

## Organic Poultry: Developing Natural Solutions for Reducing Pathogens and Improving Production

Annie M. Donoghue<sup>1</sup>, K. Venkitanarayanan<sup>2</sup>, K. Arsi<sup>3</sup>, A. Woo-Ming<sup>3</sup>, I. Upadhyaya<sup>2</sup>, A. Kollanoor-Johny<sup>4</sup>, M.J. Darre<sup>2</sup>, A. Upadhyay<sup>3</sup>, A. C. Fanatico<sup>5</sup> and D.J. Donoghue<sup>3</sup>

### Abstract

Organic poultry production is one of the fastest growing segments of organic agriculture with a 20% average annual increase since the establishment of the National Organic Program (NOP). Although most management practices in organic production are designed to promote bird health and prevent disease, the lack of consistently effective organic therapeutics for enteric diseases can adversely influence bird health and wholesomeness of poultry products. Enteric diseases such as necrotic enteritis, and food safety hazards caused by the pathogens *Salmonella* and *Campylobacter*, are high priority issues for organic poultry producers. Therefore, there is a critical need for developing strategies to promote gut health and limit disease/pathogens in organically-raised birds. Research from our laboratories indicates that natural compounds such as medium chain fatty acids and plant-derived compounds possess antimicrobial efficacy against poultry enteric pathogens, and could address food safety and disease concerns in organic production systems. These selected compounds are all natural, listed as “Generally Recognized As Safe” (GRAS) by the Food and Drug Administration (FDA), and exert significant antimicrobial properties. Data from our laboratories have shown that natural compounds such as caprylic acid, a medium chain fatty acid found in coconut oil and milk; *trans*-cinnamaldehyde from cinnamon (*Cinnamomum verum*); thymol from thyme (*Thymus vulgaris*) or oregano (*Origanum glandulosum*) and eugenol from clove (*Syzygium aromaticum*), offer potentially safe and effective strategies for controlling food borne pathogens in organic poultry and comply with NOP standards.

### Introduction

Consumer demand for organically produced foods has shown a growth of over 20% a year for over a decade in the U.S. (Fanatico 2009; ERS 2014). Interest in these products has shifted from being a lifestyle choice for a small share of consumers to being consumed at least occasionally by two-thirds of Americans (Hartman Group 2004; Green and Dimitri 2014). Organic poultry meat is the most commonly available and consumed organic meat product, followed closely by eggs, preferred by over 70% of consumers (AgMRC 2013). Furthermore, organic poultry is often one of the first organic foods that people will purchase. Organic poultry meat and egg sales reached \$350 million in 2008 (USDA 2012). Industry analysts estimate that annual sales will continue growing. Although certified organic poultry production nearly tripled in the U.S. between 2005 and 2009, to over 32 million certified broilers, 2.4 million certified layer hens (that produced over 80 million dozen organic eggs) and over 300 thousand certified organic turkeys (USDA 2012), supply still lags behind demand.

### Challenges for organic poultry producers

Unfortunately, organic poultry producers face numerous challenges. Even though outdoor access offers many benefits to the birds, it has been demonstrated that this can increase exposure to a wide variety of microorganisms that are commonly present in the environment (Berg 2001; Engvall 2001). Birds can become exposed to pathogens such as *Clostridium*, *Salmonella* and *Campylobacter* through multiple avenues. These organisms can cause deleterious effects to the bird's health and are also a source of

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<sup>1</sup>Poultry Production and Product Safety Research Unit, ARS, USDA Fayetteville, AR; <sup>2</sup>University of Connecticut, Storrs, CT; <sup>3</sup>University of Arkansas, Fayetteville, AR; <sup>4</sup>University of Minnesota, Saint Paul, MN; <sup>5</sup>Goodnight Family Sustainable Development Department, Appalachian State University, Boone, NC. This program is funded by USDA-NIFA-OREI 2011-01955.

potential foodborne infections for humans. Each year 25% of all Americans are estimated to become ill from consuming foods contaminated with pathogenic microbes and their toxins (Mead et al. 1999). Both conventional and organic poultry products have been identified as important vectors for the transmission of three of the major sources of bacterial foodborne gastroenteritis: *Salmonella*, *Campylobacter*, and *Clostridium* (Andrews and Baumler 2005; Nachamkin and Guerry 2005; Novak et al. 2005). Although organic food products may represent a safer alternative in regards to chemical contamination of the product, control of these foodborne pathogens in organic poultry is particularly important because consumers of these products tend to perceive them as being safer and choose them for children, the elderly and immunocompromised people (Magkos et al. 2003). This is a concern for organic producers because they cannot control proper cooking and other food safety practices of consumers once the poultry products are sold.

