Efficacy of Natural Cranberry Extracts against *Campylobacter* Colonization in Poultry

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Abstract—*Campylobacter* is the leading causes of bacterial foodborne illness in humans and is associated with poultry consumption. Currently there are no effective treatments to eliminate *Campylobacter* from poultry. Extracts from the American Cranberry (*Vaccinium macrocarpon*) containing proanthocyanidins have inhibited other foodborne pathogens. In this study, we evaluated two different cranberry extracts, either containing low (1%, L-PAC) or high concentrations (30%, H-PAC) of proanthocyanidins, to reduce *Campylobacter* counts, in vitro or in vivo. Although effective in vitro, when fed to 70 day-of-hatch birds at either 0% (control), L-PAC (0.5%, 1% or 2%) or H-PAC (0.5%, 1% or 2%) in replicate trials, *Campylobacter* counts were not reduced. Although highly effective in vitro, further evaluation is needed to determine optimum concentrations of cranberry proanthocyanidins to reduce *Campylobacter* in poultry.

Index Terms—natural extracts, cranberry, *Campylobacter jejuni*, poultry

I. INTRODUCTION

One of the leading bacterial causes of food borne illness worldwide is contamination of food products with *Campylobacter* spp. [1]. Estimates of food borne illness attributed to *Campylobacter*, based on data collected from the Centers for Disease Control (CDC), put the rate at approximately 850,000 cases per year [2]. Epidemiological studies have indicated that the most frequent routes for human infection with *Campylobacter* are eating improperly cooked chicken, handling chicken and exposure to animals including poultry [1]. *Campylobacter* is a commensal in chickens, naturally colonizes in the lower intestine, preferentially in the crypts within the ceca, where they are able to reach levels as high as $10^6-10^8$ CFU/gram of cecal material [3]. The prevalence of *Campylobacter* spp. within poultry flocks in the United States is reported to be as high as 90% [4]. The Food Safety and Inspection Service has estimated the percent of chicken carcasses positive for *Campylobacter* in the processing plant to be approximately 46%, which can be attributed to the feathers, the skin and the gastrointestinal tract having a high *Campylobacter* load which cannot be completely eliminated during processing [5], [6]. High levels of *Campylobacter* on and in the bird at the time of slaughter have a significant impact on carcass contamination; so a reduction of *Campylobacter* in poultry pre-harvest should lead to a reduction in human campylobacteriosis cases from contaminated poultry products [7], [8]. Multiple strategies have been tried to reduce *Campylobacter* colonization in poultry with limited success and additional treatments need to be developed to reduce this food borne pathogen in poultry [9].

Renewed interest has been placed on plant extracts for the control of pathogens in food animals due to part to restrictions by the U.S. Food and Drug Administration (FDA) on antibiotic usage in livestock production. This challenges food animal producers to find alternatives to antibiotics and still maintain animal health and welfare [10]. Alternatives to antibiotics may come from phytogenics, or plant derived compounds, some of which have the benefit of being designated as Generally Recognized as Safe (GRAS) by FDA. Compounds with GRAS designation as deemed safe to be used in foods and require no lengthy approval process, which allows them to be adopted for use quickly (Code of Federal Regulation 21CFR 184.1025, 1981). Plant extracts from the American Cranberry (*Vaccinium macrocarpon*) are GRAS and have many bioactive compounds, some of which demonstrate antimicrobial activity [11]-[16]. Cranberry extract has documented in vitro antibacterial activity to the food borne pathogens *E.coli* O157:H7, *Salmonella Typhimium*, and *Listeria monocytogenes* [14],[17].

To our knowledge, cranberry extracts have not been evaluated for their ability to reduce *Campylobacter* in poultry. For this study two commercially available GRAS designated cranberry extracts were tested. These extracts are standardized to contain a lower (1%) or higher concentration (30%) of proanthocyanidins by the manufacturer (L-PAC or H-PAC, respectively). The aim of this research was to determine if cranberry extracts, L-PAC and H-PAC, are inhibitory to *Campylobacter jejuni*, in vitro and if efficacious, examine their potential for use in young chickens to prevent *Campylobacter* colonization.

