Use of plant-derived antimicrobials for improving the safety of poultry products[1]

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ABSTRACT *Salmonella* Enteritidis and *Campylobacter jejuni* are the 2 major foodborne pathogens transmitted through poultry products. Chickens are the reservoir hosts of these pathogens, with their intestinal colonization being the most significant factor causing contamination of meat and eggs. Effective preslaughter strategies for reducing the colonization of birds with these pathogens are critical to improve the microbiological safety of poultry products. An antimicrobial treatment that can be applied through feed represents the most practical and economically viable method for adoption on farms. Additionally, a natural and safe antimicrobial will be better accepted by producers without concerns for toxicity. This symposium talk discussed the potential use of plant-derived, GRAS (generally recognized as safe)-status molecules, caprylic acid, *trans*-cinnamaldehyde, eugenol, carvacrol, and thymol as feed supplements for reducing cecal populations of *Salmonella* Enteritidis and *C. jejuni* in chickens. Additionally, the effect of plant molecules on *Salmonella* virulence genes critical for cecal colonization in chickens was also discussed.

Key words: *Salmonella* Enteritidis, *Campylobacter jejuni*, chicken, cecum, plant-derived antimicrobial

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INTRODUCTION

Salmonella Enteritidis and *Campylobacter jejuni* are the 2 major foodborne pathogens causing human infections in the United States, and are linked to the consumption of raw or undercooked poultry (Heres et al., 2004; Scallan et al., 2011). *Salmonella* Enteritidis is the most common serotype of *Salmonella* isolated from poultry products (Antunes et al., 2003; Shah et al., 2012). On the other hand, epidemiological investigations have shown a significant link between human *C. jejuni* infections and consumption of raw or undercooked poultry meat (Friedman et al., 2004). Annually, several millions of dollars are being lost due to human infections caused by these pathogens. The recent epidemiological data suggest that *Salmonella* causes an estimated 1 million cases of nontyphoidal salmonellosis annually in the United States, resulting in 19,226 hospitalizations and 378 deaths, whereas *Campylobacter* results in an estimated 0.8 million cases resulting in 8,463 hospitalizations and 76 deaths (Scharff, 2012). Further, it is estimated that salmonellosis and campylobacteriosis results in annual economic loss of approximately $4.4 and $1.5 billion, respectively (Scharff, 2012). Poultry and poultry products are considered to be critical sources of these pathogens leading to foodborne outbreaks (Marcus et al., 2007). Poultry products constitute a significant portion of human diet, and their per capita consumption has increased during the last decade (USDA-ERS, 2012). Thus, the microbiological safety of poultry and poultry products is of immense importance, especially because of the public health and economic outcomes.

Chickens serve as natural reservoir hosts for *Salmonella* Enteritidis and *C. jejuni*, and they colonize the intestinal tract of chickens, with cecum being the primary colonization site (Van Immerseel et al., 2004b). Once colonized, the pathogens are excreted to the environment via droppings, thereby potentially contaminating the environment, especially the grow-out houses infecting healthy flock (horizontal transmission). In addition, the pathogens present in an infected/contaminated carcass could cross-contaminate other carcasses during slaughter or subsequent processing operations (Keller et al., 1995). In slaughter houses, many opportunities exist for the transfer of pathogens from the outside surface of chickens to the meat during mechanized processing of carcasses. In addition, it is not easy

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to decrease populations of pathogens on carcasses to less than that which existed on the external surfaces of live birds when they arrived at the processing plants (Bailey et al., 1991). Moreover, mechanical evisceration can cause gastrointestinal spillage of these pathogens, further contaminating the edible carcasses (Byrd, 2005). The USDA Food Safety and Inspection Service data on *Salmonella* Enteritidis in broiler chicken carcass rinses collected from 2000 to 2005 indicated a more than 4-fold increase in the annual number of isolates and a 3-fold rise in the proportion of establishments with *Salmonella* Enteritidis–positive rinses (Altekruse et al., 2006). In addition, consuming chicken was reported as a risk factor for sporadic human *Salmonella* Enteritidis in the United States (Kimura et al., 2004; Marcus et al., 2007). On the other hand, colonization of broiler chickens by *C. jejuni* is widespread and is difficult to prevent. In chickens, *C. jejuni* primarily colonizes the mucus overlying the epithelial cells in the ceca and small intestine. L-Fucose, the major carbohydrate component present in the mucin of chicken cecal mucus, can be used by *C. jejuni* as a sole substrate for growth. Thus, the cecal environment in chickens is favorable for the survival and proliferation of *C. jejuni* (Beery et al., 1988) and selects its colonization in the birds.

