentioned above, this group secured a multi-million dollar research grant from USDA - the Poultry Respiratory Disease Coordinated Agricultural Project (http://www.prdcap.com). This project includes 38 investigators from all states in the NC1180 group. The collaborative goals and accomplishments of this project are listed below.

2.We are continuing to define the microbiome in the respiratory tracts of broilers, layers, and turkeys in relation to health status and performance. This work will help us to understand the microbial communities that inhabit the respiratory tracts of poultry raised for meat and eggs. By understanding the microbial community, we can better predict and prevent disease in these animals, and identify alternatives to antibiotics. This work ultimately contributes to a sustained poultry supply in the United States in the face of increased demand and consumer pressures for change. In addition, we are collaborating with the investigators in this project to study the respiratory microbiome using models of respiratory infection. We are determining the changes that microbial communities undergo over the course of respiratory pathogen infection. We are also identifying specific microbial populations that might be favored, altered, or reduced during the infection, which can serve as critical information for development of intervention strategies. In the absence of antibiotics, alternative approaches are needed to maintain health and prevent disease, and probiotics have great promise as one such approach. Respiratory microbiome project which initially led by MN group in the current NC1180 created strong collaboration among MN, OH, and DE groups to cover different types of birds (broilers, layers, and turkeys) for comparative studies. In addition, the collaboration is essentially being expanded to all the groups working on pathogenesis to better understand the multifactorial etiology of respiratory diseases.

3.Respiratory diseases involve multiple pathogens, and they interact with each other. For example, our study highlighted the role of avian Mycoplasma in exacerbating the clinical outcome of poultry co-infected with respiratory viruses (LPAIV and IBV) and also inducing side effect from normal vaccination (eg. ILTV). Thus, researchers cannot study one pathogen but must look at how the host reacts; that can vary depending on the health condition of the host. The environment, including the air quality on the farm might affect the disease. Our co-infection studies in different environmental conditions provide important and much needed information on the interaction of respiratory pathogens in poultry, which will help improve diagnostics and vaccination strategies needed to control respiratory syndromes in poultry. Specifically, our studies provide practical information on what to expect in regards to clinical outcomes of co-infections with respiratory pathogens in different environmental and host conditions. Future studies will address the role of co-infections on susceptibility and transmission of respiratory pathogens in poultry. SEPRL, GA, OH, PA groups have been the key groups addressing this challenging area in collaboration with microbiome group mentioned above.

Objectives

3.Elucidate the pathogenesis of poultry respiratory diseases

Comments: This objective includes experimental characterization of field isolates. The selection of the isolates and poultry species will be coordinated among NC participants as described in Aim 1. Research on pathogenesis of respiratory diseases involves exploration of the intricate and complex interactions among pathogen, host, and environment. The collaborative efforts will emphasize contemporary approaches to understanding multifactorial interactions of infections impacting respiratory disease of poultry. AVIAN INFLUENZA VIRUS (AIV). Pathogenicity and transmission potential of avian, swine and human influenza viruses in chickens and turkeys will be continued. In addition, virus histochemistry will be validated to determine the attachment patterns of avian, swine, and human origin influenza viruses including recent H3N2 variant of swine-origin in turkey respiratory and reproductive tissues. We will also test tissues from chickens for comparison and also to apply this in vitro system to evaluate the potential risk of emerging strains in chickens. In vivo characterization of selected strains will be conducted in turkey poults and hens to validate the in vitro data. It is expected that the optimized and validated virus histochemistry will be a useful tool to screen different influenza viruses to evaluate their potential replication and host tropism in birds. The new tool will expedite identifying the strains for further investigation in vivo for pathogenesis and vaccine efficacy studies. Investigation of host-specific factors associated with the infectivity, pathogenicity and transmissibility in different poultry species of current and emerging avian influenza viruses will be continued. In addition, virus-specific factors and viral molecular markers associated with infectivity, pathogenicity and transmissibility of influenza viruses in poultry species will be investigated with the objective of elucidating the genetic basis for the differences in pathogenesis observed with influenza viruses in poultry species. Effect of co-infections of avian influenza viruses with common poultry respiratory viruses on the pathogenicity and detection of avian influenza in poultry species will be evaluated. Co-infection studies in SPF chickens and turkeys using low and highly pathogenic AIV, lentogenic, mesogenic and velogenic NDV, IBV, and ILTV strains will be conducted. The viruses will be given simultaneously or sequentially and in different combinations. Metrics that will be evaluated include the outcome of infection (clinical signs, lesions), presence of the viruses in tissues, duration and titer of virus shedding (for each virus), transmission to contacts, and seroconversion. Samples collected from these studies will be used to evaluate the impact of mixed infections on the detection of each virus when conducting virus isolation in embryonating eggs. These studies will provide important and much needed information on the interaction of respiratory viruses in poultry which will help improve diagnostics and control strategies. INFECTIOUS BRONCHITIS VIRUS (IBV). Novel IBV variant (S1 gene) viruses will be evaluated for their pathogenicity in chickens. Also vaccination-challenge of immunity studies will be performed to assess the potential protection provided by commercial vaccines. In addition, evolutionary pathways of IBV populations will continue to be evaluated. Both in vitro and in vivo experiments will be performed to understand the relevance of emerging subpopulations on pathogenicity and immunogenicity. Variants causing variety of clinical signs will be full genome characterized and the basis of the clinical outcomes investigated. Immune responses elicited from IBV genotypes will be also investigated using molecular, immunological, clinical and genetic tools. Special focus will be given to innate responses their cells and chemical signals. INFECTIOUS LARYNGOTRACHEITIS VIRUS (ILTV). In order to better characterize the virulence of ILTV isolates the minimum infective dose, latency, incubation period, and duration of shedding of currently circulating field strains will be determined and compared to standard challenge USDA strain. In addition, comparative genome analysis of vaccine strains and virulent isolates allowed a more accurate prediction of potential virulent determinants of ILTV. So far, vaccine strains or isolates

