Latest Research Results

organic SCIENCE CLUSTER 3

Optimization of berry by-products use in organic poultry production

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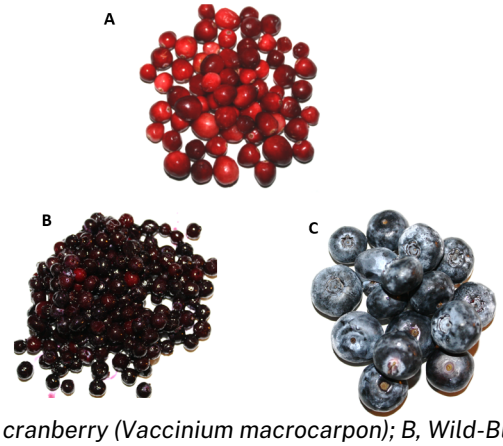
Antibiotics have been used to prevent diseases such as intestinal necrosis called necrotic enteritis in broilers. The emergence and spread of antimicrobial resistance led to the recent restriction of antibiotic use in food for animal production, and increasing consumer demand for antibiotic-free and organic broiler meat. Thus, there is an urgent need to develop cost-effective strategies to maintain and/or improve the health, productivity and safety of antibiotic-free and organic broilers. Berry pomaces, by-products of the fruit processing industry, contain several compounds with heath promoting and antimicrobial properties against pathogenic bacteria regardless of their antibiotic resistance profile. Our research team evaluated, for the first time, impacts of berry fruit pomaces on multiple integrated facets of food safety, health and productivity endpoints including performance, metabolism, general and gut health, modulation of microbiota and immunity in broiler birds, which would significantly improve production efficiency in the absence of antibiotic-feed supplementation.

1. Food Safety and Bird Health*:*

Salmonella enterica serovars Typhimurium, Enteritidis and Heidelberg are important causes of foodborne poisoning (salmonellosis) outbreaks in humans worldwide. The control of these *Salmonella* is difficult, because of their ability to grow and survive in the food production chain and the emergence of antimicrobial resistant isolates. Cranberry fruits contain several antimicrobial compounds that could be developed for application to improve the food safety. In Organic Science Cluster 2 (OSC2) we evaluated the ability of extracts (KCOH) from organic cranberry fruit wastes (pomace) to affect *Salmonella enterica* serovars Typhimurium, Enteritidis and Heidelberg isolated from broiler chickens. Exposure of these antimicrobial resistant Salmonella to 8 mg/ml of KCOH or 4 mg/ml or its sub-fractions, anthocyanins (CRFa20) and non-anthocyanin polyphenols (CRFp85), induced a substantial growth inhibition of these pathogens. *Salmonella Enteritidis*, the most common serovars from poultry implicated in human salmonellosis in Canada, responded to its exposure to sub-lethal concentrations (2 and 4 mg/ml) of KCOH by changing its mechanisms to uptake and assimilate essential nutrients such as minerals and carbohydrates from the medium. These changes affected the survival mechanisms, virulence and motility in *Salmonella*. This study provided information on the response of *Salmonella* exposed to cranberry fruit by-products, which could be useful in developing control strategies for this important foodborne pathogen.



A, American cranberry (Vaccinium macrocarpon); B, Wild-Blueberry (Vaccinium angustifolium) and C, Highbush blueberry (Vaccinium corymbosum).

Salmonella in the intestine can be transferred to liver through blood circulation. The multiplication of *Salmonella* in the liver can lead to the infection of this organ. Liver from chicken infected by *Salmonella* has been implicated in several reported human salmonellosis outbreaks. A promising strategy to reduce liver infection by *Salmonella* in chickens could be through supplementation of feed with natural products. Extracts from cranberry pomace (CPOH) is an excellent source of bioactive polyphenolic compounds having antioxidant and antimicrobial activities. In our Organic Science Cluster 3 (OSC3) project, we investigated the ability of CPOH to protect chicken liver against *Salmonella*. Results showed that CPOH preserve the integrity of liver infected with S. Enteritidis. Furthermore, CPOH reduced the adhesion of *Salmonella* to chicken liver cells by affecting not only the mechanisms of *Salmonella* entry into the cell but also by increasing the antioxidant of liver. Our data show that CPOH effectively protected chicken cell liver from colonization and damage which could offer opportunities to develop sustainable, safe, and economic strategies to reduce *Salmonella* in chicken.