Besides poultry meat, eggs contaminated with *Salmonella* Enteritidis potentially constitute a major public health hazard. It is possible that the eggs are contaminated by these pathogens either by retrograde transmission (from cloaca to oviduct) or transovarian (from ovaries/ovarian follicles/oviduct to eggs) route (Timoney et al., 1989; Shivaprasad et al., 1990). Patrick and coworkers (2004) reported that 80% of the known-source of *Salmonella* Enteritidis outbreaks during 1985 to 1999 in the United States were egg-associated. Similarly, another study revealed that among the *Salmonella* Enteritidis outbreaks with a confirmed food vehicle during the period from 1985 to 2003, 79% were egg-based or contained egg ingredients (Braden, 2006). Another study reported an estimated 700,000 cases of egg-borne salmonellosis in the United States, which accounted for ~47% of total food-borne salmonellosis, and costing more than $1 billion annually (Frenzen et al., 1999). Yet another study published by the Food Safety and Inspection Service estimated that consumption of *Salmonella* Enteritidis-contaminated shell eggs caused 182,060 illnesses in the United States in 2000 (Schroeder et al., 2005). Moreover, several epidemiological studies have revealed an association between salmonellosis and consumption of eggs (Centers for Disease Control and Prevention, 1988; Angulo and Swerdlow, 1999; Braden, 2006; Hald et al., 2007). In 2010, a nationwide outbreak of *Salmonella* Enteritidis consisting of 3,578 cases associated with the consumption of shell eggs was reported in the United States (Centers for Disease Control and Prevention, 2010). In light of the evidence of linking human salmonellosis with shell eggs, the Food and Drug Administration (**FDA**) in 2009 announced that eggs constitute an important source of *Salmonella* Enteritidis infections, and issued a final rule that requires shell egg producers to implement measures to prevent *Salmonella* Enteritidis from contaminating eggs on the farm and further growth during storage and transportation.

SALMONELLA AND *CAMPYLOBACTER* CONTROL STRATEGIES

Because humans contract *Salmonella* Enteritidis or *C. jejuni* infections by consuming contaminated chicken meat or eggs (Marcus et al., 2007), it is critical that the intestinal colonization of these pathogens in chickens be reduced to decrease the level of contamination of the products. Several critical control points have been identified at production sites to prevent or reduce colonization of chickens with *Salmonella* Enteritidis and *C. jejuni* (Bailey, 1993). The most important critical control point is the delivery of pathogen-free chicks to the grow-out houses. In addition, contaminated feed, litter, water, rodents, and insects can also play a role in the spread of *Salmonella* Enteritidis. Likewise, movement of colonized birds, contaminated equipment, egg flats, feed truck, and service personnel can also facilitate flock-to-flock transmission. A study from the United Kingdom on the sources of *C. jejuni* in broiler flocks revealed presence of the pathogen in the feed, water, and air of the farm. Moreover, *Campylobacter* was also detected in the air up to 30 m downstream of the broiler house, highlighting the potential role of airborne transmission in the spread of the pathogen. In addition to water, feed, and air, vectors, including flies, wild birds, and rodents can also act as sources of *C. jejuni* (Newell and Fearnley, 2003). Due to the multitude of sources of *Salmonella* Enteritidis and *C. jejuni*, implementation of strict biosecurity measures at the farm/production sites is critical to reduce the spread of these bacterial pathogens.