derived from commercial poultry have been analyzed. To further identify additional determinants of ILTV virulence, full genome sequences of strains from non-commercial poultry (backyard flocks) will be obtained. The hypothesis is that sequencing the full genome of these isolates will more precisely identify determinants of virulence and possible hot spots in the GaHV-1 genome that may trigger recombination events. This information is pivotal to developing safer live attenuated vaccine candidates. INFECTIOUS BURSAL DISEASE (IBDV). The pathogenicity of new IBDV strains will be determined in specific-pathogen-free (SPF) chickens. Point mutations, reassorting of genome segments and genetic homologous recombination events have been shown to affect the virulence of IBDV. Inoculating SPF chicks at 4 weeks of age will be used to assess the relative virulence of IBDV strains that are found to have new or unique nucleotide sequence mutations. Pathogenic strains will then be inoculated into maternally immune broilers to determine if they can break through immunity produced from a typical breeder vaccination program in the U.S. In addition, effect of IBDV infection on the pathogenicity of avian respiratory infectious diseases, particularly avian influenza, infectious laryngotracheitis, or avian mycoplasmosis will be evaluated. Specific-pathogen-free (SPF) chickens or commercial broiler or layer chickens will be orally inoculated with variant IBDV or its mutants generated by reverse-genetic IBDV and followed by intra-nasal or intra-tracheal challenge of chickens with the respiratory pathogen, individually or in combination. Histopathology, immunohistopathology, ELISA, virus neutralization assay, cytokine gene mRNA profiling by real-time RT-PCR, and viral or bacterial loads by real-time PCR or RT-PCR will be performed on various immune and respiratory tissues. METAGENOMIC APPROACH. NC participants will continue collaborating to determine co-infecting viral and bacterial agents involved in respiratory disease using metagenomic approaches. This approach will first be optimized at UMN using models of colibacillosis in broilers and turkeys, then applied to other models of disease. Procedures for examining bacterial community composition using 16S rRNA amplicon sequencing on the Illumina MiSeq platform and examining DNA and RNA viral populations using shotgun metagenomic sequencing on the Illumina HiSeq platform will first be applied to study E. coli-caused colibacillosis in commercial birds and experimental models of infection. In commercial birds, samples will be collected from diseased birds that display signs of colibacillosis. Birds positively identified as affected by colibacillosis will be retained, and tissue samples from the respiratory tract will be collected for total DNA, viral DNA, and viral RNA extraction using published methods. The goal of this approach is to identify correlations between colibacillosis and common co-infecting microbial flora that may be missed due to culturing bias, inability to culture, or non-comprehensive culturing (all of which likely occur in commercial settings). Experimental models of infection using commercial-source birds will also be used to study the contributing factors in the establishment of colibacillosis in broiler chicks and turkey poults, since it is understood that even in controlled conditions the reproduction of disease is inconsistent. Using a well-characterized E. coli challenge strain, we will challenge healthy and stressed birds and subsequently examine their indigenous microbiota and correlations between existing respiratory microbiota and the manifestation of disease. Once again, viral metagenomics and bacterial microbiome analyses will be used to identify the correlations between presence/absence of clinical lesions and indigenous flora. Once the procedures for respiratory metagenomic analyses have been established using the colibacillosis model, they will be applied in a collaborative effort with other researchers in the group. Some examples of etiologic agents that will be studied include IBV, IBDV, ILTV, NDV, and mycoplasmas. In addition to correlating the respiratory microbiota with disease state, mathematical modeling will be used to

determine generalized microbial markers of disease susceptibility. In addition, comprehensive culturing of bacterial flora inhabiting the respiratory tracts of healthy commercial birds will be performed. The goal of this approach is to generate genomic sequences of bacterial strains associated with “health.” This again will be a collaborative effort between UMN and other participating institutions. Samples from healthy turkeys and chickens of differing ages will be cultured to isolate organisms growing on non-selective aerobic media, or other media of interest for specific bacterial taxa. These colonies will be picked and archived using a Q-bot machine at UMN’s Biotechnology Institute. Archived samples will be speciated using 16S rRNA sequencing. Once dominant species inhabiting the respiratory tract have been identified, populations of these bacteria will be further characterized using molecular approaches such as MLST, PFGE, or MLVA. Representative isolates from major bacterial taxa associated with healthy birds will be sequenced using Illumina MiSeq technology or 454 Roche sequencing to generate draft genomic sequences. We will use 7-kb paired-end libraries where appropriate to effectively produce genomic scaffold that will be useful for the community of avian researchers. These isolates and genomic sequences will be publicly available for further use. The outcome of this approach is a community resource of sequenced isolates available to better understand how the respiratory microflora drives bird health.

Methods

OBJECTIVE # 1. Understand the ecology of poultry respiratory diseases

MICROBIOME AND METAGENOMICS. The primary goal of microbiome-based approaches in this Aim are to define the baseline poultry respiratory microbiome. To do so, we will sample from a variety of poultry flocks across the US. Tracheal swabs/washes and choanal swabs will be aseptically collected using standard approaches. Samples will be subsequently processed for nucleic acid extraction for microbiome analysis. Swabs will also be used for standard diagnostic assays to detect potential respiratory pathogens. The flock history including vaccination, daily mortality, etc. will be collected.

OBJECTIVE #3. Elucidate the pathogenesis of poultry respiratory diseases

Metagenomic approach.

