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Potential Role of Essential Oils in Organic Poultry Production[1]

Anup Kollanoor Johny BVScAH, MVSc, PhD[2,*], Kumar Venkitanarayanan DVM, MVSc, PhD[3], Annie Donoghue PhD[4], Dan Donoghue PhD[5] & Michael Darre PhD[3]

[2] Department of Animal Science, University of Minnesota, Saint Paul, MN 55108

[3] Department of Animal Science, University of Connecticut, Storrs, CT 06269

[4] USDA-ARS, Center of Excellence for Poultry Science, University of Arkansas, Fayetteville, AR 72701

[5] Center of Excellence for Poultry Science, University of Arkansas, Fayetteville, Arkansas, AR 72701

* Contact information of the speaker – 405B, Haecker Hall, 1364 Eckles Ave, Saint Paul, MN 55113. Phone: 612-625-5277; Email: anupjohn@umn.edu

Organic poultry – opportunity and challenge

Organic farming is one of the fastest growing agricultural sectors in the United States. The demand for organically-raised foods, including animal products, has increased several folds since the 1990s, with U.S. consumers being a major market driving force. It is estimated that the US organic food sales reached $35 billion in 2014, up from $28.4 billion reported in 2012 (USDA-ERS, 2009; 2014, Greene, 2013). Since the demand for organic foods out-grew supplies in the last few years, the U.S. retailers imported several billion dollars' worth of organic foods to the American market (Crandall et al., 2009). Among the organic animal products, poultry meat and eggs are available widely across the country and are well-accepted by the consumers (AgMRC, 2013). The latest NASS (2010) survey indicated that the sale of organic poultry meat and eggs fetched ~$350 million in 2008 (NASS, 2010). However, even with 9 million certified broilers, 5.5 million certified layer hens and 400,000 certified organic turkeys (NASS, 2010; AgMRC, 2013), the industry has not been able to meet the increasing demand for organic poultry. This underscores tremendous opportunity for the growth of the organic poultry sector in the coming years, as predicted by the Organic Trade Association (Greene, 2013). Although there is significant scope for the expansion of the organic poultry, concerns over the safety of organic meat and eggs potentially contaminated with foodborne pathogens could curtail the opportunity (Mogelonsky, 2008). This situation warrants rapid response to identify alternative and applicable antimicrobial intervention methods of pathogen control in organic poultry.

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Current gaps in pathogen control

The National Organic Program (NOP) restricts the use of antibiotics, hormones, herbicides and pesticides from organic agricultural activities to safeguard the environment, people and animals, thereby potentially improving the sustainability of the sector (Fanatico, 2008). In addition, consumers of organic products generally tend to perceive the products as safer alternatives due to less/no added preservatives/chemicals and choose them for their families. In line with NOP compliance, naturally-derived enzymes, antioxidants and botanicals can be used in organic poultry farming to combat infections, improve growth and enhance quality of the products. Vaccines against Marek's disease virus, Newcastle disease virus, infectious bronchitis virus and coccidia are allowed. Probiotics, the beneficial bacteria that are capable of fighting pathogens in the digestive tract of chickens, could also be used to improve general health and disease prevention in birds. In case of severe infections where antibiotics are used for treatment; birds cannot be marketed as organic (Fanatico, 2008). However, the lack of sufficient and reliable research data on the use of the aforementioned methods on improving the microbiological quality of organically raised poultry is a limiting factor. In addition, supporting the inadequacy of the current methods, several studies have indicated the presence of similar levels of pathogen contamination in organic and commercial poultry products (Sato et al., 2004; Cui et al., 2005; Stone et al., 2013; Noormohamed & Fakhr, 2014). This situation presents a unique challenge for the organic sector to advise its beneficiaries, including producers and processors, on potential antimicrobials that can be used for rendering their products safe from infectious agents. In addition, as per the NOP standards, poultry must have outdoor access, an environment that may have pathogenic organisms such as *Salmonella* and *Campylobacter*. Similar factors that could pose challenge to safe organic poultry production include the use of slow-growing breeds and very small slaughtering facilities – both potentially increasing propensity for pathogen contamination in the products (Cui et al., 2005).

Major food-borne bacterial pathogens in poultry

Among the bacterial pathogens transmitted through poultry products, *Salmonella* and *Campylobacter* are the most common infectious agents capable of causing disease in humans. Although there are multiple sources of these organisms on farms; poultry, including broilers, layers and turkeys serve as the major sources of product contamination. The primary colonization site of *Salmonella* in poultry is the ceca, with cecal carriage of the pathogen leading to horizontal transmission of the infection, contamination of carcasses and eggshell with feces, and contamination of ovaries (Gantois et al., 2009; Upadhyaya et al., 2015). Egg contamination with *Salmonella* results by penetration through the eggshell from contaminated feces during or after oviposition. Similarly, *Campylobacter* primarily colonizes the mucus overlying the epithelial cells in the ceca and small intestine of poultry. Once birds in a flock become colonized, the bacteria disseminate to the entire flock rapidly by high levels of fecal shedding and fecal–oral transmission through water and feed (Lee and Newell, 2006).

