CALS Proposals to the

NC Agricultural Foundation, Inc.

Fiscal Year 2025

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Foundation Proposal
to the
NC Agricultural Foundation, Inc.

NCSU Number: 25-01

Proposal Title:

[25-01] Topdressing Poultry Litter on Growing Soybean and Corn to Increase Nitrogen Use Efficiency and Reduce Scheduling Conflicts [linked to Proposal 23-23]

Leader: Kulesza, Stephanie
Department: Crop and Soil Science

Co-PIs and Departments:
Rachel Vann, Crop and Soil Sciences
Luke Gatiboni, Crop and Soil Sciences
Ron Heiniger, Crop and Soil Sciences

Materials and Supplies	$2,500
Other Direct Costs	$2,708
Total Additional Expenses	**$5,208**

Percentage Research	75
Percentage Extension	25

Total of all costs for this proposal **$5,208**

Objectives

We are requesting operating funds for the project entitled, "Topdressing Poultry Litter on Growing Soybean and Corn to Increase Nitrogen Use Efficiency and Reduce Scheduling Conflicts". Additional funds are needed for sample analysis and fuel. Last year, the fuel costs associated with this project were much higher than anticipated due to the need to transport litter via our dump trailer to the stations multiple times. This is a request for a total of $10,416, split evenly over the remaining two years of the project.

Justification and Description

Research Approach:

Site Locations

Field sites will be located across the state at the Piedmont Research Station in Salisbury, NC and the Caswell Research Farm in Kinston, NC. These sites are representative of the major corn production areas of the state and encompass the wide variation in soil types and production systems encountered in North Carolina. The field study will be initiated in 2024 and conclude in the 2025 growing season.

Experimental Design

There will be four replications of four litter application rates at three timings and one inorganic N rate at two field locations (56 total plots) over a three-year study. Plots will be arranged in a split plot design. Poultry litter will be surface applied the day of planting and topdressed at V2 and V5 growth stages. There

will be four rates of poultry litter determined by the greenhouse study, with one rate above the toxicity response level if found in the greenhouse study.

Manure Testing

Each year, poultry litter will be subsampled and analyzed for macro and micro nutrients to determine actual nutrient application rates for the study period. The same poultry litter will be on both sites to ensure continuity.

Soil Sampling and Analysis

An initial background soil fertility sample will be collected prior to study initiation and after the crop has been harvested. General fertility samples will be analyzed by the NCDA&CS lab. Additionally, five times over the growing season, ten soil samples will be collected to a depth of eight inches in each plot and combined to form a composite sample. Subsamples collected from the composite samples will be transported to the lab for analysis at NCSU. Soil subsamples will then be frozen until extraction for ammonium and nitrate using potassium chloride.

Plant Tissue Testing

All plant tissue samples will be sent to NCDA&CS for macro and micro nutrient analysis. Most recent mature leaf and corn ear leaf samples will be taken twice during the growing season and analyzed to determine macro and micronutrient content. Additionally, tissue damage will be assessed weekly for a minimum of two weeks after each litter application. Each plot will be given a damage assessment score (1-5) to compare treatment effects over time.

Corn Yield and Quality

Corn yield will be collected using a small-plot combine, and grain sub-samples will be collected from each plot for analysis. After harvest, protein and oil content will be determined with a near infrared spectrometer. This should provide valuable information on the benefit of poultry litter within the growing season and allow for comparison of soil nutrient status to plant yield and quality measurements.

Assessment from the first year:

We achieved very promising results in the first year of this project, with no impact of timing on corn yield. This indicates that topdressing doesn't negatively impact corn and could increase the realistic application window by one to two months! We are very excited to continue this project and generate valuable recommendations for growers.

Explanation of Other Funding

We have requested funding for materials, supplies, and sample analysis for this project three times to each the soybean producers and corn growers associations, but it has yet to be funded.