Participants will collaborate to determine the co-infecting viral and bacterial agents involved in respiratory disease using metagenomic approaches. Experimental models of infection using commercial-source birds will be used to study the contributing factors in the establishment of respiratory disease in broiler chicks and turkey poults. We will challenge healthy and stressed birds and subsequently examine their indigenous microbiota and correlations between existing respiratory microbiota and the manifestation of disease. Once again, viral metagenomics and bacterial microbiome analyses will be used to identify the correlations between the presence/absence of clinical lesions and indigenous flora. Some examples of etiologic agents that will be studied include IBV, IBDV, ILTV, NDV, and mycoplasmas. In addition to correlating the respiratory microbiota with disease state, mathematical modeling will be used to determine generalized microbial markers of disease susceptibility. (Initially, DE, OH, MN, later participants will be include – AL, CT, GA, IN, SEPRL, TX)

S1083: Ecological and genetic diversity of soilborne pathogens and indigenous microflora

Duration: 10/01/2018 to 09/30/2023

Statement of Issues and Justification

Given the need for sound integrated pest management, approaches coordinating chemical and biological controls are needed. Recently emphasis has been placed on understanding the phytobiome of plants or microbiome of production soils. While metagenomic techniques, in theory, should allow for identification and association with soil-borne diseases, more importantly, these techniques offer the opportunity to understand biological suppressiveness (Weller et al., 2002). However, there are limitations to these methods (Nesme et al., 2016) so evidence must be combined with spatial analysis (Liu, Griffin, and Kirkpatrick, 2014) or analyzed across multiple locations and years to limit sampling error and bias (Paul et al., 2011).

Recently, the use of indigenous vs. synthetic microbiomes to control soil-borne diseases was explored (Mazzola and Freilich, 2017). There are clear advantages with respect to survival and likely efficacy when microorganisms adapted to the specific environment or competing for a similar niche in the phytobiome are used. Numerous examples are presented in the literature (albeit determined with more traditional laboratory and field techniques). For example, fungi antagonistic to Rhizoctonia have been identified and have been shown to have the ability to reduce the severity of disease on numerous crops. Of these, some are other nonpathogenic Rhizoctonia solani, binucleate Rhizoctonia or fungi in other genera such as Trichoderma spp. or a sterile white basidiomycete. Recently, R. solani AG11 was shown to be associated with reduced soybean seedling disease caused by Pythium spp. (Spurlock et al., 2016). Ichielevich-Auster (Ichielevich-Auster et al., 1985) showed that a nonpathogenic isolate of R. solani AG4 reduced damping off in seedlings of cotton, radish, and wheat by R. solani and R. zea by 76-94%. Cardoso and Echandi (Cardoso and Echandi, 1987b) reported binucleate Rhizoctonia protected bean seedlings from a virulent root rot-causing isolate of AG4. At least one T. harzianum isolate also lessened disease. In another study (Cardoso and Echandi, 1987a), binucleate Rhizoctonia isolates protected snap bean seedlings from an isolate of AG4 causing root rot by what was deemed a metabolic mechanism of protection. Snap bean seedlings were exposed to the binucleate isolate and then replanted. Replanted seedlings maintained a level of suppression of the pathogen. Root exudates from the binucleate treated seedlings were also inhibitory to the pathogen in vitro. Burpee and Goulty (1984) also reported disease suppression by binucleate Rhizoctonia on brown patch disease caused by R. solani AG2-2 III B on creeping bentgrass. Sumner and Bell (1994) reported a significant efficacy of a binucleate Rhizoctonia AG-2 and T. hamatum against R. solani AG4, and recently Spurlock (2009) found an unidentified sterile white basidiomycete that protected zoysiagrass from R. solani AG2-2.

Methods

Objective 1. Evaluate the biology and diversity of soil-borne pathogens, associated antagonistic microorganisms, and environmental conditions in the context of the whole-system phytobiome. This objective includes traditional, metagenomics, and

spatial/temporal methodologies to understand microbial community dynamics that determine soil-borne disease incidence and severity on economically important crops in the U.S.

Determination of the fungal community compositions using Illumina sequencing methods. Over the last decade next generation sequencing has become an important tool for conducting culture-independent surveys (Hibbet et al., 2011; Brown et al., 2013). High percentage of the fungal sequences has never been identified or are unknowns since they have never been found in nature or unculturable. Unfortunately some of the species may be important or critical to understanding agricultural and forest microbial dynamics over time and subsequence impacts of continuous cropping, artificial management and natural inputs being added to cropland and forest health due to environmental changes such as nutrition. Therefore, in order to track changes in the pathogen and mycorrhizal communities associated with environmental causes or specific management practices with those ecosystems, whole-community (Illumina MiSeq) data is most accurate approach for understanding microbiome fluxuations within farm sites and forest habitats. As an example, new peanut production areas in Mississippi currently have minimal soil-borne diseases impacts and baseline data from these sites will be used to monitor continuous cropping systems to determine the microbiome community spatiotemporal changes as they impact yields.

Spatial/temporal analysis from diversified cropping systems. Plant and soil sampling schemes will be designed to evaluate spatial variation within and between field sites, as well as within and between seasons (temporal variation and microbial succession). These diversity surveys, coupled with soil and agronomic data, will provide information on field management and soil conditions at which particular microbial taxa, or groups of taxa, either pathogenic or beneficial, are predominant. Further, research will be focused on microbial groups consistently showing responses across field sites and years, and will relate to dynamics of core plant microbiome establishment. Rhizospheric and endophytic bacterial and fungal isolates will be used in order to monitor dynamics of plant infection by different organisms. These experiments will be performed in greenhouse settings and microbial colonization will be measured through molecular approaches such as quantitative PCR. This work will likely include multiple crops across participating states. Exact locations and production systems have yet to be determined.

NE1834: Genetic Bases for Resistance and Immunity to Avian Diseases

Duration: 10/01/2018 to 09/30/2023

Methods

2. To identify factors and agents affecting poultry immune development, function, dysfunction, and pathology.

There are numerous genetic, environmental, nutritional, physiological, management, and microbial factors that stimulate, regulate, and shape the immune response of poultry. The members of NE-TEMP 1834 have been at the forefront of research to understand basic mechanisms and unique features of

