

# NYWGF RESEARCH - FINAL REPORT

**Funding for fiscal year:** 2024-2025

## SECTION 1:

**Project title:** Upcycling grape pomace as dietary alternative to antibiotic growth promoters in broiler production-Phase 2

**Principal Investigator with contact info:** Dr. Elad Tako, Associate Professor, Department of Food Science, Cornell University (et79@cornell.edu)

**Co-PI Collaborators with contact info:**

**New Research**  **Continued Research**

**Amount Funded** \$54,000 with \$30,000 coming from EJ Gallo and \$24,000 from NYWGF

## SECTION 2:

**Project Summary Impact Statement:** Grapes have the highest production volume by weight of any fruit in the U.S.; however, 20% of volume remains as pomace after processing, and its disposal can cause environmental and economic issues. Antibiotic growth promoters (AGPs) added to poultry feed pose a threat of antibiotic resistance entering the food system. In this project, we propose utilizing GP as an alternative to AGPs in broiler feed. Our goal is to repurpose GP as a novel broiler feed additive to improve growth performance, enhance meat quality, and reduce feed costs and mortality. Building on our current observations, that demonstrated the nutritional benefits of GP in poultry feed, the further investigation of GP as a natural alternative to AGPs in poultry feed, and its assessment in a large scale, dose response, long-term feeding trial, will allow the development of optimized processes and implementable guidelines for pomace use and waste management, potentially resulting in a new market for grape pomace This proposal is directly associated to this year's priority theme of vineyard sustainability.

**Impacts:** **1.** More sustainable use of GP reduces disposal and environmental burdens, **2.** Using stilbenes from GP improves poultry health and productivity, **3.** GP utilization limits emergence of antibiotic-resistant bacteria, and, **4.** Additional economic vitality of the grape and wine industry through sales of GP to feed producers.

**Objectives:** The overall goal of this project includes repurposing grape pomace (GP) as a novel feed additive to reduce feed cost, offset environmental pollution caused by discarding it, and improve broiler performance and immune function. The specific research objectives identified to help achieve this goal are listed below (phase II):

- Objective 1: Evaluating grape pomace for its potential to replace dietary antibiotic growth promoters in poultry feed, via large scale, long-term feeding trial (dose response).
- Objective 2: Evaluating the potential of dietary grape pomace to improve intestinal functionality, morphology, microbiome, and beneficial metabolites (SCFA), in broilers (inflammation conditions).

## Materials & Methods:

**Objective 1:** We hypothesize that the dietary inclusion of grape pomace (5%) can improve broiler performance, food conversion ratio, and meat quality compared to antibiotic growth promoter in a healthy vs. inflamed model.

**i. In vivo study.** A multidisciplinary analysis will be employed to investigate the effects of Concord grape pomace on intestinal functionality, and growth performance in poultry. Cornish Cross broiler fertile broiler eggs will be obtained from a local hatchery (Moyer's chicks, Quakertown, PA) and incubated under optimal conditions at the Cornell University poultry farm (IACUC protocol code #2020-0077). The broilers will be maintained for 42 days as this is the standard time broilers take to achieve marketing size.

**ii. Dietary Antibiotic Growth Promoter (AGP).** Zinc Bacitracin is the most common AGP used in broiler production, aimed at reducing disease outbreak, and to improve animal yields. These significantly increase feed costs and drive up the price of chicken meat. GP could potentially replace AGPs and qualify under USDA organic certification.