A major control strategy for *Salmonella* Enteritidis and *C. jejuni* in poultry is the preslaughter approach. Intervention aimed at reducing pathogen colonization in chickens is the key idea behind this approach. Reducing the populations of *Salmonella* Enteritidis and *C. jejuni* in the chicken intestinal tract can potentially decrease contamination of poultry meat and eggs. A variety of approaches for reducing the colonization of the aforementioned pathogens in chickens has been explored, but with varying degrees of success. These include feeding chicks with competitive exclusion bacteria (Nurmi and Rantala, 1973; Blankenship et al., 1993; Stern et al., 2001; Filho et al., 2003), probiotic bacteria (Pascual et al., 1999; Tellez et al., 2001), bacteriophages (Wagenaar et al., 2005; Atterbury et al., 2007), mannanoligosaccharides (Spring et al., 2000; Fernandez et al., 2002), fructooligosaccharides (Bailey et al., 1991), chicory fructans (Yusrizal and Chen, 2003), organic acids such as acetic, propionic, and formic acids (Hinton and Linton, 1988; Al-Tarazi and Alshawabkeh, 2003;

Heres et al., 2004), tannic acid (Kubena et al., 2001), and antibiotics and other antimicrobials (Chadfield and Hinton, 2003). Additionally, several vaccination approaches to control *Salmonella* Enteritidis and *C. jejuni* in chickens has been undertaken (Barrow, 1997; Dueger et al., 2001; Khan et al., 2003; Inoue et al., 2008). Despite intense control programs, the CDC reported a 3% increase in the incidence of laboratory-confirmed cases of *Salmonella* in the United States (Centers for Disease Control and Prevention, 2012), highlighting the need for alternate strategies to control this foodborne pathogen. In addressing this, we have been investigating the potential of several plant-derived molecules for controlling *Salmonella* Enteritidis and *C. jejuni* in chickens. The following is a review of research undertaken for determining the efficacy of several plant molecules as preslaughter treatments to improve the microbiological safety of poultry products.

CAPRYLIC ACID ON *SALMONELLA* ENTERITIDIS AND *C. JEJUNI*

Caprylic acid (octanoic acid) is a natural, 8-carbon medium-chain fatty acid (**MCFA**) present in coconut oil, palm kernel oil, caprine milk, and bovine milk (Jensen et al., 1990, Sprong et al., 2001; Jensen, 2002), and is a food-grade chemical approved by the FDA as generally regarded as safe (**GRAS**). One of our first reports (Vasudevan et al., 2005) examined the anti-*Salmonella* potential of caprylic acid on rapid reduction of *Salmonella* Enteritidis (>5.0 log cfu) in chicken cecal contents in vitro. Interestingly, caprylic acid was minimally inhibitory ($P < 0.05$) toward the endogenous cecal population of anaerobic bacteria. Van Immerseel and coworkers (2004a) reported that MCFA, particularly caproic acid supplemented to chicks (at 3 g/kg of feed), resulted in significant reductions in the colonization of the *Salmonella* Enteritidis in the ceca and internal organs of the birds. They found that MCFA suppressed the expression of *hilA*, a key gene regulator involved in the invasion of *Salmonella*, thereby resulting in its decreased colonization in vivo. In pigs, several studies have revealed that dietary caprylic acid reached bactericidal concentrations in the stomach and intestine, thereby significantly reducing intestinal bacterial loads without any adverse effect on the performance (Dierick et al., 2002, 2003, 2004).

Based on our previously published data on the in vitro antimicrobial efficacy of caprylic acid on *Salmonella* Enteritidis (Vasudevan et al., 2005), a study was undertaken to determine the prophylactic potential of caprylic acid for controlling the pathogen in commercial day-old broiler chicks. Birds were fed with either 0.7 or 1% caprylic acid supplemented through feed for 18 d. Caprylic acid supplementation at 0.7 and 1% consistently decreased *Salmonella* Enteritidis populations on d 7 and 10 after challenge in cecum, cloaca, liver, spleen, intestine, and crop ($P < 0.05$). Feed intake and BW did not differ between the treated and control groups. Histological examination revealed no pathological changes in the cecum and liver of caprylic acid-supplemented birds. In addition, caprylic acid supplementation did not significantly affect the cecal pH or the cecal endogenous bacteria. The results suggested that prophylactic caprylic acid supplementation through feed could potentially reduce *Salmonella* Enteritidis colonization in chickens without adverse effects (Kollanoor Johny et al., 2009).