II. MATERIALS AND METHODS

A. Cranberry Plant Extracts

Two extracts of the North American Cranberry (*Vaccinium macrocarpon*) were used for both the in vitro
and in vivo trials; (1) cranberry concentrate powder standardized to contain 1% proanthocyanidins (L-PAC) and (2) dried cranberry extract powder standardized to contain 30% proanthocyanidins (H-PAC) obtained from Decas Botanical Synergies (Carver, MA).

B. In Vitro Antimicrobial Activity of Cranberry Extracts

Assessment of in vitro antimicrobial activity was determined using a mixture of three wild type Campylobacter jejuni strains previously isolated and identified from poultry. Campylobacter inoculum was prepared as previously described by Aguiar and coworkers [18]. For each assay, duplicate samples were tested which included eleven treatments, a positive control (no cranberry extract) and five concentrations (4%, 2%, 1%, 0.5% or 0.1%) of each cranberry extract. Each assay was replicated two times. To prepare the various concentrations of cranberry extract, 900 µL of fresh CEB was aliquoted into sterile test tubes and appropriate weights of L-PAC and H-PAC were added to the CEB and resuspended by vortexing. A 100 µL aliquot of the three strain C. jejuni culture was inoculated into each cranberry extract tube and a positive control tube (no cranberry extract). Both cultures were then incubated at 42°C under microaerophilic conditions for 8 hours or 24 hours post-inoculation. A 100 µL sample of each of the C. jejuni plus cranberry extracts was serially diluted (1:10) in Butterfield’s Phosphate Diluent (BPD) and direct plated onto Campy-Line Agar (CLA) [19] then incubated at 42°C under microaerophilic conditions for 48 hours. Presumptive Campylobacter colonies were directly enumerated and converted to cfu/mL of broth culture. Bacterial colonies were confirmed by latex agglutination test (PANBIO Inc., Columbia, MD) and by API Campy (Biomeriuex Durham, NC).

C. Animal Studies

Day of hatch broiler chicks was obtained from a commercial hatchery. Birds were weighed individually, placed in floor pens and provided free access to feed and water during the entire duration of the trials. For each trial, 70 chicks were randomly assigned to one of seven treatment groups (n=10 per treatment group). Treatment groups for each trial included a positive Campylobacter control (no cranberry extract) or 0.5%, 1%, or 2% of either H-PAC or L-PAC added to the feed. The same dosages were used for Trial 2. For each trial, all birds were given feed supplemented with H-PAC and L-PAC, except for positive Campylobacter control, starting at day of placement and continuing through the entire 14 day trial. At day 7 all birds were challenged with a mixture of three wild type Campylobacter jejuni strains by oral gavage. Each bird received 0.25 mL of approximately 10^8 cfu/mL Campylobacter jejuni mixture. The Campylobacter challenge was prepared as described by Aguiar and coworkers [18]. On day 14, cecal contents were collected for enumeration of Campylobacter as per the procedure described in our earlier studies [20]. All the birds were individually weighed at the end of the study period and the feed consumption data was also recorded.

D. Statistical Analysis

Data were analyzed using PROC GLM procedure of SAS [21]. Campylobacter counts from the ceca were logarithmically transformed (log cfu/mL) before analysis to achieve homogeneity of variance [22]. Treatment means were partitioned by LSMEANS analysis and probability of P<0.05 was required for statistical significance.

III. RESULTS

A. In Vitro Antibacterial Activity of Cranberry Extracts

There was no consistent reduction (>1 log) in Campylobacter counts for the 0.1 or 0.5% treatments at 8 or 24 hours post-inoculation for either the L-PAC or H-PAC (Table I) treatments when compared with the controls in either trial.

TABLE I. THE EFFECT OF DIFFERENT CONCENTRATIONS OF CRANBERRY EXTRACT L-PAC OR H-PAC ON IN VITRO GROWTH OF CAMPYLOBACTER JEJUNI IN TRIALS 1 AND 2