**iii. Grape Pomace Processing.** The grape pomace variety chosen is the Concord grape (*Vitis labrusca* L.), as we have previously performed an intra-amniotic preliminary study with it (Agarwal et al., 2022), and a preliminary feeding trial (Agarwal et al., in preparation). This will allow us to build on previous data and experience. Concord grapes are widely cultivated around the NY Finger Lakes District, Lake Erie, Lake Ontario, Southwestern Michigan, and the Yakima Valley in Washington. Although we believe this study would also apply to other grape varieties as their phytochemical composition is largely the same. **Processing.** Previous studies on GP have not clearly described the manufacturing process for preparing the GP. This is crucial as this would determine the phytochemical profile and microbial load of the feed. For this project, we will oven dry the GP at a maximum of 60°C until moisture is less than 10%. This is to ensure phytochemical stability and future scalability. This oven drying can be done commercially, irrespective of sunlight availability. The concord GP will be provided by Prof. Vanden Heuvel (chair of Horticulture Plant Science Dept. of Cornell University) from Cornell University Vineyards, and by industry collaborators: Gallo E& J, and National Grape/Welch's CO-OP. Prof. Padilla Zakour (Director at Cornell AgriTech), will conduct the GP processing to be done in the Cornell AgriTech Food Venture Center in Geneva, NY. **GP quality testing.** In addition to testing moisture content, we propose testing total polyphenol, monomeric anthocyanin, crude protein, condensed tannin, neutral detergent fiber, and non-fiber carbohydrates content in the processed GP (similar to our preliminary study, Table 1). This would allow us to make predictions for the outcome in the broilers and help us understand the physiological effects we see at the end of the study. We will test for microbial activity following industrial standard testing methods, including total plate count, total coliforms, yeast, and mold. The microbial profile of the feed can have a significant impact on the gut microbiome and the overall health of the broilers. Testing would ensure the physiological effects are due to the phytochemistry of GP (which can be consistent to an extent) and not due to its microbial profile (which varies drastically based on the environment).

**iv. Dose/percent Dietary Inclusion.** We propose testing 3 doses of GP (0.5, 2.5 and 5% GP). This is justified based on previous data that included large-scale poultry studies (Table 1).

**Table 1.** The treatment groups (poultry long-term feeding trial) selected to meet Objective 1\*.

Group #	Treatment
1	Standard diet (Normal conditions)
2	High NSP diet (inflammation condition)
3	(2) + AGP
4	(2) + 0.5% GP
5	(2) + 2.5% GP
6	(2) + 5% GP

\* A total of 240 broilers will be maintained with n = 40 in each treatment group. NSP, non-starch polysaccharide (30% rice bran), AGP (Zn bacitracin, anti-bacterial growth promoters), GP, grape pomace

**v. Grape Pomace Testing.** Total polyphenol content will be tested using the Folin-Ciocalteu method described by Waterhouse. Total monomeric anthocyanin content will be determined using the pH differential method. The non-fibrous carbohydrate, acid detergent fiber, and neutral detergent fiber analyses will be conducted according to AOAC 962.09 and 973.18 at Dairy One Co-Op Inc (Ithaca, NY, USA). Total plate count, total coliforms, yeast, and mold will be AOAC 966.23, 991.15, and 2014.05, respectively. Heavy metals Hg, Cd, As, and Pb will be tested using ICP-MS using USP <231> (USA Pharmacopeia).

**vi. Experimental Design (poultry feeding trial).** A total of 90 fertile broiler eggs will be incubated under optimal conditions at the Cornell University poultry farm (IACUC protocol code #2020-0077). On hatch, the hatchlings will be sexed and randomly allocated to one of six groups (n=15, Table 2), ensuring an even number of males and females. The birds will be kept in confinement (1 animal = 1 m<sup>2</sup> metal cage), with controlled temperature, humidity, and 16 h light/day. The confinement area will have an automatic watering system and a manual self-feeder. The broilers will be fed a corn-soy-rice bran diet with at least 30% rice bran except for group 1. Group 1 will be provided the standard corn-soy diet throughout the experiment to serve as a control. The diets will be formulated to meet the nutrient requirements for poultry (NRC, 1994), with *ad libitum* access to feed/ water.

**Table 2.** Sample/data collection plan during and at the end of the feeding trial.

Sample collection and analysis		Experimental day						
Sample	Specific analyses	00	07	14	21	28	35	42
Mortality*	Number	✓	✓	✓	✓	✓	✓	✓
Body Weight	Weight gain	✓	✓	✓	✓	✓	✓	✓
Feed intake*	Feed conversion ratio	✓	✓	✓	✓	✓	✓	✓
Blood	FITC-d			✓		✓		✓
	Inflammatory cytokines			✓		✓		✓
	Enzyme activity			✓		✓		✓
	Alpha 1 acid glycoprotein			✓		✓		✓
	Ceruloplasmin			✓		✓		✓
	Short Chain Fatty Acids							✓
Duodenum	Gene expression and histomorphology							✓
Jejunum								✓
Cecum	Microbiome metagenomics							✓
Breast	Meat quality parameters							✓
Thigh	Meat quality parameters							✓

Day 00 – baseline; Day 42 – Endpoint. \*Mortality and feed intake will be monitored daily.

