

Botanical alternatives to antibiotics for use in organic poultry production¹

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ABSTRACT The development of antibiotic resistant pathogens has resulted from the use of sub-therapeutic concentrations of antibiotics delivered in poultry feed. Furthermore, there are a number of consumer concerns regarding the use of antibiotics in food animals including residue contamination of poultry products and antibiotic resistant bacterial pathogens. These issues have resulted in recommendations to reduce the use of antibiotics as growth promoters in livestock in the United States. Unlike conventional production, organic systems

are not permitted to use antibiotics. Thus, both conventional and organic poultry production need alternative methods to improve growth and performance of poultry. Herbs, spices, and various other plant extracts are being evaluated as alternatives to antibiotics and some do have growth promoting effects, antimicrobial properties, and other health-related benefits. This review aims to provide an overview of herbs, spices, and plant extracts, currently defined as phytobiotics as potential feed additives.

Key words: botanicals, phytobiotics, extracts, essential oils, poultry, *Salmonella*, *Campylobacter*

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INTRODUCTION

With the European Union ban of antibiotics as growth promoters (**AGP**) in animal feed, on January 1, 2006 (EC regulation No. 1831/2003¹) (see: <http://eur-lex.europa.eu/LexUriServ.do?uri=OJ:2003:268:0029:0043:EN:PDF>), alternative methods are being evaluated to improve the performance of agricultural livestock, especially in swine and poultry production (Windisch et al., 2008). In some countries, including the United States, where the use of AGP is still permitted, the increased risk of antibiotic resistant pathogens and consumers concerns about antibiotic residues have resulted in the implementation of some recommendations to reduce AGP use in livestock (Institute of Medicine, 1980, 1989; a Council for Agricultural Science and Technology report 1981; Committee on Drug Use in Food Animals; Dibner and Richards, 2005). For organic poultry production, United States Department of Agriculture (**USDA**) organic regulations do not allow the use of antibiotics in animals raised in these rearing systems to prevent diseases and or as growth promoters. For this reason, organic poultry producers need non-antibiotic treat-

ments as alternatives to prevent diseases and improve bird performance. Many studies are evaluating the use of botanicals that have been used traditionally in humans as feed additives. Most of the beneficial effects attributed to plants and plant extracts are related to health properties that include stimulating endogenous digestive enzymes and antioxidants (Lee et al., 2004b).

Compared with synthetic antibiotics or inorganic chemicals, plant-derived products are natural, less toxic than antibiotics, and typically residue free. Many are certified as Generally Recognized As Safe (**GRAS**) by the Food and Drug Administration (**FDA**) and therefore, make them ideal candidates to use as feed additives in organic poultry production (Wang et al., 1998). Although the term “phytobiotic” comprises a wide range of substances with respect to biological origin, formulation, chemical description and purity, they can be classified into four groups (Windisch and Kroismayr, 2006): 1) herbs (products from flowering, non-woody and non-persistent plants); 2) botanicals (entire or processed parts of a plant, e.g., roots, leaves, bark); 3) essential oils (**EOs**) (hydrodistilled extracts of volatile plant compounds); and 4) oleoresins (extracts based on non-aqueous solvents).

Several growth and health promoting properties have been attributed to phytobiotics usage in poultry. These benefits are derived by improving gut health including increasing digestibility (Mitsch et al., 2004; Kroismayr et al., 2008), modifying digestive secretions, and sustaining and improving gut histology (Williams and Losa, 2001; Kreydiyyeh et al., 2003; Jamroz et al., 2003). Furthermore, some phytobiotics stabilize the

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microbiome, which reduces microbial toxins (Windisch et al., 2008; Perič et al., 2010; Steiner, 2006). This, in turn, reduces inflammation and; therefore, protein production can be allocated to growth as opposed to production of immune modulators (Steiner, 2006; Kroismayr et al., 2008).