In an organic setting, the likelihood of birds being exposed to *Salmonella* and *Campylobacter* is relatively high since they have outdoor access. These pathogens could be present in the soil and water, and can infect birds. Organic poultry products could be critical vehicles in the transmission of these pathogens to humans (Sato et al., 2004; Cui et al., 2005; Stone et al., 2013; Noormohamed & Fakhr, 2014). There is ample evidence demonstrating that organic poultry harbor similar or higher pathogens levels, compared with conventionally raised poultry. Cui et al. (2005) studied the prevalence of the pathogens in organic chickens and found that 76% and 61% of the samples were contaminated with *Campylobacter* and *Salmonella*, respectively. They also observed a similar prevalence for *Campylobacter* on both organic and commercial chickens, but found more *Salmonella* on organic chicken samples. In a similar study, Noormohamed and Fakhr (2014) reported almost similar *Campylobacter* prevalence in both organic and conventional chickens. In yet another study, Lestari et al. (2009) reported similar (21-22%) prevalence of *Salmonella* from organic and conventional chicken carcasses sampled from Louisiana. Van Overbeke et al. (2006) also observed a similar prevalence of *Salmonella* from conventional and organic chicken samples. A noteworthy study by Consumer Reports (2007) concluded that a major percentage (>74%) of all the poultry carcasses sampled from both conventional and organic sources were contaminated with *Campylobacter*, and 27% of the USDA organic-certified carcasses had *Salmonella* contamination. With conditions of lower bird density, outdoor access and restrictions on pesticides and antibiotic use, consumers may conclude that organically raised poultry are free or have a reduced food borne pathogen carriage compared to conventionally raised birds. This assumption may increase the potential of contamination through mishandling of raw product. This situation warrants the necessity for effective organic-friendly antimicrobial strategies at the farm and processing levels for improving the microbiological safety of poultry meat and eggs. Unfortunately, organic poultry operations have limited effective interventions against these harmful pathogens.

Essential oils & active compounds

Among the few emerging and recently explored alternatives, essential oils or their constituent compounds have proven antibacterial potential against the aforementioned pathogens. Essential oils have been used in medicine, food preservation, cosmetics and perfumery for centuries. Originating from plants, essential oils, the distilled extracts of volatile plant compounds, and most of their active constituent components present an array of safe alternatives that are natural, less toxic, environmentally-friendly, GRAS status (generally recognized as safe by the FDA) feed additives (Venkitanarayanan et al., 2013; Upadhyay et al., 2014). Since essential oils and/or their biological constituents contain a number of different active chemical groups in their structure, their antimicrobial activity is attributable to more than one specific mechanism (Burt, 2004); this unique property significantly reduces the likelihood of bacteria developing resistance to them. Our research group has evaluated the effect of several active essential oil compounds such as *trans*-cinnamaldehyde, eugenol, thymol and carvacrol against *Salmonella* and *Campylobacter* in commercial broiler and layer chickens. Currently our research has focused on utilizing these essential oil compounds against foodborne pathogens in organic chickens. *Trans*-cinnamaldehyde is the major component in the bark extract of cinnamon. Eugenol is a natural phytophenolic compound present in the clove essential oil. Thymol and its structural isomer carvacrol are derived from a number of plant sources such as *Thymus, Origanum* and *Carum* spp.

Effect of essential oil compounds on *Salmonella* and *Campylobacter* in chickens

