



BC Sustainable Poultry Farming Group
Project updates for May 26, 2017 meeting

2015-01 Genomic Analysis of Wetland Sediment as a Tool for Avian Influenza Virus Surveillance in Wild Waterfowl

- They are in the final stages of preparing a publication; they will be invited to the next meeting to provide an update in person.

2015-02 - The Development and Commercialization of Aerobic Digestion of Poultry Manure to Produce Bio-Active Fertilizers

- The project focuses on the aerobic digestion of poultry manure to produce plant fertilizer solutions to be used in greenhouses and field crops. Their fermentation process utilizes oxygen enhancement and pH control to minimize nutrient losses due to precipitates and ammonia off-gassing. The use of oxygen greatly reduces odours and accelerates the process.
- **Technology and Project Developments**
 - I. Supplementing Soluble Carbon to Increase Microbial Biomass**

All 13 fermentation runs to date are characterized by having a (rapid) exponential decay of soluble carbon given by BOD, Biological Oxygen Demand, graphs. The addition of methanol to the culture served to yield 1000 X more bacteria. We're up to 10^{11} bacteria per mL - that's 100 million bacteria per drop

This highlights the pathogen kill due to competition from other bacteria. It would be ideal to co-ferment a waste stream rich in soluble carbon with poultry manure.

- II. Iron Supplements** We're experimenting with adding iron sulfate to the culture in order to increase the iron concentration. The goal is to *pressure* the bacteria to uptake iron; if so, once they die the iron would likely be available to plants. Chemical analysis does indicate increased iron concentrations.

Peculiarities have been observed. Divalent cation oscillations have been observed for the first time. Iron had these oscillations for both types of analysis – dissolved iron varied between 15 and 185 / 125 ppm – the final solution assayed for 100 ppm iron – easily 5 to 10 times more iron than prior runs. *But, is it available to plants?*

Are these oscillations due to chemistry – phosphate is quite consistent it fluctuated between 750 and 1,000 ppm - (*Maybe more conjecture than hypothesis*) *Can these divalent cation oscillations be due to microbial biomass i.e. uptake during growth and release during death phase?* It's difficult to conclusively correlate runtime heat cycles to these cation concentration cycles.

One of many questions: at the valley of these oscillations where did the cations go?

- III. **pH Control Agent Development** The acid control agent is now an acid blend of sulphuric (the cheapest) and phosphoric (the most expensive) acid; doing so serves to modestly increase the phosphate levels. The caution is not to increase the phosphate concentration so much as to lose nutrients by inducing irreversible phosphate-divalent cation precipitates. Similarly, the base control agent is a blend of ammonium and caustic (potash) hydroxides; the addition of ammonium hydroxide may serve to stimulate bacteria growth especially at the beginning.

Most runs tend to go to higher pH's (near 9); at these times there's considerably more acid added to the culture than base. However, at times (possibly due to low oxygen levels and other factors) there are "runs that go acidic" – in the presence of a catalyst it may be able to produce hydrogen during these conditions.

- IV. **Vinegar as an Antifoam Agent** Commercial cleaners use vinegar as an antifoam agent; the project also tried using vinegar as an antifoam agent: It appears adding vinegar to the culture at the beginning does delay the need for antifoam addition – this allows the culture to easily attain temperatures near 70°C since antifoam addition is known to negatively impact broth temperatures. However, there appears to be a negative impact (unknown at this time) in using vinegar since the last two runs stalled and vinegar may have induced a "major Foam-Out".
- V. **CARA Soil Health Study** A producer research group will be investigating the solution's impact on soil health; soils were trialed Fall of 2016 and Spring of 2017. The group will likely be assessing the impact on a Tree Farm's three year trial of the solutions.

- For greater process / technology detail a webinar was archived at this link: [<https://gov-ab.webex.com/gov-ab/onstage/g.php?PRID=af09e48e11e118d1f4c659631e98c4df>] select, 14 February, 2017
- They presented at the "Cultivating Connections Forum" Edmonton Northlands, the display booth was well received; the project also had a display booth at Edmonton's Green Industry Show & Conference.