the the avian immune response, to develop novel and effective means to promote poultry health and production. Over the past 4 years the members of NE-TEMP1834 have produced over 250 peer reviewed publications, and awarded approximately 15 competitive NIFA grants worth over $6 million. The majority of these efforts are related to our understanding of factors and agents affecting poultry immune development, function, dysfunction, and pathology, and represent some of the most influential studies related to avian immunity over the past 5 years. As this highly successful project continues, the members of NE-TEMP1834 will continue to use a variety of poultry systems, immunomudulatory approaches, and pathogens to expand our understanding of the avian immune response. AR will determine immunopathology, immune system dysregulation, and the role of environmental factors in multifactorial, non-communicable diseases such as fibrosis/scleroderma, vitiligo, thyroiditis and other (auto-) inflammatory diseases. AR will determine basic innate-and adaptive immune system mechanisms in poultry and immunomodulatory effects of nutrients on immune system development and function. Methods will include a range from whole animal studies to histological, cellular and molecular examinations, including gene-expression at the transcriptome and protein level. UCD will investigate the effects of viral respiratory infection, specifically IBV, on the upper respiratory microbiome. UCD will examine the difference in immune function and development in genetically distinct chicken lines using RNA-seq and flow cytometry. Additionally, the molecular mechanism of disease resistance will be further investigated using CRSPR-cas9. DE will continue working with Industrial partners to test innate immune inducers that increase the resistance of poultry to various microbial agents. DE will use transcriptomic analysis of the effects of these inducers on innate signaling and ultimate patterning of acquired immune responses. DE will also employ a kinomic approach to study poultry health and disease from an immunometabolism perspective. This approach broadens our view of health, metabolism, disease pathogenesis and potential intervention strategies, and identify metabolic intermediates or immune modulatory compounds to be used therapeutically. GA will study the development of T-regulatory cells and the contribution of T-regulatory cells to Salmonella persistence, with ultimate goal of developing a nanoparticle based vaccine against Salmonella. UMD will generate deep sequencing libraries from avian immune cells in order to identify epigenetic markers, patterns of alternative splicing, and noncoding RNA such as microRNA and long intergenic noncoding RNA (lincRNA). Most importantly, UMD will ascertain the factors affecting avian immune development such as enhancer, repressor, insulator and transcription binding sites (TFs) and explore their influences on chromatin and the association with immune function, dysfunction and pathology. NC will investigate the interaction between the host's intestinal microbiome and development/function of the avian immune system. As a part of these investigations, NC will focus on how differences in host genetics affect this process, identifying key members of the microbiome, their metabolites, and ultimately their role in helping the bird resist colonization and infection by avian pathogens and foodborne pathogens that reside in poultry. NL will use homozygous SNP-typed TLR1A variant chickens, challenged with various types of infectious agents to understand its role in immunity and the production of natural (auto-) antibodies. Additionally, NL will investigate transgenerational epigenesis of specific immunity and innate immunity. OH use in ovo inoculation of d18 embryos with various types of bacteria to investigate the impact of different pioneer bacteria on GIT immune system development. As part of this work the microbiome and proteome will be analyzed up to 10 days of age, leading to deeper insights into which types of bacteria promote appropriate immune development and improved sustainability. PEI will focus on the nutritional immunological factors regulating immune

responses, animal health, and food safety, with the ultimate goal to advance our understanding of the nutritional, microbiological; and molecular components affect the chicken immune response. VT will focus on the impact of in ovo and in vivo delivered supplements on the gut microbiota and development of the immune system in poultry (chickens and turkeys). VT will assess how these treatments affect gut physiology (tight junction disassembly/restructure), the impact on cellular and body metabolism, feed intake and performance, and gut immune responses. As part of these studies VT will examine these effects under specific challenges such as; necrotic enteritis, coccidiosis, APEC, salmonellosis, in addition to blackhead and cellulitis in turkeys. WU will analyze epigenetic modifications during macrophage development and investigate what influence immune stimulants (liposomal vaccines, adjuvants and others) have on training of macrophages and other immune responses in different B haplotypes.

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.

When paired with techniques such as next generation sequencing, SNP analysis, and qPCR, highly inbred chicken and turkey lines, sets of MHC-congenic lines, random breed lines and lines with distinct phenotypes allow the detection and selection of functional genetic elements that are related to immune function. To the extent that facilities and research budgets allow, IA will maintain, study and share with collaborators, several unique genetic stocks of chickens for research on the genetic basis of immune response and response to pathogens. The stocks include highly inbred lines, sets of MHC-congenic lines, and advanced intercrosses of lines with distinct phenotypes. UCD Regulatory elements such as enhancer, insulator, promoter in chicken genome will be functionally annotated and functional elements related to immune function will be identified. Genetic variants associated with disease resistance to virus infection will be used to genetically enhance broad immunity and resistance to specific pathogens in poultry. BRI MHC-Y was originally identified through polymorphic restriction fragments revealed in Southern hybridization. Until recently Southern hybridizations were the only means for revealing MHC-Y genotypes. BRI has developed simpler PCR-based methods for distinguishing MHC-Y haplotypes. These simpler methods make it easier to MHC-Y type large numbers of birds. BRI will continue to improve these methods and make them available for those wishing to define the MHC-Y haplotypes within genetic stocks and for use in defining the role of MHC-Y in genetic resistance to disease. NC To further the long-term goal of understanding how the genetic makeup of poultry determine it response to parasitic infections such as Histomonas meleagridis, experiments will be performed to identify SNPs associated with turkeys that can and cannot survive infection with a virulent strain of H. meleagridis. NL Chickens will be selected and bred such that homozygous genotypes will be obtained for a TLR1A variant on chromosome 4 using SNP-typing in combination with high natural antibody (Nab) levels to KLH (CC variant) and low NAb levels to KLH (GG variant). ARK To aid in research related to cellular and humoral immunity, genetic lines that spontaneously develop autoimmune diseases will be maintained and shared with project collaborators and techniques will be developed to monitor responses to antigen in blood and tissues. WV Congenic lines 003.R2 and 003.R4 will be maintained for use by project collaborators. Line 003.R4 differ from 003.R2 by a 225 bp insert in the BG-1 gene3’ UTR resulting in variation in immune responses to various diseases. ADOL Experiments