Natural plant compounds and extracts has been important component of most traditional medical systems. Extracts from cinnamon (*Cinnamomum verum*), thyme (*Thymus vulgaris*), oregano (*Origanum glandulosum*) and clove (*Syzygium aromaticum*) being the most important (Burt 2004, Siragusa et al. 2008; Venkitanarayanan et al. 2013; Upadhyay et al. 2014). The specific extracts of these plants that possess the highest activity, include *trans*-cinnamaldehyde from cinnamon, thymol from thyme or oregano and eugenol from clove. Another alternative evaluated was caprylic acid, which occurs naturally in milk and coconut oil. These extracts have shown *in vitro* and *in vivo* efficacy against the pathogenic bacteria *Escherichia coli*, *Staphylococcus aureus*, *C. jejuni*, *Salmonella* spp. and *Clostridium* spp. (Cosentino et al. 1999; Dorman and Deans 2000; Mitsch et al. 2004; Johny et al. 2010; Traul et al. 2000; Vasudevan et al. 2005; Skřivanová et al. 2006; De los Santos et al. 2008, 2009; Johny et al. 2009).

Our team of researchers evaluated these natural, effective and safe alternatives to address health issues in birds, along with the prevention of foodborne diseases associated with poultry products. We tested these compounds due to their potential antimicrobial efficacy, their GRAS status, and NOP allowed status.

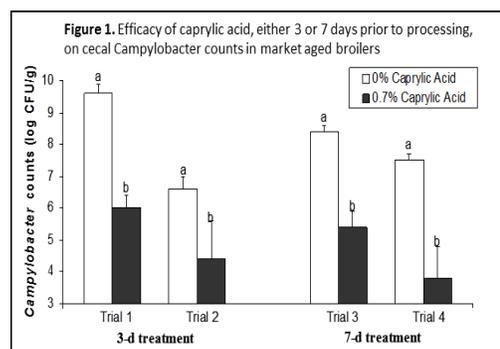
## Research studies

Four trials were conducted to evaluate the effectiveness of caprylic acid administration in market-age birds already colonized with *Campylobacter* (therapeutic effectiveness). Birds were challenged with  $\sim 1 \times 10^6$  cfu *Campylobacter* at three weeks of age. In the first two trials, birds received either 0% (positive controls), 0.7 or 1.4 % caprylic acid in feed for the last 3 days of the trial. Treatments were similar for Trials 3 and 4, except that caprylic acid was given during the last 7 days of the trial. On day 42, ceca were collected and *Campylobacter* counts determined.

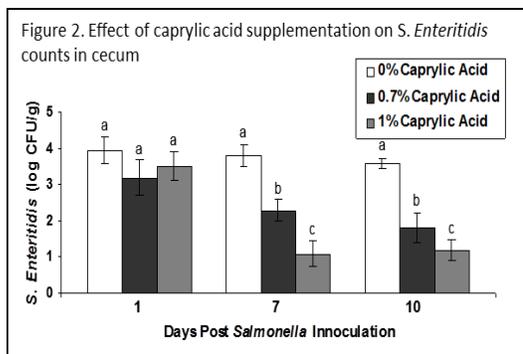
Caprylic acid at 0.7% consistently reduced ( $P < 0.05$ ) the colonization of *C. jejuni* in the chicken ceca compared to positive control treatment (Fig. 1).

The results suggest that therapeutic supplementation of caprylic acid in the feed can reduce *Campylobacter* in market-aged chickens, and may be a potential treatment for reducing pathogen carriage in poultry (De los Santos et al. 2009).

We investigated the prophylactic effectiveness of feed supplemented with caprylic acid for reducing *S. Enteritidis* (SE) colonization in chicks. Day old chicks were divided into six groups that consisted of 1 negative control (No SE challenge, birds fed normal diet), 2 negative caprylic acid controls (No SE challenge, birds fed with 0.7, 1% CA in feed), 1 positive control (SE challenged, fed normal diet), 2 positive caprylic acid controls (SE challenged and fed with CA at 0.7, 1% respectively). On day 7, chicks were inoculated with  $5.0 \log_{10}$  cfu of SE by crop gavage. Six chicks from each group were sacrificed on day 1, 7 and 10 after challenge and SE populations in the cecum, small intestine, cloaca, crop, liver and spleen were enumerated. The study was replicated three times.



Caprylic acid at 0.7 and 1% consistently decreased SE populations recovered from the treated chicks in comparison to positive control chicks (Fig. 2). SE counts in ceca, small intestine, cloaca, crop, liver, and spleen of caprylic acid treated chicks were substantially lower ( $P < 0.05$ ) than those of control chicks on day 7 and 10 after challenge. Feed intake, body weight and cecal endogenous bacterial populations did not differ between the caprylic acid treated and control groups. Histological examination revealed no pathological changes in the ceca and liver of birds supplemented with caprylic acid in comparison to controls. The results suggest that prophylactic supplementation of caprylic acid through feed can effectively reduce SE colonization in day-old chicks, and may be a potential treatment for reducing the pathogen carriage in poultry (Johny et al. 2009).