The *in vitro* efficacy of the essential oil compounds, *trans*-cinnamaldehyde, eugenol, thymol and carvacrol were tested in a modified cecal medium. Each compound was added at different concentrations (ranging from 0.1 to 1.2%) to chicken cecal contents inoculated with 7.0 $\log_{10}$ CFU/ml of *S*. Enteritidis (one of the common serotypes of *Salmonella*) or 5.0 $\log_{10}$ CFU/ml of *Campylobacter*. The pathogen populations in the cecal contents after specific periods of incubation were determined. All essential oil compounds were bactericidal on both pathogens, with *trans*-cinnamaldehyde and eugenol being most effective (Kollanoor-Johny et al., 2010). Sequentially, we conducted a series of experiments in broiler chickens determining the efficacy of essential oil compounds against *S*. Enteritidis in broiler chicks. Birds were supplemented with either 0.5 or 0.75% *trans*-cinnamaldehyde, and 0.75 or 1% eugenol through feed for 20 days. On day 8, birds were inoculated with 8.0 log CFU *S*. Enteritidis. The pathogen populations in the cecum were determined at the end of the study. *Trans*-cinnamaldehyde at 0.5 and 0.75% and eugenol at 1% reduced *S*. Enteritidis in the cecum (3 $\log_{10}$ CFU/g) after 10 days of infection in challenged birds. Neither compound altered the pH nor endogenous cecal microflora counts. Feed intake and body weight were not significantly different for *trans*-cinnamaldehyde supplemented groups. However, eugenol-treated groups had significantly lower body weight compared to the control (Kollanoor Johny et al., 2012a). In follow up studies, we determined the antibacterial efficacy of *trans*-cinnamaldehyde and eugenol for decreasing *S*. Enteritidis in market-age broiler chickens. *Trans*-cinnamaldehyde was added at 0.75% and eugenol at 1% as an antimicrobial additive in the feed for 5 days prior to slaughter. Both essential oil compounds reduced cecal colonization of *S*. Enteritidis by 1.5 $\log_{10}$CFU/g (Kollanoor Johny et al., 2012b). Recently, we reported that the in-feed supplementation of *trans*-cinnamaldehyde reduced egg-borne transmission of *S*. Enteritidis in commercial layer chickens (Upadhyaya et al., 2015). Supplementation of *trans*-cinnamaldehyde for 66 days at 1 and 1.5% to 40-week and 25-week old layer hens decreased *S*. Enteritidis on the egg shell and in the yolk without causing any deleterious effect on the growth, egg production and consumer acceptability of eggs. In addition, Upadhyaya et al. (2013) also reported on the rapid inactivation potential of essential oil compounds against *S*. Enteritidis on shelled eggs. These studies indicate that the tested essential oil compounds have the potential to reduce contamination of organic meat and eggs as well.

We also tested the efficacy of the essential oil compounds carvacrol and thymol on *Campylobacter* populations in broiler chickens. These molecules reduced *Campylobacter* colonization, although consistency in the antimicrobial effect across the experiments was a problem (Arsi et al., 2014). Thymol at 0.25 and 1%, or carvacrol at 1%, or a combination of the molecules at 0.5% was effective against *Campylobacter* colonization in broilers (Arsi et al., 2014).

Mechanisms of action of essential oil compounds

A critical property of essential oils or their compounds is their hydrophobicity that leads to bacterial cell membrane damage and leakage of ions and other cell contents. Specifically, *trans*-cinnamaldehyde affects glucose uptake and ATP synthesis, two critical growth/survival mechanisms in bacteria. Another mechanism by which the essential oils/compounds kill microorganisms is by inhibiting key enzymes such as amino acid decarboxylases. In addition to the aforementioned mechanisms of action, *trans*-cinnamaldehyde and eugenol target virulence in *S*. Enteritidis. Our research revealed that these molecules resulted in decreased *S*. Enteritidis motility and invasion of avian abdominal epithelial cells, chicken oviduct epithelial cells and chicken macrophages by down-regulating critical virulence genes (Kollanoor Johny et al., 2012a, b; Upadhyaya et al., 2015). Moreover, our DNA microarray results indicated that several genes, including those involved in the regulation of *Salmonella* Pathogenicity Island 1, type 3 secretion system, outer membrane proteins, metabolic pathways, and electron acceptors under anaerobiasis were down-regulated in *S*. Enteritidis by these molecules (Kollanoor Johny et al., 2011).

Application of essential oils/compounds in organic poultry production

Our current research is focused on the application of the aforementioned essential oil compounds and other newly screened compounds to control pathogens in organic poultry. These molecules have not only shown antibacterial effect against *Salmonella* and *Campylobacter*, but also on several other economically important and hazardous infectious agents such as *Clostridium jejuni*, *E. coli* O157: H7 and *Listeria monocytogenes* on several non-poultry food matrices (Baskaran et al., 2013; Upadhay et al., 2013; Mooyottu et al., 2014). Since these compounds are safe for animals and humans, and are environment-friendly, we are exploring their potential in organic poultry production for both pre-harvest and post-harvest applications. Apart from salmonellosis and campylobacteriosis, the organic industry faces challenges with coccidiosis, clostridia infections, internal and external parasites and high mortality from diseases for which the potential of essential oils and their compounds has to be explored. Moreover, economic considerations for inclusion of these compounds in organic poultry feeds need to be determined (Darre et al., 2014).

Our research group is currently developing comprehensive strategies for pathogen control using essential oils/compounds in organic poultry production. As organic poultry producers have a limited number of safe, effective and approved organic strategies to prevent and treat food borne contamination problems in their flocks our findings have produced effective solutions. Our research indicates that essential plant extracts have antimicrobial efficacy against poultry enteric pathogens, are permitted under NOP and address food safety and disease concerns in organic production systems.

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