will be performed to develop assays to assess epigenetic modifications in a commercial or inbred chicken line, followed by analyzing the genetic variations associated with differential immune responses in B2 and B19 haplotypes. These assays will include ChiP seq, ATAC seq, PLAC seq and others. The genomic data will be made available to the poultry community at large as a resource for further research. (ADOL, NC, WVC) To study Salmonella colonization in broilers, two immunologically divergent lines of broilers based on selecting for a high and low phenotype of key innate immune markers in both sires and dams will be generated. Additionally, the role of the gut microbiome and intestinal mucosal response (secretory IgA [sIgA]) will be examined in the founder and the selected High and Low lines to determine the interplay between host genetics, the gut microbiome and local immune response, selection pressures, and S. Enteritidis colonization. (UA) To assess immune function in chicken cells, they will make an interferon reporter construct, mCherry-tagged IRF1 and IRF7 constructs, and many cloned genes and will also develop qRT-PCR primers for many chicken immune genes including IFN-beta, Mx, OASL. (VT) To continue improving the turkey transcriptome by further sequencing of additional tissue RNAs, The data will help in refining the global turkey transcriptome during early development, updating tissue-specific and overall annotations of both transcriptome and genome, and providing public tools for comparative analyses in poultry and other avian species. (UMD) By integrating different “OMICS” data, advanced methods on host-virus interaction with small number of chicken immune cells will be developed. In addition, methods on immune response modeling analysis will be established and share with project collaborators.

NC1208: Biology, Etiology, and Management of Dollar Spot in Turfgrasses

Duration: 10/01/2019 to 09/30/2024

Related, Current and Previous Work

Several biological products are currently registered for dollar spot control, including Rhapsody (Bacillus subtilis), root and turf shield (Trichoderma harzianum), and EcoGuard (Bacillus licheniformus). Other products have been evaluated in the past, including BioJect Spot-Less (Pseudomonas aureofaciens). To date, these products can reduce dollar spot during times of low disease pressure, but typically fail to provide adequate control when disease pressure increases. In addition, researchers at the University of Maryland, Rutgers University, and the University of Wisconsin – Madison are currently researching the turfgrass microbiome and the potential for an ‘antagonistic microbiome’ that would provide natural suppression of dollar spot.

NC1182: Management and Environmental Factors Affecting Nitrogen Cycling and Use Efficiency in Forage-Based Livestock Production Systems

Methods

Objective 1: Quantify environmental and economic effects of forage- and pasture-based management strategies and climate change on N-use efficiency by ruminant animals, N cycling in herbage and soils, aquatic N losses, and GHG and other pollutant emissions from grassland agro-ecosystems. (AR, GA, KY, MI, NE, TN, UT, WA, TN). Specific

objectives: (i) Investigate effects of management strategies that alter spatiotemporal distribution of soil N pools, grazing and nutritive value of forage on ruminant performance, protein metabolism, and N harvest efficiency; (ii) Evaluate environmental and economic effects of management strategies and climate change on herbage mass and accumulation, nutritive value, botanical composition, and N use efficiency across growing seasons and pasture landscapes; (iii) Determine N pool and cycling (soil, plant, atmosphere, and water), N-use efficiency and biological activity, and economic responses to management strategies in forage-based ruminant production systems with or without forage legumes across variable soil environments and climatic conditions; (iv) Determine the impact of legumes on the GHG footprint and economic returns of livestock production systems.

(iii)

In Arkansas, urine and feces from lambs offered forages from different management scenarios will be applied individually or as a slurry (urine + feces) to individual plots of tall fescue or bermudagrass that are separated by metal strips embedded to 30 cm to provide a containment border for each plot. Commercial N fertilization controls and negative controls (no fertilization) will be included. Within each plot, static chambers (30 cm diameter × 45 cm length PVC pipe) will be inserted into the soil (30 cm deep with 15 cm headspace for grass growth). Nitrous oxide, NH3, carbon dioxide (CO2), and CH4 emissions will be measured for each enclosure using static chambers consulting GRACEnet project protocol recommendations (Parkin and Venterea, 2010). Measurements will be made immediately after application, daily during the first 5 days, and at specified time intervals afterward. Grass will be clipped (5-cm) inside each enclosure 1 wk prior to the beginning of gas measurements to maintain a consistent forage height during initial gas emissions measurements. Forage will be harvested (5 cm above the soil surface) from each enclosure immediately prior to fertility addition and on days 21 and 42 to determine forage mass. Soil will be collected at the surface (0-5 cm) for each treatment immediately prior to fertilization (day 0) and at day 14, and analyzed for electrical conductivity, pH, NO3, ammonium, dissolved organic carbon (DOC) and N (DON), particulate organic matter (POM), and the soil microbiome to determine treatment effects on soil properties. At the end of each static chamber experiment, soil will be sampled at the 0-5, 5-15, and 15-30 cm depths for bulk density, particle size analysis, electrical conductivity, pH, NO3, ammonium, Mehlich-III extractable nutrients, microbial biomass C and N, DOC, DON, POM, total soil C and N, and the soil microbiome to determine treatment effects on soil properties. The soil microbiome will be analyzed at the soil surface for each treatment immediately and at day 14, and at each depth for each treatment at day 42.

NE1942: Enhancing Poultry Production Systems through Emerging Technologies and Husbandry Practices

Duration: 10/01/2019 to 09/30/2024

Related, Current and Previous Work

OBJECTIVE 2

Alternative Ingredients and Feedstuffs.

Researchers in Hawaii have been working on a project entitled “Evaluation of local feedstuffs for sustainable animal farming in Hawaii” and have been able to develop a large dataset of nutrient profiles of local feedstuffs (e.g., macadamia nut cake, microalgae, cassava root chips, sweet potato, taro, yam, wheat bran, barley brewer’s grain, and others) to test productivity and efficiency of animals (Berrocoso et al., 2017; Malabad et al., 2016; Tiwari and Jha, 2015, 2016, 2017; Yadav and Jha, 2017; Yadav et al., 2017). These data reveal that some feedstuffs are rich in energy and protein, which can serve as potential feedstuffs for poultry diets when appropriately formulated. However, these alternative feedstuffs are typically rich in fiber. The fiber is not well utilized by endogenous enzymes of monogastric animals and reduces the utilization of other nutrients as well (Jha and Berrocoso, 2015). The fiber in the feedstuffs, however, can be degraded by use of exogenous enzymes to enhance its nutritional value and gut health (Jha et al., 2015; Tiwari et al., 2018), leading to improved performance. However further concerns include the effects on the health of the poultry; there are limited data on the impact of alternative feedstuffs and pasture-based poultry production on the intestinal microbiome and gut health parameters. This is an important area for future research as they play a crucial role in immune regulation, digestion, metabolism, and general health and well-being (Jha and Berrocoso, 2016).