**Objective 2:** We hypothesize that the dietary inclusion of grape pomace at a higher dietary dosage (2.5% 5%), can reduce inflammation biomarkers, and improve intestinal functionality, morphology, microbiome, and beneficial blood metabolites (SCFA), of broilers.

**i. Intestinal Barrier Integrity.** Fluorescein isothiocyanate dextran (FITC-d) (MW 3–5 KDa; Sigma Aldrich Co., MO, USA) will be used as a marker to determine the extent of paracellular transport and

intestinal barrier dysfunction. For the assessment, the optimized protocol specifically for broiler gut permeability will be used. In addition, duodenum and jejunum gene expression of tight junction proteins (Occludin, Zonula occludins-1, and Claudin-3,-4) will be assessed to verify intestinal barrier integrity using an RT-qPCR.

**ii. Inflammatory Status-related Biomarkers.** Superoxide dismutase (SOD), glutathione peroxidase (GPx), and malondialdehyde (MDA) will be measured in the plasma using commercially available ELISA kits to determine the antioxidant status of the birds. SOD is a detoxification enzyme component of the first line of defense against reactive oxygen species. Similarly, GPx is an important intracellular enzyme that breaks down H<sub>2</sub>O<sub>2</sub> to H<sub>2</sub>O and lipid peroxides to their corresponding alcohols. MDA is one of the final products of PUFA peroxidation, and an increase in its levels indicates excess free radicals. Additional pro-inflammatory cytokine (IL-6, NF- $\kappa$ B, TNF- $\alpha$ , and IFN  $\gamma$ ) gene expression will be determined. Further, calprotectin (CP) and alpha1-acid glycoprotein ( $\alpha\alpha$ 1-AG) have been identified as important non-invasive novel methods to assess the gut inflammation process. Plasma levels of  $\alpha\alpha$ 1-AG will be determined using a commercial radial immunodiffusion kit. Serum levels of calprotectin will be quantified using a commercial ELISA kit per its instructions.

**iii. Cecal Microbiome (MiSeq) Metagenomics.** Cecum microbial genomic DNA will be extracted using the PowerSoil DNA isolation kit. Bacterial gene sequences will be PCR-amplified using primers for the V4 hypervariable region of the 16S rRNA gene. PCR products will be quantified using a Quant-iT PicoGreen dsDNA assay. Equimolar ratios of total samples will be pooled and sequenced using a MiSeq Sequencer (Illumina). Sequences that pass quality filters will be analyzed using the QIIME software package, demultiplexed by per-sample barcodes and Illumina-sequenced amplicon reads errors corrected by Divisive Amplicon Denoising Algorithm (DADA2). Sequences will be classified taxonomically using the Greengenes reference database at a confidence threshold of 99%. The Greengenes taxonomies will generate summaries of the taxonomic distributions of features across different levels (phylum, order, family, and genus). After filtration of low abundant features (observed > 2 samples per group),  $\alpha$  and  $\beta$  diversity analyses will be calculated. Microbial richness, an  $\alpha$  diversity parameter, will be calculated using Faith's Phylogenetic Diversity.  $\beta$  diversity will be analyzed using Jaccard similarity distances. Special attention will be given to the number of commercially relevant pathogens (*C. perfringens*, *E. coli*, *Mycoplasma*, *Salmonella*, *Staphylococcus*, *Streptococcus*, *Listeria*).

**iv. Short Chain Fatty Acids analysis.** Cecal and blood samples were homogenized in HCl (2 ml, 3%, 1 M), centrifuged and combined with ethyl acetate (100  $\mu$ L) and acetic acid-d<sub>4</sub> (1  $\mu$ g/mL) before collecting the organic phase to determine short chain fatty acid (SCFA) composition. Samples were quantified via GC-MS using a TRACE™ 1310 gas chromatograph (Thermo Fisher Scientific, Waltham, MA, USA) and a TraceGOLD™ TG-WaxMS A column (Thermo Fisher Scientific, Waltham, MA, USA).