Additional Expenses Explanation

- Materials and Supplies: $1500 for gasoline to field sites, $500 for field supplies, such as flags, stakes, bags, etc., and $500 for lab supplies, such as gloves, pipette tips, chemicals, etc. = $2500 in year 1 and 2
- Other Direct Costs:
    - Manure storage and disposal and trailer sanitation fee: $800 in year 1 and 2

- Vendor/Contracted Services
    - Soil analysis: 104 general fertility samples analyzed for macro and micronutrients at NCDA&CS at $5/sample = $520 in year 1 and 2
    - Manure testing: manure samples will be collected at each site each year and analyzed for macro and micro nutrient concentration: 2 sites at $22/sample at NCDA&CS = $44 in year 1 and 2
    - Plant tissue samples will be analyzed annually in year 2 and 3 at one sampling event: 112 plots with sampling time at $12/sample at NCDA&CS lab = $1,344 in year 1 and 2

Total: $5,208 PER YEAR for two years (total $10,516)

Foundation Proposal
to the
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NCSU Number: 25-02

Proposal Title:

[25-02] Development of a Robotic System for Automated SweetPotato Transplanting [linked to Proposal 24-56]

Leader: Xiang, Lirong
Department: Biological and Agricultural Engineering

Materials and Supplies	$5,000
Total Additional Expenses	**$5,000**

Percentage Research	85
Percentage Extension	15

Total of all costs for this proposal **$5,000**

Objectives

The long-term goal of this project is to develop a fully automated system for sweet potato transplanting, making use of cutting-edge robotics, machine vision, and machine/deep learning techniques. Automatic transplanters capable of pulling transplants from float trays are now available that can plant 10+ acres per day with one operator and a tractor driver. In order to take advantage of this technology, we must automate the process of plant singulation. The specific objectives of the proposed work are to

1. Customize a high-resolution and high-speed 3D imaging system through an offline-online co-design strategy for 3D reconstruction of sweet potato segments.
2. Develop intelligent image analysis algorithms for skeleton and keypoint identification and robotic grasping posture determination.
3. Determine the optimal moving path of robotic arms and adjust the gripper's control parameters to reliably grasp the sweet potato segments and insert them into paper chain pots.

Justification and Description

The project was selected for funding in year 2023. In the first year of this project, we developed customized camera sensors and collected a dataset of sweet potato slips. The dataset was labeled for training deep learning algorithms. Both the camera system and the deep learning model showed promising results in building accurate 3D models of sweet potato slips and identifying keypoints for robot grasp.

We have hired one PhD student for the project. The project needs further investigation before its completion. Therefore, we are requesting funds for the second year of the project to support the student and to purchase materials (such as paper chain pots, soil, etc.). More importantly, our second stage, focusing on robotic control, will be essential for the success of the entire project.

Budget justification

A PhD student (with a base stipend of $32,000 annually) will devote 12 calendar months to this project. Funds requested in support the 2nd year of the student will also include tuition support and total $32,000. The student responsibilities include but not limited to (1) sensor and robotic platform development, (2) hardware system integration and control, (3) data processing and analysis.

Materials and supplies including paper chain pots, 3D printing materials, plant materials, soil, etc. $5,000.

Explanation of Other Funding
The project receives partial funds from the NC Sweetpotato Commission. This portion of funds is mainly used for purchasing supplies and materials.

Additional Expenses Explanation
Additional expenses are requested for materials and supplies including but not limited to paper chain pots, 3D printing materials, plant materials, soil, etc. $5,000.

Foundation Proposal
to the
NC Agricultural Foundation, Inc.

NCSU Number: 25-03

Proposal Title:

[25-03] Mining a single cell protein, Corynebacterium glutamicum cell mass, from by-products of amino acid production as a novel feed additive to enhance gut health and growth of newly weaned pigs [linked to Proposal 23-27]

Leader: Kim, Sung Woo
Department: Animal Science

Materials and Supplies	$3,600
Publication Costs	$1,800
Total Additional Expenses	**$5,400**

Percentage Research	70
Percentage Extension	30

Total of all costs for this proposal **$5,400**

Objectives

Pig production is largely affected by health status of pigs especially upon weaning. Their intestinal health is compromised upon weaning due to production environments especially in this era of antibiotics free production. Feed additives to enhance intestinal health are in immediate needs. Corynebacterium glutamicum cell mass (CGCM) is obtained after producing amino acids by fermentation. However, the use of CGCM as a feed additive is rather limited because nutrients in intact cells are rather poorly digestible. Proper processes can lyse of CGCM by breaking the cell wall and these cell wall components can be wisely utilized for functions modulating intestinal immune responses, whereas the cell contents can be exposed to enhance nutritional values. Based on current experience of using CGCM in feeding livestock, it is hypothesized that processed (lysed) Corynebacterium glutamicum cell mass would provide nutritional and functional benefits as a feed additive in nursery pig diets by increased nutrient utilization and proposed functions as immune modulator in the intestine of newly weaned pigs. To test our hypothesis, the proposed study is to determine the functional and nutritional values of Corynebacterium glutamicum cell mass from lysine production as a novel feed additive to enhance gut health and growth of newly weaned pigs in antibiotics free production program.