<table>
<thead>
<tr>
<th>Treatments</th>
<th>8 hours</th>
<th>24 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Trial 1</td>
</tr>
<tr>
<td>0% (Control)</td>
<td>4.8x10^7</td>
<td>5.7x10^7</td>
</tr>
<tr>
<td>0.1% L-PAC</td>
<td>4.3x10^7</td>
<td>1.1x10^8</td>
</tr>
<tr>
<td>0.5% L-PAC</td>
<td>3.0x10^7</td>
<td>3.8x10^7</td>
</tr>
<tr>
<td>1% L-PAC</td>
<td>3.2x10^7</td>
<td>5.4x10^7</td>
</tr>
<tr>
<td>2% L-PAC</td>
<td>3.2x10^7</td>
<td></td>
</tr>
<tr>
<td>4% L-PAC</td>
<td>1.0x10^7</td>
<td></td>
</tr>
<tr>
<td>0.1% H-PAC</td>
<td>3.9x10^7</td>
<td>3.0x10^7</td>
</tr>
<tr>
<td>0.5% H-PAC</td>
<td>5.8x10^7</td>
<td>8.1x10^7</td>
</tr>
<tr>
<td>1% H-PAC</td>
<td>3.7x10^7</td>
<td>3.2x10^7</td>
</tr>
<tr>
<td>2% H-PAC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4% H-PAC</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1. L-PAC or H-PAC was inoculated with a three strain mixture of wild type Campylobacter jejuni and incubated at 42°C under microaerophilic conditions for 8 hours or 24 hours and the Campylobacter counts were expressed in CFU/mL.
2. ND = not detectable; detection limits of assay are 1.0x10^5 CFU/mL.

For the 1% treatment, there was at least a one log reduction in Campylobacter counts for both compounds at 8 or 24 hours post dosing for both trials. For the 2 or 4% doses, there was a greater reduction in counts for both time points and trials when compared with control for the L-PAC treatment (Table I). For H-PAC, the 2 or 4% doses eliminated detectable Campylobacter in both trials (Table I).

B. Cecal Campylobacter Counts, Body Weights and Feed Consumption

Cecal Campylobacter counts in 14 day old broiler chicks were not reduced by administration of L-PAC or H-PAC in Trial 1 or Trial 2 when compared with the Campylobacter positive control (Table II).

Body weights were not affected by feeding any dose of L-PAC in either trial when compared with controls (data not shown). Body weights were, however, reduced in Trial 1 at the highest concentration and for the 1% and 2% H-PAC treatments in Trial 2 when compared with controls (data not shown). Although feed consumption was only determined for each pen at the end of the trials,
the only consistent change for both trials was an increase in feed consumption in the 0.5% H-PAC treated birds versus the controls.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose</th>
<th>Trial 1</th>
<th>Trial 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0%</td>
<td>7.01±0.23&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.97±1.05&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>L-PAC</td>
<td>0.5%</td>
<td>7.37±0.67&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>6.55±0.76&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>1%</td>
<td>5.64±0.81&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.43±0.74&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>2%</td>
<td>7.00±0.31&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.7±0.54&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>H-PAC</td>
<td>0.5%</td>
<td>5.97±0.66&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>7.25±0.23&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>1%</td>
<td>7.05±0.20&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.69±0.26&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>2%</td>
<td>6.90±0.22&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.10±0.33&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Means within columns with no common superscript differ significantly (P<0.05).

In conclusion, the two cranberry extracts tested in this study were able to inhibit the growth of *Campylobacter jejuni* in broth culture and, if efficacious, to test these compounds for their ability to reduce or eliminate *Campylobacter* colonization in young broiler chickens. The *in vitro* antimicrobial susceptibility assays demonstrated that L-PAC and H-PAC at the lowest concentrations had little effect on *Campylobacter* growth. However, the higher concentrations of L-PAC were able to reduce *Campylobacter* by greater than 5 logs at 8 hours and to undetectable levels 24 hours after treatment. For the H-PAC treatment, at both 8 and 24 hours, *Campylobacter* was reduced to undetectable levels. These results indicated an apparent dose response relationship between the increasing concentrations of cranberry extracts and increasing efficacy against *Campylobacter* in *vitro*. Furthermore, it appears that H-PAC is more effective against *Campylobacter* than L-PAC in these *in vitro* trials. H-PAC has a higher concentration of proanthocyanidins than L-PAC which may explain its increased antibacterial activity against *Campylobacter*. Studies with Proanthocyanidins have shown to have antioxidant and iron scavenging properties [23]-[26] and may play a role in the *in vitro* anti-*Campylobacter* activity observed in our study. Although these compounds were effective *in vitro*, they were not able to consistently reduce cecal *Campylobacter* counts in young broiler chickens.