**v. Intestinal Gut Morphology.** Duodenal and jejunal brush border membrane characteristics, including villi surface area, crypt depth, and intestinal wall thickness, will be determined. Transverse sections of the two samples will be fixed, paraffin-embedded, stained with H&E, and imaged under the light microscope (BX3M series, Olympus Waltham, MA, USA). To count and measure, the CellSens Standard Software will be used.

**vi. Statistical Analysis.** The mean difference between treatment groups will be calculated using ANOVA followed by a Duncan post-hoc test using SPSS software version 27 (IBM, Armonk, NY, USA). Differences with *p*-values  $\leq$  0.05 will be considered statistically significant. The values will be represented as means  $\pm$  standard deviation. Power analysis will be done using G\*Power software (version 3.1.9.7) to calculate the sample size needed for each parameter.

### **Results/Outcomes/Next Steps:**

The intent of this project is to promote the use of natural feed inputs to improve environmental and agricultural industry performance. Our work is especially relevant given the state's large grape industry, converting GP from a costly byproduct to a value-added feed input that is locally available to NY poultry farms. Specific outcomes and impacts include:

**Outcomes:** 1. Demonstration of dietary GP to improve inflammation biomarkers, poultry growth and performance, 2. Demonstration of nutritional and economic benefits of GP as a poultry feed input, and, 3. Development of industry guidelines for GP processing and best management practices for its use in poultry feed

**Impacts:** 1. More sustainable use of GP reduces disposal and environmental burdens, 2. Using stilbenes from GP improves poultry health and productivity, 3. GP utilization limits emergence of antibiotic resistant bacteria, and, 4. Additional economic vitality of the grape and wine industry through sales of GP to feed producers.

**Results:**

1. Complete analysis of the concentrations of bioactive compounds in Concord grape pomace (*Vitis Labrusca* L.), revealed significant concentrations of the following bioactives: Trans-Resveratrol, Cis-Resveratrol, Pterostilbene, Tannin, gallic acid, and Quercetin Dihydrate (Table 1).
2. The nutritional composition of Concord grape pomace (*Vitis Labrusca* L.) detected significant values of the following nutrients (Amino Acids): Lysine, Methionine, Arginine, Leucine, Isoleucine, and Valine (Table 2).
3. Duodenal Morphometric Parameters (Figure 1): Dietary Grape Pomace (GP), at a level of 2.5%, and 5%, increased villus surface area (the intestinal nutrients digestive and absorptive surface), relative to controls ( $p < 0.05$ ). Further, dietary Grape Pomace (GP), at a level of 2.5%, and 5%, increased crypt depth (indicator of cellular proliferation), and muscularis thickness ( $p < 0.05$ ). Overall, the dietary inclusion of GP at the levels of 2.5%, and 5% significantly improved small intestinal inflammation symptoms. GP (5%) increased villus surface area, crypt depth, and muscle tissue, despite induced inflammation. This indicated on increased cellular proliferation, and improved digestive and absorptive capacity.
4. Duodenal Gene Expression: Figure 2 illustrates the differences in gene expression of proteins related to BBM functionality, and tissue permeability. The expression of all tested genes (Claudin 3, Claudin 4, ZO-2, OCLN), was significantly ( $p < 0.05$ ) down regulated in the 2.5%, and 5% GP dietary inclusion, and despite induced inflammation. This indicates on significant improvement in intestinal inflammation, and reduced intestinal leakiness
5. Analysis of the Gut Bacterial Populations: The 16s rDNA analysis of cecal bacterial populations showed significant changes in the relative abundance of the Lactobacillus, Klebsiella., E. coli, and Bifidobacteria In the 2.5%, and 5% dietary GP treatment groups, when compared to controls. Specifically, significant increased in probiotics populations abundance, of Bifidobacteria, and Lactobacillus (Figure 3). This indicates on the prebiotic effects of dietary GP, and despite induced inflammation conditions.