2015 07b Reducing Salmonella and Campylobacter contamination of poultry

- The goal of this proposal is to reduce the load of both *Campylobacter jejuni* and *Salmonella enterica serovar Typhimurium* (*S. typhimurium*) in the digestive tract of chickens using different methods.
- Garlic-derived organosulfur compounds will be identified and the treatment to either chicken house or chickens with the microencapsulated sulfur compounds will reduce the numbers of broilers colonized with *C. jejuni* and *Salmonella*.

- A de novo approach will be developed to identify lactobacilli with probiotic activity to decrease or reduce the load of *C. jejuni* and *S. typhimurium* in the intestines of chickens.
- They will construct an engineered *Lactobacillus* bacteria strain that displays immunogenic epitopes of *C. jejuni* and *S. typhimurium* colonization associated proteins. This vaccine strain will stimulate the production of protective antibodies, resulting in a reduction in pathogen colonization.
- Results
 - **Section 1: Microencapsulation of garlic oil and diallyl sulfide –**
 - The weight of the oil microcapsules was less than β -cyclodextrin and oil added. The microcapsule yield result indicated that the 8:92 GO: β -CD had a higher recovery compared to that of 4:96 and 12:88 ratio. For DS: β -CD microcapsules, the 4:96 ratio had the highest recovery. Along with the increase in the amount of garlic oil added, the recovery increased from 85% to 90% then dropped down to 70%. This may be due to either the loss of mixture during experiment or the loss of some volatile compounds during freeze drying. Some compounds in garlic oil especially the sulfide compounds tend to evaporate easily and can lead to the decrease in recovery rate.
 - The highest efficiency for diallyl sulfide was achieved at the ratio of 96:4. For garlic oil, it was achieved at the ratio of 92:8. This is probably due to the saturated microencapsulation capacity of β -cyclodextrin. It suggested that the saturation point was between 96:4 and 92:8 for diallyl sulfide, while the range for garlic oil was 92:8 and 88:12.
 - To further access the microencapsulation capacity of β -cyclodextrin of diallyl sulfide and garlic oil, the amount of microencapsulated oil (mg) per gram of β -cyclodextrin was analyzed. The result indicated that the highest amount of encapsulated oil was achieved at the ratio of 88:12 and 92:8 for diallyl sulfide and garlic oil, respectively.
 - **Section 2: Microencapsulation of *Lactobacillus* under acidic conditions**
 - The efficacy of bacteria that released from alginate microcapsules versus free-suspended bacteria in PBS (control sample) was tested over 24 hours at room temperature. The microencapsulated sample showed slightly higher viability than the test control sample and overall stability.
 - The viability of encapsulated *L. casei* versus non-encapsulated *L. casei* was assessed over a 10 day period at room temperature. As shown in Figure 8, the microencapsulated bacteria showed a higher stability over the course of 10 days. At day 10, the non-encapsulated bacteria were no longer viable and the encapsulated bacteria had a 40% reduction from the starting level.
 - The number of viable encapsulated bacteria in various pH conditions was compared. Each separate test group was analyzed over different time periods. All the tested conditions were repeated in biological triplicate. As shown in Figure 9, there is almost no change in viability for the encapsulated bacteria subjected to pH 3.5 or pH 7 over 120 min.

- **Section 3: Determine the effect of garlic-derived organosulfur compounds on C. jejuni colonization of chickens**
 - In brief, ajoene treatment did not reduce colonization of Campylobacter in chickens. Based upon this result, we decide not to further test the effect of other garlic-derived organosulfur compounds on C. jejuni colonization of chickens because these compounds have lower in vitro antimicrobial effect against C. jejuni and Salmonella compared to ajoene.
 - The major possibility to explain this result is that garlic compounds are relatively volatile. During its delivery to chicken gastrointestinal tract, the majority of the garlic compounds are degraded with a significant loss of bioactivity. Therefore, microencapsulation is highly required to protect the bioavailability of these compounds during the delivery to chicken gastrointestinal tract.
- **Section 4: Construct a Lactobacillus vaccine strain**
 - The rationale for is to narrow the site of interest within a C. jejuni surface-exposed colonization protein (SECP) to 30 residues and to test if a combination of fragments from different proteins would generate enhanced protection (i.e., a reduction in C. jejuni colonization) versus injection with an individual C. jejuni SECP. We defined the 'site of interest' for the CadF, FlaA, and FlpA proteins to be the site most likely to be surface-exposed, immunogenic, and protective (i.e., required for protein function). Our strategy also involved generating both GST-tagged 90 mers and His-tagged full-length proteins (minus the signal peptide) for CadF, FlaA, FlpA, and CmeC (Figure 12). The trifacta (CadF-FlaA-FlpA) peptide, composed of three 30 mers, was generated only as a GST-tagged protein. The rationale for using two distinct peptides is to generate an antibody response against a specific region of the protein using the first injection with the GST-90 mers. The booster injection then used a full-length version of the peptide to enhance the immune response without including the 26 kDa GST tag, resulting in specificity against the 90 mer fragment (and not GST). The 90 mer regions used were chosen based on putative surface exposure (i.e., hydrophilicity predictions), conservation amongst C. jejuni strains, and the presence of sequences required for protein function.
 - The design of this vaccine strain is still in progress.