Justification and Description

[Original plan]

Historically, single cell protein (SCP) from yeast has been limitedly used in feeding swine and chicken. More recently, application of bacterial SCP become available with commercially available products. The source of SCP tested in feeding pigs was from dried yeast or bacterial after inactivation. These products are believed to have intact cells which may have limitation with releasing cell contents that are retained in the cell. Use of SCP (regardless of sources) seems to be limited (less than 5% in the diet) which may be

due to low nutrient availability. Processing (lysis) of SCP would provide opportunities for its nutritional values and for its physiological (or immunological) functionality. Cell wall components include carbohydrates such as arabinogalactan which is known to be recognized by lectins expressed on the gut mucosa participating as innate immune defense proteins. Cell contents include protein/peptides, organic acids, nucleotides, vitamins, etc. which can feasibly be digested and available to pigs. These cell wall components and cell contents can be attractive feed additives to enhance intestinal immune status and provide essential nutrients to support fast growth of newly weaned pigs. Therefore processed (lysed) CGCM can be used as a novel feed additive (or supplement) for potential intestinal immune modulation and nutrient sources which can be beneficial for nursery pigs under intestinal challenge condition immediately after weaning.

CGCM will be processed by a large scale homogenizer (200 L/hr capacity, BTEC, Raleigh, NC) to rupture Corynebacterium glutamicum cells. After processing, CGCM will be checked to check % of cell ruptured using a Flow Cytometer. Our target is to rupture minimum 70% of cells in CGCM. Analysis will be conducted at Diagnostic Testing Lab of Comparative Medicine Institute at North Carolina State University (Raleigh, NC). After homogenization, processed CGCM will be freeze dried (the research purpose: freeze drying is not needed for a commercial application).

Nutrient composition including carbohydrate structure will be determined before the use in the study. CGCM with or without processing will be analyzed for proximate nutrient analysis as well as for amino acid content to consider in diet formulation and to confirm if nutrient content is not altered after processing. If nutrient content is modified, supplementation levels of both CGCM to experimental diets will be adjusted. Analysis will also include glycosyl composition and degree of polymerization. In the proposed study, 60 newly weaned pigs at 3 weeks of age (5.5 to 6.5 kg body weight) will be used in a randomized complete block design with sex and initial body weight as blocks. There will be 60 pens where pigs will be housed individually. Within a block, pigs will be randomly assigned to 5 dietary treatments (n=12). The first 4 treatments will have increasing supplemental levels of lysed CGCM from 0, 2, 4, and 6%. The 5th treatment will be intact CGCM (4%).

In order to test the functional role of CGCM, supplementation of CGCM will replace soybean meal in the diets based on equal crude protein content. Supplemental amino acids (L-Lys, L-Met, L-Thr, L-Trp, and L-Val) will be used to match the content of essential amino acids among experimental diets. Pigs will be fed for 21 days based on 2 phase feeding program: phase 1 (initial to 7 kg body weight) and phase 2 (7 kg body weight to the end of the study). Experimental diets will be produced and processed at Feed Mill Educational Unit.

Feed intake and body weight will be measured at the end of each phase. Fecal scores (based on 1 to 5 scale) will be recorded at d 3, 5, 7, 9, and 11. On d 18, blood samples will be taken to obtain plasma. Experimental diets will include 0.4% titanium oxide as an indigestible external marker and fed during the last 7 day period. After 21 day feeding, all pigs will be euthanized to take tissue, mucosa, and digesta from proximal jejunum and distal jejunum. Digesta will also be collected from ileum. Mucosa will be used to measure pro-inflammatory cytokines, oxidative stress markers, and immunoglobulins. Tissues will be used to measure tight junction proteins to test gut integrity, enterocyte proliferation, and gut morphology. Mucosa and digesta will be used to determine microbial population changes at species levels by microbiome sequencing. Diets and ileal digesta will be analyzed to measure titanium oxide, dry matter, amino acids, crude protein, and gross energy to measure apparent ileal digestibility of dry matter, amino acids, crude protein, and energy.