NC1192: An integrated approach to control of bovine respiratory diseases (NC-1027)

Duration: 10/01/2016 to 09/30/2021

Methods

Objective 1:To elucidate pathways by which host characteristics, pathogen virulence mechanisms, and environmental impacts interact to produce BRD, and to develop strategies to mitigate detrimental factors and enhance protective mechanisms.

MS and SD will characterize host immune responses to infection. MS will collect blood samples for isolation of RNA from white blood cells. Cellular RNA will be subjected to deep sequencing of messenger RNA (RNA seq) to evaluate relative expression of genes related to immune response, inflammatory state, metabolism, and other physiologic functions. Gene expression in cattle that complete a 60-day backgrounding period without being treated for BRD will be compared to that in cattle that are treated for BRD once, and cattle that are treated for BRD three times. Differences in gene expression between these populations will be assessed to determine whether major changes in expression of any gene is related to likelihood of completing the 60-day backgrounding period without developing BRD. SD will confirm findings in previous pilot work. In collaboration with WI they will measure the effect of BVDV strains on the neutrophil, NK and macrophage cytokine expression including TNF-?, interleukin-1? (IL)-1?, interleukin-8 (IL-8) and interleukin-6 (IL-6) through quantitative reverse transcription polymerase chain reaction (q-RT-PCR), and measure the effect of BVDV strains (cp

and ncp) on neutrophil and macrophage phagocytic activity. SD will also measure the effect of BVDV strains on the macrophage's and neutrophils bacterial killing ability and oxidative burst. The effects will be measured using different virulence strains of BVDV. SD will assess the role of dietary fiber in inflammation. The study will consist of 10, 150-200 kg Holstein or Holstein-Jersey steers. The entire experiment will be repeated using 4-5 animals/replicate (4-5 animals X 2 replicates= 8-10 animals). The intestinal-lymph cannulation (ILC) surgery will be performed on all the animals. These animals will be used in three experiments. First, lymph and microbiome profile will be determined in cattle fed a diet based on soy hulls, a rumen-friendly digestible fiber source. Second, lymph and intestinal microbiome profile will be determined in cattle fed conventional corn/soybean based diet. Third, the effect of inclusion of beta-glucans in the conventional corn/soybean based diet on the lymph and microbiome profile will be determined.

NE1640: Plant-Parasitic Nematode Management as a Component of Sustainable Soil Health Programs in Horticultural and Field Crop Production Systems

Related, Current and Previous Work

The loss of multi-purpose soil fumigants, such as methyl bromide, as well as several traditional nematicides from the market due to environmental concerns and the costs of re-registration has focused attention on the development of host resistance, nematode antagonistic rotation or cover crops, soil amendments and biological agents (Hirunslee et al., 1995; McSorley and Dickson, 1995; McSorley and Gallaher, 1992; Rodriguez-Kabana and Kloepper, 1998; Weaver et al., 1995). The development and deployment of plant resistance to nematodes may be the single most effective means of managing these plant-parasites. Fumigant nematicides have 'sterilized' the soil and given favor to pathogens to have a competitive advantage in recolonizing over the beneficials. This may be analogous to the gut microbiome and how the whole suite of beneficial microbes is important for a well-functioning and healthy agricultural ecosystem (Chaparro et al., 2012). Conversely, harnessing the beneficial biology in soils requires a different approach. It is more knowledge intensive. We have to understand the ecology of the beneficial organisms so they are given a competitive advantage over the pathogen (Philippot et al., 2013). Plant resistance limits reproduction of the nematodes in the target crop and in some cases can be as effective as chemical control (Cook and Evans, 1987; Starr and Roberts, 2004). In one study of the economic benefits, the $1 million cost of developing a soybean cultivar resistant to cyst nematodes was far surpassed by $400 million in benefit (Brady and Duffy, 1982). Most resistance has been investigated against root-knot and cyst nematodes, and that will be the primary thrust in these efforts. Deployment strategies for utilizing resistance will also be investigated. RKN resistant vegetable cultivars as rotational crops may limit damage in subsequently planted susceptible crops. Cucumber and muskmelons double-cropped after a RKN resistant tomato produced increased yield, had reduced root-galling, and lower densities of M. incognita second-stage juveniles (J2) in soil than the same cucumber and muskmelon cultivars grown after a susceptible tomato (Colyer et al., 1998; Hannah et al., 1994; Hannah, 2000). The RKN resistant pepper Carolina Cayenne was effective as a rotation crop for managing M. incognita in susceptible bell peppers (Thies et al., 1998). Yield of squash grown in the spring following a rotation crop of castor, cotton,

velvetbean, or crotalaria, produced heavier yields than squash grown after RKN susceptible peanut (McSorley et al., 1994). When eggplant was grown following resistant 'Mississippi Silver' southernpea, root galling by M. incognita race 1 was less severe than when eggplant was grown following susceptible 'Clemson Spineless' okra (McSorley and Dickson, 1995). While the above studies demonstrate the concept and utility of plant resistance, most resistance genes are not effective against northern root-knot nematodes, requiring unique screening efforts and research.

NC2042: Management Systems to Improve the Economic and Environmental Sustainability of Dairy Enterprises.

Methods

Objective 1. Optimize calf and heifer growth and development by improving feeding strategies, management systems, well-being, new technologies, and environmental impacts for productivity and profitability.