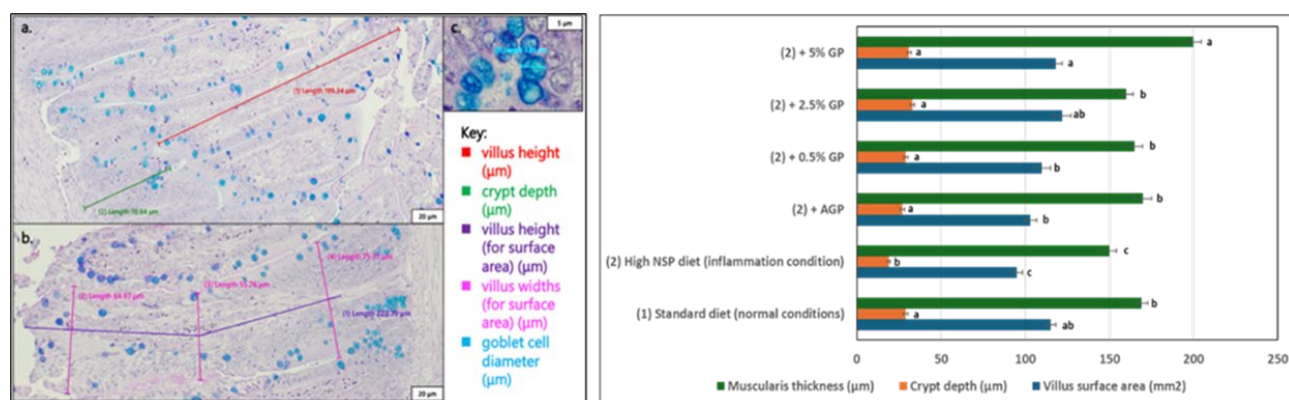
**Table 1.** The concentrations of bioactive compounds in Concord grape pomace (*Vitis Labrusca* L.)

Bioactive compound	Value	Unit
Total Anthocyanins	34.9	(mg/L)
Total Polyphenols	4534.0	(mg/L)
Trans-Resveratrol	0.45	(µg/L)
Cis-Resveratrol	0.19	(µg/L)
Pterostilbene	0.25	(µg/L)
Tannin	2001.1	(mg/L)
Gallic Acid	15.26	(mg/L)
Quercetin Dihydrate	6.28	(mg/L)

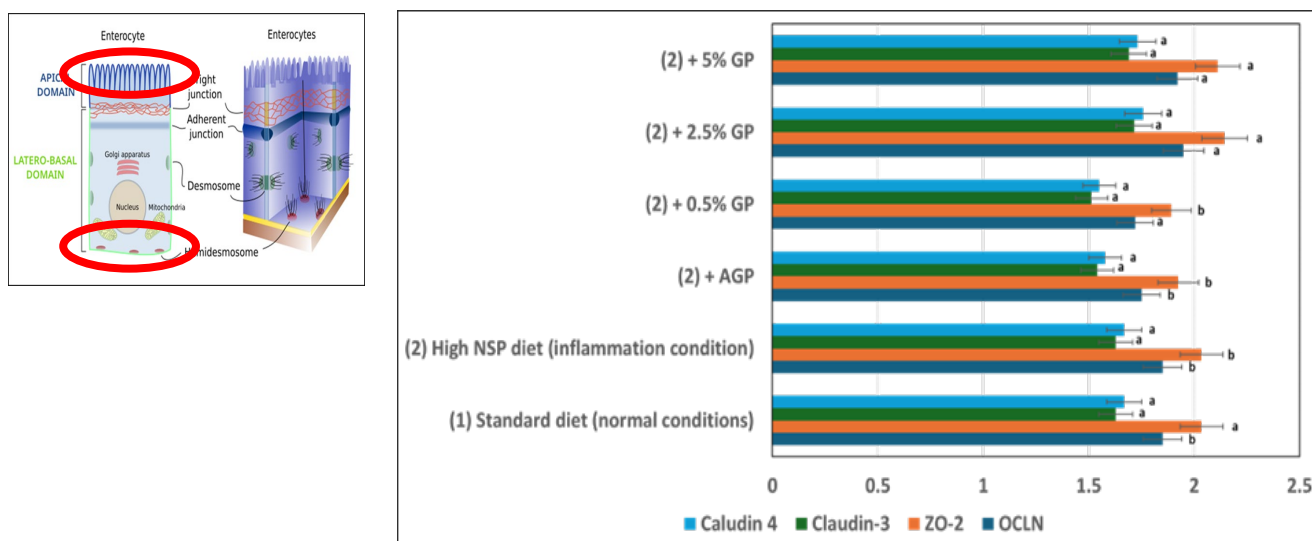
**Table 2.** The nutritional composition of Concord grape pomace (*Vitis Labrusca* L.)