Based on the findings from the prophylactic supplementation study, we determined the therapeutic efficacy of caprylic acid for reducing *Salmonella* Enteritidis in chickens. Caprylic acid was supplemented through feed at 0.7 or 1% for the last 5 d before slaughter at 3 or 6 wk of age. As observed with the prophylactic supplementation, caprylic acid at 0.7 or 1% significantly decreased *Salmonella* Enteritidis populations in the cecum, small intestine, cloaca, liver, and spleen in both 3- and 6-wk experiments ($P < 0.05$). In addition, the feed intake and BW did not differ between the bird groups ($P > 0.05$; Kollanoor Johny et al., 2012c). Results confirmed that caprylic acid could be used as a potential antibacterial feed additive to reduce *Salmonella* Enteritidis colonization in broiler chickens.

Further, to elucidate potential antibacterial mechanisms of action of caprylic acid on *Salmonella* Enteritidis, we investigated if a subinhibitory concentration (**SIC**; the highest concentration below the minimum inhibitory concentration that does not inhibit the bacterial growth) of caprylic acid could reduce *Salmonella* Enteritidis invasion of budgerigar abdominal tumor cells (**BATC**), an avian intestinal epithelial cell line (Dodson et al., 1999), and the expression of *Salmonella* invasion genes, *hilA* and *hilD*. Results indicated that caprylic acid reduced invasive abilities of *Salmonella* Enteritidis strains by ~80% ($P < 0.05$). Gene expression studies revealed that caprylic acid downregulated ($P < 0.05$) *Salmonella* invasion genes, *hilA* and *hilD*. These results suggest that supplementation of caprylic acid potentially reduces the pathogen's ability to invade intestinal epithelial cells by downregulating the key invasion genes, *hilA* and *hilD* (Kollanoor Johny et al., 2012c). These results are in agreement with Van Immerseel et al. (2004a) who found that MCFA suppressed the expression of *hilA* thereby resulting in decreased *Salmonella* colonization in chicks. These investigators suggested that MCFA could potentially reduce invasive abilities of *Salmonella* in T-84 epithelial cell lines. Additionally, Boyen et al. (2008) reported that caprylic acid at 2 m*M* reduced invasion of *Salmonella* Typhimurium on porcine epithelial cells and downregulated the expression of *hilA* and *fimA* in the bacterium.

In addition to these studies, other research conducted at the University of Arkansas at Fayetteville investigated a series of studies on determining the potential of caprylic acid on *C. jejuni* colonization in broiler chickens. In a study that involved day-old chicks supplemented with caprylic acid in feed, Solis de los

Santos et al. (2008b) reported that 0.7% caprylic acid reduced *C. jejuni* colonization. Further, the therapeutic potential of caprylic acid for reducing *C. jejuni* was also explored with supplementation of caprylic acid to 15-d-old chicks 72 h before necropsy. It was found that caprylic acid at 0.7 and 1.4% reduced cecal *C. jejuni* counts by 3 to 5 $\log_{10}$ cfu/g (Solis de los Santos et al., 2008a). In addition, the therapeutic efficacy of caprylic acid for decreasing cecal *C. jejuni* was demonstrated in older chickens. Solis de los Santos et al. (2009) observed that 0.7% caprylic acid reduced *C. jejuni* colonization consistently in market-age (42 d) broiler chickens when supplemented during the last 3 or 7 d of the experiment without any adverse effects. Interestingly, when 0.7% caprylic acid was supplemented for last 3 d of the experiment, *C. jejuni* colonization was reduced by 3 $\log_{10}$ cfu/g, even after a 12-h feed withdrawal period. These results collectively suggested that caprylic acid at 0.7% concentration when supplemented through feed could reduce *C. jejuni* colonization in commercial broiler chickens prophylactically and therapeutically, and can also be used to reduce the pathogen carriage during feed withdrawal periods.

PHYTOPHENOLICS ON *SALMONELLA* ENTERITIDIS AND *C. JEJUNI*

Plant-derived essential oils are a group of natural molecules that have been traditionally used as dietary constituents, especially to preserve foods and enhance food flavor (Wollenweber, 1988). The antimicrobial properties of several plant-derived essential oils have been demonstrated previously (Burt, 2004; Holley and Patel, 2005), and a variety of active components in these oils has been identified. These compounds are helpful to plants as a defense mechanism against several invading microbes and predators. Although considerable information is available on the antimicrobial properties of essential oils, most the studies determining their antimicrobial activity have been conducted in vitro using model broth systems (Knight and McKellar, 2007), highlighting that their specific applications for improving food safety need to be identified and validated. Based on the in vitro screening of the antibacterial activity of several plant-derived antimicrobials (**PDA**) on *Salmonella* and *Campylobacter* (Kollanoor Johny et al., 2010a,d), we found 4 molecules, namely *trans*-cinnamaldehyde, eugenol, carvacrol, and thymol, of potential use to control these pathogens in poultry, which are discussed in this review.