To continue to advance the management and performance of growing replacement heifers research will be conducted both at individual and a by collaboration include ID, LA, MN, MO, NC, NH, PA, SC, SD, and WI. As mentioned previously dairy replacement heifers represent a huge investment of resources for producers and additionally must be grown to reach optimal performance as a lactating cow. Several challenges are universal among producers raising replacement calves and heifers such as increasing nutrient utilization and efficiency, improving implication and understanding of how to use new technologies, and issues with decreasing resources are national in scope.Standard procedures for measurements and data collection will be used by researchers on the project to allow for integration of data and results among stations and allow us to make use of larger impact recommendations for dairy producers. More specifically, measurementsof passive immunity transfer in postnatal calves will be documented where possible, as well as calf morbidity and mortality. Calf and heifer performance, feed intake, housing and health management will be documented. Water intake and quality will be documented wherever feasible. Additional measurements may include blood metabolites and nutrient digestibility parameters. Where possible, heifer performance will be followed through first lactation. Efforts will be made to integrate animal welfare, environmental impact and economic comparisons to be included across collaborating states. Relationships of dietary energy and protein will be described. Pre-partum dry cow management strategies to improve colostrum quality, immunoglobulin absorption, calf health and growth. NH will lead, in collaboration with PA, MN, and SD, research with focus on pre-partum dry cow nutritional strategies and their effects on colostrum quality and absorption in newborn calves in both conventional and organic grazing systems. Optimizing IgG intake through manipulation of colostrum and colostrum feeding management will be addressed. PA plans further work on consistency of heat-treated colostrum and effects on calf health. Best management practices will be evaluated for milk (whole milk and milk replacers) and starter feeding and the impact on growth, gastrointestinal development, economic efficiencies and well-being under varying environmental conditions (LA, MN, NC, NH, PA, SD, WI). Refinement and impact of feeding strategies of conventional, moderate, and intensive milk replacers and on-farm processed milk will be addressed by all states under different environmental stressors. MN will lead efforts in collaboration with SD and WI,

to refine calf starter formulations and impact on calf performance. Efforts across all states will be to evaluate alternative supplements in place of antibiotics for disease and pathogen control in dairy calves and heifers. Some research will be conducted evaluating calves with different milk feeding systems, i.e., automatic calf feeding units versus more traditional feeding systems. Work will continue on aspects of calf feeding programs that impact gastrointestinal development (IN, LA, MS, NH, PA, SD). Assessment of nutrient utilization, metabolism, microbiome, development and growth in calves and heifers to determine measures of increased efficiency and performance under different feeding systems will also be conducted (ID, MN, NC, NH, PA, SC, SD, WI). Precision feeding of heifers will be a continuing emphasis in conventional systems that optimize feed efficiency and productivity, and minimize nutrient (i.e., N and P) excretion (NH, PA, SC, SD, WI). WI and MN, will be lead states in evaluating efficiency of grazing systems. Evaluation of feed ingredients and feed management strategies on calf and heifer growth, nutrient excretion, and subsequent effects on lactation performance (ID, MN, NH, PA, SD, WI). Specifically evaluating utilization of high- and low-quality forages, co-products or alternative feed ingredients from the biofuels and other industries and feeding fat to heifers will be assessed (ID, MN, NH, PA, SC, SD, WI). Cost effective feed ingredients will be prioritized. In the next five years methods will be evaluated on incorporating genomic data into design of nutritional studies and management practices. In addition, we will evaluate correlations of genomic data and actual performance data of heifers (ID, MN, SD, WI).

Objective 2. Optimize dairy cow performance and well-being by improving nutrition, forage utilization, technology, and management.

Research in IL, NE, NH, SC and WI will conduct studies to understand the relationship among diet, the rumen microbiome, and host metabolism as it related to milk composition. Research will attempt to establish how changes in concentration and physical form of fiber influences the rumen environment (PA, VA, WI). Production of methane, a greenhouse gas linked to animal production systems, will be measured in response to changes in diet formulations or through the addition of feed additives that impact the rumen microbial population (NE, NH, SC, WI). Studies in CA, NE, SD, and VA will study nutrient utilization of feeds under conventional dairy production systems while work in NH and MN will do complimentary work under an organic dairy production system.

Measurement of Progress and Results

Outputs

Objective 2: Peer-reviewed scientific publications. Comments: Several manuscripts relevant to this objective (i.e., feeding strategies on cow performance and health, feeding strategies on transition cow management, feeding strategies on rumen metabolism and microbiome, feeding strategies on nutrient utilization and output to the environment, etc) will be prepared and submitted for publication to Journal of Dairy Science, Journal of Nutrition, the Professional Animal Scientist, or similar scientific journals.

NC2040: Metabolic Relationships in Supply of Nutrients for Lactating Cows

Duration: 10/01/2018 to 09/30/2023

Related, Current and Previous Work

Influencing ruminal metabolism (PA and VA stations)

PA investigated the effect of diet on induction of milk fat depression including the effect of monensin on recovery from milk fat depression, ability of a methionine analog to inhibit milk fat depression, and the alterations of rumen microbial populations during diet-induced milk fat depression (Rico et al., 2015a,b). Research in VA is being conducted to assess the effects of varying dietary fiber, starch, protein, and fat on de novo VFA production in the rumen and interchange among VFA. These flux measurements are being linked to mRNA expression patterns and microbiome composition. The overarching goal of the work is to synthesize a matrix of observations that can be used to test existing models of volatile fatty acid production within the Molly cow model and develop new models if the old, largely empirical models prove to be insufficient. This work, along with the efforts at FL and OH described above, are directly integrated with efforts in Objective 3.

NE1943: Biology, Ecology & Management of Emerging Disease Vectors

Duration: 10/01/2019 to 09/30/2024

Methods

Objective 2: Research the ecology and geographic distribution of invasive and native disease vectors under changing environmental conditions to enhance our ability to predict conditions leading to existing and novel animal and human diseases.