Bioactive compound	Value	Unit
Metabolizable Energy	1250-2080	(Kcal/L)
Crude Protein	8.50-13.9	(%)
Total Lysine	2.30-8.30	(%)
Total Methionine	0.51-1.40	(%)
Total Arginine	6.20-8.00	(%)
Total Leucine	5.20-7.70	(%)
Total Isoleucine	2.90-4.80	(%)
Total Valine	3.50-6.00	(%)

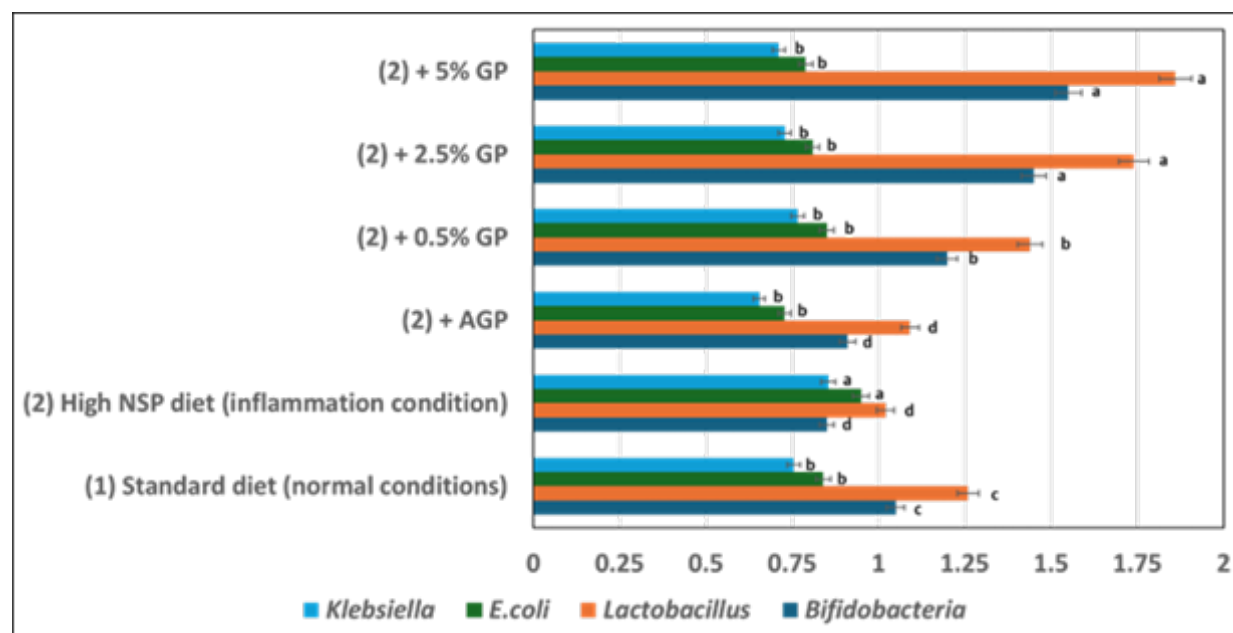
**Figure 1.** Effect of dietary grape pomace (GP), on the duodenal villi surface area, crypt depth, and muscularis thickness. Values are the means  $\pm$  SEM (n = 20). <sup>a-c</sup>Treatment groups not indicated by the same letter are significantly different (p < 0.05).



**Figure 2.** Effect of dietary grape pomace (GP), on duodenal brush border membrane functional proteins. Values (AU) are the means  $\pm$  SEM (n = 20). <sup>a-c</sup>Treatment groups not indicated by the same letter are significantly different (p < 0.05).



**Figure 3.** Effect of dietary grape pomace (GP), on cecal microbial populations. Values (AU) are the means  $\pm$  SEM (n = 20). <sup>a-c</sup>Treatment groups not indicated by the same letter are significantly different (p < 0.05).



**Technology Transfer Plan:** Associated research is linked to US non-provisional patent Application No. 63/159,109 (filed March 10, 2022), Title: “Methods for improving poultry health” (Inventor: E. Tako), Cornell CTL Reference No: 9587-01-US.