Trans-cinnamaldehyde is a major component of the bark extract of cinnamon. It is a GRAS compound approved for use in foods by the FDA. The US Flavoring Extract Manufacturers' Association reported that *trans*-cinnamaldehyde has a wide margin of safety between conservative estimates of intake and no observed adverse effect levels, from subchronic and chronic studies (Adams et al., 2004). The report also indicated no genotoxic and mutagenic effects due to the compound. In addition, Michiels et al. (2008) reported that oral supplementation of *trans*-cinnamaldehyde in piglets at 13.0 mg/kg of BW did not result in any toxic effects. Moreover, published data from our laboratory indicated that the trans-cinnamaldehyde at 0.5 and 1.5% did not produce any cytotoxic effect on human epithelial cell lines and urinary tract cells in vitro, respectively (Amalaradjou et al., 2009, 2010). *Trans*-cinnamaldehyde possesses antimicrobial activity toward a wide range of foodborne pathogens, including gram-positive and gram-negative bacteria (Bowles and Miller, 1993; Bowles et al., 1995; Friedman et al., 2002). The antibacterial activity of *trans*-cinnamaldehyde against *Clostridium botulinum* (Bowles and Miller, 1993), *Staphylococcus aureus* (Bowles et al., 1995), and *Escherichia coli* O157:H7 has been previously reported. In another study, *trans*-cinnamaldehyde was found to be inhibitory on various pathogenic bacteria in vitro without exerting any harmful effect on the natural microflora in pigs. The compound was found to reduce the *S.* Typhimurium DT104, *E. coli* O157: H7, and other coliforms with little inhibition toward beneficial bacteria, lactobacilli, and bifidobacteria in vitro (Si et al., 2006). In bioavailability studies conducted earlier, Nutley (1990) fed groups of male Fischer-344 rats with a single oral dose of 2.5 mmol of cinnamaldehyde that resulted in 16% recovery of the compound in excreta, with 0.7% retained in the carcass. In another similar study in rats, Sapienza et al. (1993) reported that a single oral dose of 500 mg/kg of radioactive cinnamaldehyde resulted in 3.2% recovery in excreta after 24 h. Radioactivity of the compound was seen primarily in the gastrointestinal tract, kidney, liver, and fat. In addition, these investigators reported that a 7-d supplementation of cinnamaldehyde resulted in 4.5% recovery of the PDA in feces, indicating that continuous supplementation might result in higher recovery.

Eugenol is a natural molecule present as an active ingredient in the oil from cloves (*Eugenia caryophillis*; Ali et al., 2005). The antibacterial activity of clove oil and eugenol has been documented by many researchers (Stecchini et al., 1993; Menon and Garg, 2001; Suhr and Nielsen, 2003; Ali et al., 2005). Si et al. (2009) reported that eugenol was inhibitory on various pathogenic bacteria without exerting any harmful effect on the natural microflora in pigs using an ex vivo model. Similar to *trans*-cinnamaldehyde, eugenol also reduced *Salmonella* Typhimurium DT104, *Escherichia coli* O157:H7, and other coliforms with little inhibition toward lactobacilli and bifidobacteria (Si et al., 2006). In another study, Michiels et al. (2008) observed that supplementation of eugenol at 12.5 mg/kg of BW in piglets did not result in any toxic effects.

Carvacrol and thymol are major ingredients in oregano oil obtained from *Origanum glandulosum* (Bendahou et al., 2007). The oil has been found effective against bacterial and fungal infections of the gastrointestinal and genitourinary tract (Blumenthal et al., 2000; Chun et al., 2005).