[Supplemental Plan]

During the recent years, our lab has been engaged in developing a PCR based identification of bacterial sensing receptors (TLR2, TLR4, NOD1, NOD2, CD14, GCP) on the animals enterocytes (epithelial cells lining the mucosal layer) which regulate immune response to release IFN-r, IL-8, and IL-6. This response may turn on mTOR signaling pathway to activate intestinal stem cell in crypts to proliferate. Investigation

of these mechanisms are pioneering in pig nutrition and pig production. This supplemental investigation will bring innovative new knowledge showing how to evaluate and select postbiotics or probiotics to help the intestinal health of young animals including pigs.

Explanation of Other Funding

This project (under the same objectives) is not currently funded by other sources. However, the PI plans to seek for additional funding from National Pork Board, North Carolina Pork Council, and allied industry to support the proposed studies.

Additional Expenses Explanation

Materials ($3600)

- PCR reagent: $800
- Microtubes: $700
- cDNA synthesis and kit: $1200
- qRT PCR kit and supplies: $900

Publication ($1800)

- One peer reviewed paper

Foundation Proposal
to the
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NCSU Number: 25-04

Proposal Title:

[25-04] Development of Novel Value-Added Food and Feed Products and Processing Techniques to Improve Viability, Profitability and Sustainability of Industrial Sweetpotato Processing [linked to Proposal 23-62]

Leader: Simunovic, Josip
Department: Food, Bioprocessing and Nutrition Sciences

Co-PIs and Departments:
Fernanda Santos, Food, Bioprocessing and Nutrition Sciences

Materials and Supplies	$15,000
Publication Costs	$5,000
Travel	$6,000
Total Additional Expenses	**$26,000**

Percentage Research	90
Percentage Extension	10

Total of all costs for this proposal **$26,000**

Objectives

- Development of Novel Value-Added Food and Feed Products and Processing Techniques to Improve Viability, Profitability and Sustainability of Industrial Sweetpotato Processing
- Collaborate with the currently actively operating, pending and anticipated processing facilities for sweetpotato processing and preservation in North Carolina
- Analyze the processing and production methods and operations in order to locate and identify the sources and streams of waste and secondary biomaterial sources, including pre-process, in-process and post-process material sources
- Particularly focus on facilities with multiple potential sources of materials for further processing and commercial value addition such as frozen sweetpotato fries processing facilities in North Carolina
- Multiple sources of waste / processing materials in swetpotato frozen french fry facilities include:

    a. Pre-process rejected roots, due to a variety of criteria, particularly general geometric properties like size and shape
    b. In-process surplus of mechanically removed skins and peels, generating a slurry rich in bioactives and nutrients, like antioxidants, proteins, fibers and minerals, which is currently recovered only to be spread back to the fields as a fertilized, while it could be profitable processed particularly into poultry feed materials
    c. In-process surplus/waste of short small edge and corner pieces after the cutting of longer rectangular pieces of french fries to be frozen. This stream could be particularly useful in the

production of clean, nutritious purees and blends since it has been pre-cleaned and pre-processed so would be easy to adapt as an ingredient for further processing

- Propose and develop novel food and feed products based on one or more of the waste/surplus streams noted above
- Test the viability and acceptability of the developed foods and feeds
- Propose, assemble and demonstrate the processing, preservation, packaging and storage techniques for developed food and feed product lines
- Generate reports and publications from the results of R&D and share with the local, national and international stakeholders and the scientific / academic communities

Justification and Description

Development of Novel Value-Added Food and Feed Products and Processing Techniques to Improve Viability, Profitability and Sustainability of Industrial Sweetpotato Processing