(2.2) Make novel discoveries of the ecology and distribution of container-breeding Aedes vectors responsible for the transmission of human arboviruses (dengue, Zika, chikungunya), as well as ornithophilic vectors responsible for transmission of zoonotic viral encephalitides (WNV, western equine encephalitis, and EEE viruses).

Mosquitoes are responsible for the transmission of a number of pathogens in the U.S., which can be roughly divided into human-to-human pathogens transmitted by container Aedes spp. and zoonotic pathogens, mostly transmitted by Culex spp. While the potential for human-to-human transmission likely represents the greatest potential for large human epidemics (1000s to 100000s of new and overall cases) (Habálek 2003), zoonotic arboviruses have long been responsible for greater morbidity and mortality in the continental U.S. (Calisher 1994). Surveillance of these species focuses on trapping host-seeking adults, egg-laying females, or eggs. No trapping methodologies are completely unbiased, but most host-seeking traps can capture both container Aedes spp. and Culex spp. We will trap host-seeking adults with off-the-shelf traps, baited with CO2 and/or a chemical lure, BG-Sentinel (focused on Aedes spp.) or CDC light (a more general trap) traps (Silver 2008). Gravid trapping is usually more species-specific, although both Culex spp. and Aedes spp. that inhabit containers are often caught in standard gravid traps, with Culex spp. often more abundant (CDC or Reiter-style) (Silver 2008). We will ovitrap with black plastic cups, filled with water and baited with standard leaf infusion (Silver 2008). These approaches are effective for documenting the presence and relative abundance of these species and generate consistent data allowing for meaningful comparisons (see sub-objective 2.4). From these

surveillance data, we will make inferences about phenology, spatial variation, population genetics/genomics, and population dynamics (e.g., Little et al. 2017, Reed et al. 2018, Hopperstad and Reiskind 2016). Surveillance of Culex spp. vectors usually focus on gravid female capture and follow similar methodologies and data generation as studies on container-breeding Aedes mosquitoes. Whenever possible, we will save biological specimens for potential subsequent data collection (e.g. DNA extraction, microbiome analyses, etc.). Replicate numbers of traps will vary among indvidual trials depending on the specific study system and be subject to statistical power analyses to detect potential differences among treatment levels.

NE1748: Mastitis Resistance to Enhance Dairy Food Safety

Duration: 10/01/2017 to 09/30/2022

Methods

Objective 3: Identify and apply new strategies associated with the control of mastitis that can reduce the use of antibiotics in dairy herds (CT, ID, ME, MN, MO, NJ, TN, UT, VA, VT, WA, Canada).

The University of Missouri (MO) is aiming to explore the effects of intramammary antimicrobial usage on the fecal microbiome and resistome. This will include exploring if an increase in pathogenic bacteria are found in the feces after the administration of intramammary antibiotics, which could be a concern for dairy food safety. Future work will also identify if antimicrobial resistance patterns of fecal pathogens are affected by intramammary antibiotic administration. The University of Missouri is also evaluating antimicrobial peptides as potential therapeutics for diseases of cattle.

NE1833: Biological Improvement of Chestnut through Technologies that Address Management of the Species and its Pathogens and Pests

Duration: 10/01/2018 to 09/30/2023

Methods

Objective 2: Evaluate biological approaches for controlling chestnut blight from the ecological to the molecular level by utilizing knowledge of the fungal and hypovirus genomes to investigate the mechanisms that regulate virulence and hypovirulence in C. parasitica.

At SU, bark plugs will be collected from surface sterilized C. mollissima, dentata,and their backcross hybrids in breeding orchards of the Virginia Chapter of TACF, and placed in nutrient-free agar. Any fungi that grow from the bark plugs will be transferred to nutrient agar and maintained in culture. Each unique fungal culture will be identified based on DNA sequencing of fungal ITS regions. We will then evaluate the trees post-inoculation in order to determine the level of resistance each tree has against C. parasitica. The fungal microbiome of trees that demonstrate resistance to C. parasitica will be compared to trees that are not resistant to C. parasitica. If differences are found, we will use the list of

fungi found on resistant trees in order to select those to test as a potential bio control against C. parasitica.

W3150: Breeding Common Bean (Phaseolus vulgaris L.) for Resistance to Abiotic and Biotic Stresses, Sustainable Production, and Enhanced Nutritional

Duration: 10/01/2015 to 09/30/2020

Methods

Obective2: Analyze, document, and utilize genomic resources to enhance nutritional qualities and identify diversity within Phaseolus vulgaris to facilitate development of nutritious food products to promote human health and well-being. Subobjective 2d. Health Effects. (CO, IA, IL, MO, NE, TX). The long term goal of this research is to establish dry beans as a food system able to prevent or remediate chronic inflammation and its associated disease risk factors. Our hypothesis is that beans will protect against chronic inflammation and/or its disease risks; however, certain bean classes will be more effective than others due to their diverse chemical composition. This hypothesis will be tested by completing the two studies. In Study 1, the effects of individual components present in dry beans and combinations thereof (particularly the phenols, flavonoids and their metabolites) on chronic inflammation will initially be studied using murine RAW 264.7 macrophages (Park et al., 2008; Zbasnik et al., 2009; Zhongshi et al., 2011). These screening experiments will thus provide information on the ability of the components present in beans 1) to induce a pro-inflammatory state, 2) to maintain or remediate to an inactive state, or 3) to evoke an anti-inflammatory state. The Study 2 will involve in vivo studies to determine the effects of select bean market classes, cultivars, and/or the potent extracts, determined from Study 1, on cholesterol levels and intestinal inflammation caused by a fatty diet. A hamster model will be used for this purpose as their lipid metabolic profiles are similar to humans. Briefly, hamsters will be fed an atherogenic diet supplemented with and without beans or bean extracts at different doses for 4 weeks according to Lee et al. (2014). After four weeks, the animals will be euthanized, and evaluated for liver / plasma cholesterol markers (Lee et al., 2014) and intestinal inflammation markers, as described in Studies 1 and 2. Lastly, the effects that beans have on the microbiome of these hamsters will be evaluated as described by Martinez et al. (2009).