In light of these documented reports, we conducted a series of experiments examining the potential of these plant molecules for their anti-*Salmonella* and anti-*Campylobacter* effects using in vitro models. We used cecal contents because this menstruum closely resembles the cecum of chickens compared with synthetic laboratory media. We observed that among the PDA tested, *trans*-cinnamaldehyde was significantly effective in reducing *Salmonella* Enteritidis and *C. jejuni* populations in cecal contents without affecting the total cecal endogenous populations (Kollanoor Johny et al., 2010a). *Trans*-cinnamaldehyde at 0.2 and 0.35% inactivated *Salmonella* Enteritidis by 9 $\log_{10}$ cfu/mL, compared with the controls after incubation for 24 h. Although exhibiting slightly lower antibacterial potential compared with *trans*-cinnamaldehyde, other molecules eugenol, carvacrol, and thymol at 0.75 and 1% also brought about significant reductions in pathogen populations after 24 h of incubation. It was also observed that *C. jejuni* was more sensitive to all the PDA. For example, all PDA at ~0.1% were able to significantly reduce the pathogen after 24 h of incubation (Kollanoor Johny et al., 2010a). *Campylobacter jejuni* is considered to be a fragile bacterium that is difficult to culture especially when it is outside the host (Solomon and Hoover, 1999), thus explaining its increased sensitivity to the plant molecules.

Drinking water is a major source of *Salmonella* Enteritidis in chickens. Contamination of water with feed or fecal material from infected chickens can potentially result in pathogen colonization in birds. To determine the efficacy of *trans*-cinnamaldehyde for reducing *Salmonella* Enteritidis in chicken drinking water, a study was undertaken with tap water collected from the University of Connecticut Poultry Farm added with or without contaminating feces (1%) or feed (1%). We found that *trans*-cinnamaldehyde at 0.06% inactivated *Salmonella* Enteritidis completely after 24 h in water with 1% added feces at 12.5 and 25°C. Results indicated that *trans*-cinnamaldehyde is effective in killing *Salmonella* Enteritidis in chicken drinking water, and could be used as an additive to control water-borne *Salmonella* Enteritidis colonization in chickens (Kollanoor Johny et al., 2008).

After examining the antibacterial potential of plant molecules in vitro, studies were conducted to assess their efficacy in reducing the intestinal colonization of *Salmonella* Enteritidis and *C. jejuni* in chickens. The prophylactic efficacy of *trans*-cinnamaldehyde and eugenol was tested against *Salmonella* Enteritidis counts in commercial day-old broiler chicks. Birds were supplemented with either 0.5 or 0.75% trans-cinnamaldehyde, and 0.75 or 1% eugenol through feed for 20 d. *Trans*-cinnamaldehyde at 0.5 and 0.75% and eugenol at 1% reduced ($P < 0.05$) *Salmonella* Enteritidis in the cecum (>3 $\log_{10}$ cfu/g) after 10 d of infection. Neither compounds altered the pH nor endogenous cecal microflora counts ($P > 0.05$). Feed intake and BW were not significantly different for *trans*-cinnamaldehyde-supplemented groups ($P > 0.05$). However, eugenol-treated groups had significantly lower ($P < 0.05$) BW compared with the control birds (Kollanoor Johny et al., 2012b).

In follow up studies, 2 experiments were conducted with market-age broiler chickens to determine the therapeutic efficacy of *trans*-cinnamaldehyde and eugenol for decreasing *Salmonella* Enteritidis in birds. *Trans*-cinnamaldehyde was added at 0.75% and eugenol at 1% as an antimicrobial additive in the feed given to market-age chickens for 5 d before slaughter. It was observed that both molecules consistently reduced significant populations of *Salmonella* Enteritidis in both experiments ($P < 0.05$). The plant molecules reduced cecal colonization of *Salmonella* Enteritidis by ~1.5 $\log_{10}$ cfu/g ($P < 0.05$). In the cloacal contents, *trans*-cinnamaldehyde and eugenol decreased *Salmonella* Enteritidis populations by ~1.5 and 2 $\log_{10}$ cfu/g, respectively ($P < 0.05$; Kollanoor Johny et al., 2012d).

In addition to determining the efficacy of the PDA in reducing *Salmonella* Enteritidis colonization in broiler chickens, currently experiments are underway in our laboratory for studying the potential of these molecules in reducing the vertical transmission of the pathogen to eggs in layers. Preliminary results indicate that the molecules are effective in reducing the vertical transmission of the pathogen. Moreover, the PDA reduced the attachment and invasion of *Salmonella* Enteritidis onto chicken oviduct epithelial cells and downregulated the expression of critical genes responsible for oviduct colonization (I. Upadhyaya, University of Connecticut, Storrs, unpublished data).