2. Performance, Immunity and Gut Health

It is well known that cranberry fruit pomace contains nutrients such as carbohydrates, lipids, proteins, and fibers as well as bioactive molecules including phenolic compounds known for their health benefits in humans. Currently, the use of berry by-products as feed supplements during broiler chicken production is limited. Therefore, OSC2 studies investigated effects of feed supplementation with different doses (1 or 2%) of organic cranberry (CP1 and CP2) and wild blueberry (BP1 and BP2) pomaces as well as their extracts at 150 (COH150 and BOH150) or 300 ppm (COH300 and BOH300) in broilers raised for 30 days. Results showed that COH300 and BOH300 in feed increased the bodyweight during the first 10 days and from 10 to 20 days of age, respectively while COH150 improved the overall feed efficiency compared to control. The lowest prevalence of necrotic enteritis was observed with CP1 and BP1 compared to the traditional antibiotic bacitracin in feed. Although no clear evidence of a dose-dependent response was noted, feed supplementation with berry products improved intestinal health by modulating the abundance of gut microbes such as *Acidobacteria* and *Lactobacillaceae*, while simultaneously influencing immunity in broilers.

Effects of feed supplementation with various organic cranberry products to improve the blood metabolite profile and the immunity of broiler chickens have been investigated. Analysis of blood collected from 21-day old birds showed that feeding with cranberry products influenced their blood defense proteins (called antibodies or immunoglobulins) levels. At 21-days of age, studies on liver and bursa indicated that cranberry products in feed reduced the level of pro-inflammatory markers in the liver while promoting the anti-inflammatory markers production in the bursa, suggesting beneficial effects on broiler immunity. Since there was no increase in stress related marker levels, the results suggest that birds were not exposed to any stress conditions due to feeding treatments or other management concerns. Overall results of this study showed that feed supplementation with cranberry products re-enforce immune responses in broilers in agreement with the hypothesis that berry products could be developed in broiler feed as effective alternatives to antibiotics.

There is a complex population of microorganisms that live in the intestinal tract of chickens. This gut microbial population, also called gut microbiota, can be modified by diets and the bird's health status. As such, gut microbiota plays an important role in the ability of poultry to fight against diseases by providing immunity. We examined changes in the intestinal microbial population and the capacity to mount responses against infectious diseases in broiler chickens fed various diets. The broiler chickens received diets supplemented with **1)** a traditional antibiotic called bacitracin, **2)** cranberry pomace, **3)** wild blueberry pomace or **4)** a combination of cranberry and blueberry pomaces. The chickens were either vaccinated against coccidiosis (a parasitic intestinal disease) or left unvaccinated. Vaccination significantly decreased the incidence of intestinal diseases but affected bird's growth performance parameters particularly between 10 to 20 days of age. All feed supplementations (bacitracin, cranberry pomace and blueberry pomace) showed similar benefits on gut health. Vaccination and pomace feeding induced changes in blood composition (metabolites) and increased the production of antibodies which are proteins produced by poultry to fight against infectious microbes. Vaccination and/or dietary pomace resulted in modifications of the abundance of gut bacteria such as those belonging to the *Lactobacillaceae*, *Enterobacteriaceae*, *Clostridiaceae*, and *Streptococcaceae* groups. Overall, this study revealed that, in addition to their beneficial effects on productivity, inclusion of bacitracin and berry pomaces with enzymes in feed, modified the intestinal microbial population and blood composition in broilers