It is possible that the inability of these cranberry extracts to reduce *Campylobacter* in young birds is because it was absorbed prior to reaching the ceca in the lower intestine or did not reach the ceca in concentrations high enough to reduce *Campylobacter* counts. Although increasing the dose in the feed may be an option, it appears, at least for the higher doses of H-PAC, this extract adversely affected the birds as demonstrated by a reduction in body weights.

It is also known that pH and temperature affect the antioxidant activity of cranberry extracts [14], [27], which may not be optimal in birds. Transition through the digestive system of the bird would subject these extracts to drastic changes in pH and the elevated body temperature of the chicken may also be a contributing factor [28]. Another possible reason that these cranberry extracts were not efficacious *in vivo* may be due to the niche *Campylobacter* occupies in the intestine. Even if cranberry extracts reached the ceca at high concentrations it is possible that penetration into the crypts within the ceca may not occur. *Campylobacter* is able to sequester itself deep within the mucous filled crypts of the ceca, due to its chemotraction to mucin, where it remains protected [3], [29]-[31]. Previous research from our laboratory observed that even when antibiotic treatment eliminated *Campylobacter* within other sites along the gastrointestinal tract, the ceca remained colonized [32].

The ability of *Campylobacter* to remain protected within the mucous in the crypts may also prevent exposure to the anti-adhesive capabilities of cranberry extracts [33], [34]. Cranberry proanthocyanidins are able to prevent actin filaments of host cells from rearrangement, which is a mechanism used by some pathogenic bacteria for host cell invasion [35]-[37]. *In vitro* studies to assess host cell invasion by *Campylobacter jejuni* have determined that it utilizes adhesions and secreted proteins to alter the actin cytoskeleton leading to membrane ‘ruffling’ then invasion [38]. The significance of this mechanism is supported by *in vivo* studies demonstrating that modified strains are unable to produce adhesion proteins are not capable of colonizing chickens [39], [40]. Therefore, treatments which reduce the mucous crypt concentrations may expose *Campylobacter* to the anti-pathogenic properties of cranberry extracts. Research with bismuth compounds has demonstrated reductions in mucous viscosity and partial efficacy against *Campylobacter* colonization of the ceca [41], [42]. Follow up experiments are planned to evaluate if co-administration of bismuth and cranberry extracts can further reduce cecal *Campylobacter* colonization.

Cranberry extracts contain many flavinoid compounds including anthocyanins and proanthocyanidins [15], [43], [44]. Research into the addition of flavonoids to improve poultry nutrition have shown that gut microbiota plays an essential role in the bioavailability of flavonoids, which require deglycosylation in order to be absorbed in the gut [45]. Iqbal and coworkers [45] were able to isolate and identify three *Lactobacillus* strains from chicken cecal contents that significantly improved the bioavailability of flavonoids. This presents the opportunity for further study of cranberry extracts plus *Lactobacillus* strains as a potential pre-harvest intervention for *Campylobacter* colonization of poultry.

V. CONCLUSION

In conclusion, the two cranberry extracts tested in this study were able to inhibit *Campylobacter in vitro* but not when tested in young chickens. Follow up experiments

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are needed to increase the potency of cranberry proanthocyanidins, such as combining them with lactic acid bacteria strains or bismuth compounds, which may reduce Campylobacter jejuni colonization in chickens. Further, these compounds may have potential efficacy against this important food borne pathogen in post-harvest poultry as they showed promise in in vitro studies.

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REFERENCES


Dr. Komala Arsi, a post-doctoral fellow at the University of Arkansas. Her research is focused on pre and post-harvest food safety in poultry, with special emphasis on strategies to reduce the enteric pathogen, *Campylobacter*.

Dr. Ann M. Donoghue, ARS, USDA, has over 20 years of research experience with poultry. Her research focuses on enteric physiology and alternatives to antibiotics for pathogen intervention strategies in poultry.

Dr. Jonathan R. Moyle was a Research Associate with ARS, USDA. Currently he is working as an Extension Specialist at the University of Maryland.

Dr. Dan J. Donoghue is a professor in the Center for Excellence for Poultry Science at University of Arkansas. He is internationally reknown poultry physiologist/toxicologist with expertise in developing novel strategies, as potential alternatives to antibiotics, to control foodborne pathogens in poultry.