The efficacy of the plant compounds carvacrol and thymol was examined in reducing *C. jejuni* populations in broiler chickens. The recent report concluded that these molecules could reduce *C. jejuni* colonization, although additional research is warranted to develop consistent treatment regimens (Arsi, 2011). Thymol at 0.25 and 1%, or carvacrol at 1%, or a combination of the molecules at 0.5% was effective against *C. jejuni* colonization in broilers, although the effect was inconsistent (Arsi, 2011). In another study, Hermans et al. (2011) examined the effect of cinnamaldehyde on reducing *C. jejuni* KC40 in a seeder chick model and reported that at the 0.3% level (coated form) in the feed, the compound failed to reduce the pathogen significantly.

A critical property of essential oils or their components is their hydrophobicity, which helps them to target the lipid-containing bacterial cell membrane (Knobloch et al., 1986; Sikkema et al., 1994; Smith-Palmer et al., 2004). This makes these membranes more permeable, leading to leakage of ions and other cell contents (Cox et al., 2000; Carson et al., 2002; Ultee et al., 2002). Besides the effect on cell membrane, *trans*-cinnamaldehyde is also believed to kill bacteria by inhibiting energy generation and glucose uptake (Gill and Holley, 2006). Yet another mechanism by which *trans*-cinnamaldehyde and eugenol kill microorganisms is by their inhibitory effect on key enzymes such as amino acid decarboxylases (Wendakoon and Sakaguchi, 1995).

Because plant-derived molecules contain several chemical groups in their structure, their antimicrobial activity is attributable to more than one specific mechanism (Skandamis and Nychas, 2001; Carson et al., 2002; Burt, 2004; Smith-Palmer et al., 2004). Therefore, it is hypothesized that the potential for bacteria for developing resistance to plant antimicrobials is negligible (Ohno et al., 2003; Smith-Palmer et al., 2004; Domadia et al., 2007).

To determine the potential effect of plant molecules on bacterial virulence mechanisms, we investigated if *trans*-cinnamaldehyde and eugenol could reduce *Salmonella* Enteritidis invasion of BATC (Dodson et al., 1999). Results revealed that *trans*-cinnamaldehyde and eugenol reduced the invasive ability of *Salmonella* Enteritidis, and downregulated the expression of invasion genes *hilA*, *hilD*, and *invF*, and motility genes, *flhC* and *motA* ($P < 0.05$). Moreover, these molecules reduced the motility of the pathogen significantly ($P < 0.05$; Kollanoor Johny et al., 2012b).

Because *trans*-cinnamaldehyde and eugenol were able to reduce the invasive ability and the motility of the pathogen, we conducted a microarray with the RNA extracted from *Salmonella* Enteritidis exposed to SIC of the molecules to elucidate potential pathways downregulated by *trans*-cinnamaldehyde or eugenol. Analysis of transcriptional profiles of bacteria exposed to an inhibitor can yield new information for pathway characterization and investigating the inhibitor mechanism of action. Our microarray results revealed 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 downregulated in *Salmonella* Enteritidis by these molecules (Kollanoor Johny et al., 2012a). We are currently investigating the whole genome response of *C. jejuni* exposed to the SIC of the PDA.

CONCLUSIONS

Although substantial progress has been achieved in food safety through pathogen reduction programs, *Salmonella* Enteritidis and *C. jejuni* remain the most common foodborne pathogens transmitted to humans through the consumption of poultry products. Therefore, innovative on-farm strategies for preventing colonization of birds with these pathogens are critical to reduce/prevent the contamination of poultry products. An antimicrobial treatment that can be applied through feed or water represents the most practical and economically viable method for adoption by farmers. Additionally, a natural and safe antimicrobial will have greater acceptance by producers, including organic farmers, without concerns for toxicity. In summary, our research suggests the potential use of caprylic acid, *trans*-cinnamaldehyde, eugenol, carvacrol, and thymol as potential dietary supplements for reducing *Salmonella* Enteritidis and *C. jejuni* in chickens. Further, microarray results revealed that these molecules may be reducing the pathogen populations by downregulating bacterial virulence and colonization mechanisms. Future studies confirming the efficacy of these plant molecules under field conditions, and the sensory attributes of meat and eggs from treated birds are necessary before recommending them for adoption by farmers.

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