We conducted a study to investigate the therapeutic efficacy of plant compounds, *trans*-cinnamaldehyde (TC) and eugenol (EG) on reducing *S. Enteritidis* (SE) in commercial, market-age broiler chickens. Day-old broiler chicks were randomly divided into 6 groups of 14 birds each ( $n = 14/\text{group}$ ): a negative control (no SE, no TC or EG), EG control (no SE, 1% EG), TC control (no SE, 0.75% TC), a positive control (SE, no TC or EG), an EG challenge group (SE, 1% EG), and a TC challenge group (SE, 0.75% TC). On day 30, birds were challenged with a 4-strain mixture of SE ( $8 \log_{10}$  cfu/bird). Birds were given feed supplemented with TC (0.75%) or EG (1%) for 5 d before slaughter on d 42 ( $n = 10$  birds/group) for determination of SE populations in the cecum and cloaca. The experiment was repeated 2 times.

*Trans*-cinnamaldehyde and eugenol consistently reduced SE in the samples in both the trials ( $P < 0.05$ ). Body weights and feed consumption did not differ among the groups ( $P > 0.05$ ). On the basis of histological analysis, no abnormal changes in the liver were observed because of supplementation of plant compounds. The results suggest that TC and EG supplemented through feed could reduce SE colonization in market-age chickens.

We conducted 2 separate trials to investigate the efficacy of *trans*-cinnamaldehyde (TC) for reducing egg-borne transmission of SE and organ colonization in layer chickens. In each trial, 120 single-comb White Leghorn hens were randomly assigned to 6 treatments ( $n = 20/\text{treatment}$ ): a negative control (no SE, no TC), 2 compound controls (no SE and 1% or 1.5% vol/wt TC), a positive control (SE, no TC), a low dose treatment (SE and 1% TC) and a high dose treatment (SE and 1.5% TC). Selected doses of TC were supplemented in the feed for 66 days. On d 10, birds in the positive controls, low dose and high dose treatments were challenged with SE ( $10 \log_{10}$  cfu/bird) by crop gavage. After 7 d of challenge, eggs were collected and examined for SE in the yolk and on the shell daily until the end of the trial. On d 66 10 birds from each treatment were sacrificed to determine SE presence in the ceca, liver and oviduct.

In both trials, TC supplementation at 1% and 1.5% decreased SE on shell and in the yolk ( $P < 0.05$ ). Additionally, TC at both concentrations reduced SE in all cecum, liver and spleen ( $P < 0.05$ ) compared with control birds. Follow up mechanistic studies using cell culture and gene expression assays revealed that TC reduced SE adhesion and invasion of chicken oviduct epithelial cells, survival in chicken macrophages, and down regulated the expression of SE virulence genes critical for oviduct colonization ( $P < 0.05$ ). No significant differences in egg production were observed among the different bird groups ( $P > 0.05$ ). The results suggest that TC could potentially be used as a feed additive to reduce egg-borne transmission of SE in layer chickens (Upadhyaya et al., 2015).

The objective of this study was to determine the efficacy of different concentrations and combinations of thymol and carvacrol in feed to reduce *Campylobacter* in broilers. A total of four trials were conducted and in each trial ten 1-day-old chicks were randomly assigned to each treatment group. In the first two trials the birds were randomly divided into nine treatment groups ( $n = 10$  birds per treatment; total 90

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birds per trial) and the treatment groups included a positive *Campylobacter* control (0% thymol or carvacrol), 0.25, 0.5, 1 or 2% thymol or carvacrol. In trials 3 and 4, doses of 0.125, 0.25 or 0.5% of thymol or carvacrol and combinations of these doses were evaluated ( $n = 10$  birds per treatment; total 160 birds per trial). Each trial lasted 10 days and all the birds were provided with either control or treated feed throughout the study period. *Campylobacter* counts were reduced for 0.25% thymol (Trial 1), 1% carvacrol or 2% thymol (Trial 2) treatments, or a combination of both thymol and carvacrol at 0.5% (Trial 3) in this study. These results indicate that supplementation of these compounds in feed may control the colonization of *Campylobacter* in chickens but, additional research is required to develop treatment regimens providing consistent efficacy.

### **Conclusions**

Our research has led to the development of strategies to promote gut health and limit disease/pathogens in these birds. As organic poultry producers have a limited number of safe, effective and approved organic strategies to prevent and treat health problems in their flocks, our findings have produced effective solutions. Our research indicates that two classes of natural compounds such as fatty acids and essential plant extracts have antimicrobial efficacy against poultry enteric pathogens. These compounds are permitted under NOP and address food safety and disease concerns in organic production systems.

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