#### Manuscripts:

- Manuscript is published\* (Journal: Nutrients; Special Issue: “Emerging bioactives in health and disease”): Agarwal, N.; Shukla, V.; Kolba, N.; Jackson, C.; Padilla-Zakour, O.I.; Tako, E. Comparing the Effects of Concord Grape (*Vitis labrusca* L.) Puree, Juice, and Pomace on Intestinal Morphology, Functionality, and Bacterial Populations In Vivo (*Gallus gallus*). Nutrients 2022, 14, 3539. <https://doi.org/10.3390/nu14173539>.
- Manuscript in preparation, to be submitted to the journal of Poultry Science in June 2025.

\*NYWGF funding support was acknowledged in the manuscript.

#### Conferences:

- Data was presented in the Poultry Science Association, 2024 Meeting (July 2024, Louisville, KA).
- Data will be presented in the Poultry Science Association, 2023 Meeting (July 2025, Raleigh, NC).

### **SECTION 3:**

**Project summary and objectives:** Grapes are the most produced fruit by weight in the U.S., but processing leaves behind 20% as pomace, which poses environmental and economic challenges. Antibiotic growth promoters (AGPs) in poultry feed contribute to antibiotic resistance in the food system. This project proposes using grape pomace (GP) as a natural alternative to AGPs in broiler feed. The goal is to improve poultry growth, meat quality, and reduce feed costs and mortality by repurposing GP. A large-scale, dose-response, long-term trial will help develop practical guidelines for GP use, supporting vineyard sustainability and creating a new market for pomace.

**Objectives:** Repurpose grape pomace (GP) as a sustainable poultry feed additive to reduce feed costs, minimize environmental waste, and enhance broiler growth and immune function. **Phase II Objectives:**

- Objective 1: Assess GP as a natural alternative to antibiotic growth promoters through a large-scale, long-term, dose-response feeding trial.
- Objective 2: Investigate the effects of dietary GP on gut health, including intestinal structure, microbiome composition, and beneficial metabolite (SCFA) production under inflammatory conditions.

**Importance of research to the NY wine industry:** This research is important for the New York wine industry because it offers a sustainable solution for managing grape pomace, a major byproduct of winemaking. Currently, pomace disposal presents environmental and financial challenges for wineries, especially in regions like New York with high grape production. By converting this waste into a value-added poultry feed ingredient, the research supports circular agriculture, reducing waste and creating new economic opportunities for growers and producers. It also aligns with the industry's growing focus on sustainability and climate-resilient practices, helping New York wineries stay competitive and environmentally responsible. Ultimately, this innovation could enhance the overall sustainability profile and profitability of the state's wine industry.

### **Project Results/next steps:**

#### **Results:**

- Bioactive Compounds in Concord Grape Pomace: Significant levels of Trans-Resveratrol, Cis-Resveratrol, Pterostilbene, Tannin, Gallic Acid, and Quercetin Dihydrate were identified.
- Nutritional Composition: Concord grape pomace contains notable amounts of essential amino acids, including Lysine, Methionine, Arginine, Leucine, Isoleucine, and Valine.
- Duodenal Morphometry: Dietary inclusion of grape pomace (2.5% and 5%) increased villus surface area, crypt depth, and muscularis thickness, indicating enhanced nutrient absorption and intestinal health, even under inflammatory conditions.
- Gene Expression in Intestinal Tissue: Expression of tight junction genes (Claudin 3, Claudin 4, ZO-2, and OCLN) was significantly downregulated with GP diets, suggesting reduced intestinal inflammation and improved gut barrier integrity.

- Gut Microbiota Modulation: Grape pomace supplementation significantly increased beneficial bacteria (Bifidobacteria and Lactobacillus) in the gut, supporting its prebiotic potential, even during inflammation.

**Next steps:**

Conduct a long-term study to evaluate the efficacy of dietary grape pomace (GP) in alleviating severe disease conditions (Coccidiosis), in poultry.

**Why It's Important:** Coccidiosis is a major intestinal disease in poultry that leads to significant economic losses due to reduced growth performance, increased mortality, and the need for costly treatments. Current control strategies often rely on anticoccidial drugs, which can contribute to resistance and residue concerns. Demonstrating that GP can serve as a natural, cost-effective alternative to mitigate the impact of coccidiosis would not only improve animal health and productivity but also support the transition to more sustainable and antibiotic-free poultry production systems.

**Supporting attachments:** Graphical abstract, Summary if research