2016-01 Turkey Manure Fermentation

- The goal was to add turkey manure to project 201502 aerobic fermentation. However, matching funding was not obtained by the researcher, so SPFG has pulled their funding.

2016-02 One-day knowledge translation workshop in April 2017 on Wetland Sediment testing as a Tool for Avian Influenza Virus Surveillance

- The workshop has been delayed.

2016-04 Validation of Cedar Leaf Oil Vapor in Poultry Hatcheries and Processing Facilities

- They received \$140,000 from the Investment Agriculture Foundation of BC (IAFBC) as part of the Canadian Government's Agri-Innovation Program, to validate their system within commercial chicken farms and chicken processing facilities.
- As part of the IAFBC Program, their system was installed in a chicken broiler barn (12,000 square feet, about 12,000 chicks), which had an outbreak of the Adenovirus (IBH) (17% mortality) in its previous cycle. The next cycle with our system, mortality was reduced to 3.2% and IBH was not detected. They are now in the final stages of the second cycle and mortality is again around 3%, which is approximately 50% less than the average. The chicken production weights have also increased about 10%, compared to average.
- They have also installed a system in a chicken processing facility, which received a number of positive detection counts for campylobacter, since the installation of our system, all the test results are negative.

2016-05 Dairy/poultry manure co-digestion with pre-treatment and nutrient extraction

- The project did not receive funding from IAF.

2016-06 BCAC's Public Trust Initiative

- The majority (85%) of BCAC Members approved the Public Trust Initiative concept and committed to the special levy. At the January meeting, the BCAC Board approved the draft Terms of Reference for the steering committee, draft work plan and coordinator's job description.
- The feeling at the BCAC board was that industry has to lead on Public Trust, or someone else will do it for industry and not in a way that helps farmers. The BCAC Board wants to be in place to direct/coordinate/lead the work.
- They've discussed this idea with some of their input suppliers. They agree with the need and like the idea.
- Next steps
 - They are recruiting individuals to sit on the Steering Committee (Shar Bennie is the poultry rep) and will begin the hiring process for a provincial coordinator as soon as possible. They have had initial discussions with other potential partners (input suppliers, allied trades etc) who are interested in becoming part of the initiative.
 - As much of the work will continue being done at the commodity level, the provincial coordinator will be connecting with staff working on or have interest in helping with the Public Trust Initiative. They anticipate that this ad hoc group will develop many of the Initiative's details and facilitate getting the work done.

2016-07 Development of a Point-of-Care PCR diagnostic platform and assays for the poultry industry – Phase Two – field trials

- In the last week of April Aquila conducted their first field trials for eimeria, which causes coccidiosis and Clostridium, which causes necrotic enteritis. They used the University of

Guelph and the Animal Health Center respectively as their reference test labs. Results were 100% in agreement on all positives for both pathogens (100% specificity). Tests were run in field at Canadian Poultry Consultants by their technicians. They were very pleased with these results and have further plans for more field trials with these pathogens. Salmonella, AI and Campylobacter field trials will begin this June.

- They presented these findings at the Western Poultry Veterinary Conference in Abbotsford on May 17, 2017. In their closing remarks they disclosed the SPFG as a supporting funder for their research and gave special thanks to all our funders and partners, including SPFG.