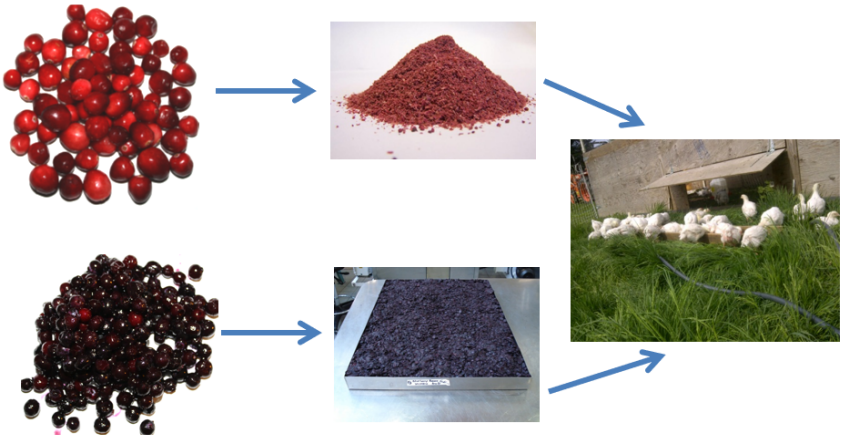
but such effects could be impacted by a vaccination against coccidiosis in chickens. We extended this study during the OSC3 project and showed that berry pomaces improved intestinal health of broiler chickens challenged with *Eimeria*.

3. Antimicrobial Resistance and Meat Quality

Results of the OSC2 project showed that ceca from birds vaccinated against coccidiosis harbored less bacteria *Lactobacillus* populations but more *Escherichia coli* population than non-vaccinated birds. The highest abundances of the bacterium *L. crispatus* were observed in birds fed cranberry pomace (CP), blueberry pomace (BP), and CP + BP compared to those from negative control (NC) or bacitracin (BAC) treatments. Coccidiosis vaccination affected the abundance of many virulence genes (VGs) with less VGs being observed in berry pomace-fed birds. Vaccination against coccidiosis also affected more than seventy-five antimicrobial resistance genes (ARGs). However, ceca from birds fed CP, BP, and CP + BP showed the lowest abundances of several ARGs, when compared to ceca from birds fed BAC. Vaccinated and non-vaccinated groups were different in the richness and diversity of genes conferring resistance to aminoglycosides, β-lactams, lincosamides, and trimethoprim. In conclusion, this study demonstrated that feeding birds with berry pomaces and coccidiosis vaccination influences cecal microbiota, virulence and resistance gene abundances and diversities in the ceca of broiler chickens.

Results of the OSC3 project showed that the dietary enzymes-mixture was associated with a decrease in the level of resistance to gentamycin and tetracycline in broilers. Cranberry pomace resulted in similar breast yield as bacitracin (BMD), while significantly lower breast yield was observed in the broilers fed with blueberry pomace than those with BMD. In addition, supplementation with the cranberry or blueberry pomace did not affect meat lightness and yellowness, while the deeper red meat (higher a* values) was observed in the birds receiving the diet containing 0.5%. Inclusion of berry pomaces in the diet did not change meat texture and proximate composition (moisture, protein, fat, ash) irrespective of their concentrations used. Although there were no obvious effects on meat antioxidant capacity and the incidence of myopathies, the upward trend of antioxidant

capacity and less severity of wooden breast were observed in birds fed with 0.5% cranberry pomace. Accordingly, dietary cranberry pomace resulted in higher mRNA levels of antioxidant genes. Taken together, cranberry pomace supplementation could potentially maintain meat quality and lessen the severity of wooden breast.



Using food science, microbiology and animal science to develop value added organic berries by-products as feed additives to improve organic broiler productivity, health and immunity.

For more information visit the OSC3 Activity 24 webpage and/or DAL.CA/OACC/OSCIII & https://organicfederation.ca/organic-science-clusters/

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