Invention, development, commercialization and implementation of an advanced thermal processing technology - i.e. Continuous Flow Microwave Sterilization and Aseptic Packaging for Foods and Biomaterials by our team of researchers at the NC State Department of Food, Bioprocessing and Nutrition Sciences and its subsequent implementation at Yamco LLS in Snow Hill, NC for production of the first microwave sterililized shelf aseptic stable food in the U.S. as well as anywhere in the world has been a major scientific, technological and idustrial success. This development was recognized with a number of major national awards for innovation, collaboration, research and development and industrial achievement. The facility was established to process the previously rejected sweetpotato roots (up to 40% of the crop sometimes) into nutritionally and organoleptically superior shelf stable sweetpotato puree packaged in large aseptic packages for further use by the industry, bakeries and restaurants. This development was followed by the addition of about a dozen other fruit and vegetable materials to Yamco's palette of shelf stable puree ingredients. The patented processing technology was also established as the technological basis for several new food processing installations, as well as several innovative food technology, process and product development paricularly in North Carolina (Wright Foods in Troy, Carolina Dairy in Biscoe, SinnovaTek, SinnoVita and FirstWave Innovations in Raleigh, Windridge Sensors in Wilmington, Mind Full Inc. in Raleigh, ThermaLytics in Raleigh, Covington Distillery in Snow Hill, Induction Food Systems in Raleigh, Ripe Revival in Rocky Mount). These new businesses are actively collaborating to introduce new products and technologies to the US and international markets. Additionally, there are active and pending international projects with private, academic and government collaborating institutions with processing facilites under construction, contracted or currently negotiated in Kenya, South Africa, Ghana, Nigeria, Honduras, Dominican Republic and Europe). In the meantime, North Carolina has increased its national and international dominance as the prime supplier of sweetpotato roots, varieties and science and technologies for their breeding, selection, production and processing. Over 200 new sweetpotato based commercial food product lines which have emerged during the last decade. There are also new generations of health-promoting, nutritionally superior products under development using microwave-sterilized sweetpotato purees like sweetpotato beta-carotene enriched bakery products in Kenya, reducing the country's dependence on imported wheat and providing a nutritional basis to combat vitamin A defficiency for children in Africa (for which SinnovaTek, another spin-out company based on technologies developed by our teams, has received the 2022 Tibbetts Award from the US SBIR Administration), and novel bioactive baby and infant food products processed by the FirstWave Innovations in Raleigh . Due to North Carolina's dominance in high quality sweetpotato production, there has been a recent growing interest in new processing facilities, particularly frozen sweetpotato french fries and other sweetpotato and vegetable based products (Trinity Foods, Cardinal foods, Natural Blend Vegetable Dehydration, Carolina Innovative Food Ingredients). All of these active

and emerging processing installations provide a basis for sourcing of surplus, waste and secondary material sources which will be considered for further processing and novel value added nutritionally superior food and feed products by this project proposal.

Two initial product lines will be investigated, developed and tested initially:

1. Shelf stable nutrient rich blend of sweetpotato puree and plant based protein and lipid / energy enriched formulations for intervention feeding of nutritionally challenged populations (particularly infants and children) in underdeveloped countries
2. Poultry feed blends/additives to improve the nutritional and health benefits of chicken and turkey feeds Additional product lines will be developed, added and introduced according to the needs and interests evolving during the project performance. One of the possibilities is the initiation and construction of a small local bakery to provide sweetpotato enriched bakery product lines.

Methods for preservation, packaging, storage and safety assurance will be developed and implemented for both listed and other pending product lines. Technological and economic effects will be studied, evaluated and reported. Results of these studies will be shared with the collaborating industrial processing companies, other shareholders and submitted for publication in reputable scientific journals.

Explanation of Other Funding

The project is primarily funded by the Ag Foundation. Since the original funding only included the graduate (PhD) student assistantship and tuition support, other sources have been used intermittently to support the work on this project. Progress report files are presently sent to Mr Lewis separately.

Additional Expenses Explanation

The additional expenses are requested annually - i.e. the additional total of $26,000 is requested for operating expenses for each year of the project performance.
This is primarily reflecting the expanded objective of in-vitro anti-cancerogenic testing of the bioactivity of polyphenolic extracts and complexes based on both established and novel varieties of purple fleshed sweet potatoes, publication expenses as well as expanded planned international collaborations, particularly in Kenya (processing plant built and in production), South African Republic (processing plant project approved for funding and the equipment under construction, with additional funding pending) and Uganda (processing plant and associated R&D projects under current negotiations.
Development and testing of new ingredients and products based on North Carolina sourced sweet potatoes will continue as planned in the original project proposal, with the added activities focused on the use of sweetpotatoes, sweetpotato based ingredients and products in European countries - additional projects and funding currently negotiated.