The positive effect of phytobiotics is mainly linked to the plant constituents including terpenoids (mono- and sesquiterpenes, steroids) phenolics (tannins), glycosides, alkaloids (present as alcohols, aldehydes, ketones, esters, ethers, and lactones) flavonoids, and glucosinolate (Wenk, 2006). For this reason, many herbs and spices can be added to food with the benefit of enhancing organoleptic properties (Wenk, 2006).

MECHANISMS OF ACTION OF BOTANICALS

In general, botanicals, also termed as phytobiotics, contain primary and secondary plant compounds. The primary compounds are considered as the principal nutrients (protein, fat, and carbohydrates), whereas the secondary are described as EOs, bitterns, colorants, and phenolic compounds. Although the precise mechanisms of antimicrobial action of phytobiotics are not elucidated yet, some mechanisms suggested to be responsible for their beneficial properties include: 1) disruption of the cellular membrane of pathogens; 2) modification of the surface of the cells affecting to the hydrophobicity and, therefore, their virulence capacity; 3) stimulating the immune system, specifically activation of lymphocytes, macrophages, and NK cells; 4) protecting intestinal mucosa from bacterial pathogens colonization; and 5) promoting the growth of beneficial bacteria such as *Lactobacilli* and *Bifidobacteria* (Vidanarachchi et al., 2005; Windisch and Kroismayr, 2007).

Among phytobiotics, essential oils have gained more attention due to their attributed antimicrobial and growth promoter properties. Essential oils are compounds obtained by distillation or solvent extraction from aromatic plants, herbs, or spices (Yang et al., 2009). Many EOs contain multiple active components and these components are primarily used to protect the plants from damage caused by insects and bacteria. Each component may have a different mechanism of action and these components can work synergistically (Senatore, 1996; Russo et al., 1998). Thus, the mechanism of action of EOs is based on its chemical composition. As an example, analogous molecules including thymol and carvacrol can exert similar antimicrobial effects, but the mechanism of action differs due to differences in the location of the hydroxyl group. Similarly, limonene and p-cymene vary in the alkyl group location and, thus, the antimicrobial efficiency (Dorman and Deans, 2000). It is difficult to predict the efficacy of the EOs, because the active components present in the EOs can vary depending on the method of extraction, geographical origin, plant genotype, the harvesting season, and length of storage (Cosentino et al., 1999; Dorman and Deans, 2000; Wenk, 2006).

More than 3,000 essential oils are known, 300 of which are commercially important and used in pharmaceutical, agronomic, food, sanitary, and cosmetic and perfume industries as effective alternatives or complements to synthetic compounds (Bakkali et al., 2008). Recently, the food industry and animal producers have increased their interest in the use of EOs, not only for their antioxidative and anti-inflammatory properties, but also for their antimicrobial, coccidiostatic, antihelminthic, and anti-viral effects (Cuppett and Hall, 1998; Hirasa and Takemasa, 1998; Nakatani, 2000; Halliwell et al., 1995; Craig, 1999; Burt, 2004; Rhodes et al., 2006; Wei and Shibamoto, 2007). Multiple oils, including carvacrol (**CAR**), thymol (**THY**), obtained from oregano (*Origanum glandulosum*) or eugenol (**EUG**) obtained from the oil cloves (*Eugenia caryophyllis*), are reported to inhibit many pathogenic bacteria (Applegate et al., 2010; Dorman and Deans, 2000; Kollanoor et al., 2010; Si et al., 2006). In view of these studies, oils also are being evaluated to improve the microbiological quality of food when used as additives.

EOs are gaining more interest in conventional and organic poultry nutrition, primarily focusing on the improvement of gut functions. The positive effects in the digestive tract include stabilizing the microflora, which improves nutrient utilization and absorption. EOs improve nutrient utilization and absorption by increasing the activity of digestive enzymes including trypsin and amylase (Lee et al., 2003; Jang et al., 2004). Additionally, active components increase intestinal secretions of mucus, which prevents the adhesion of pathogens (Jamroz et al., 2006).

BOTANICAL PROPERTIES

Antimicrobial Properties

In order to accomplish the beneficial effects associated with the AGPs in poultry production, it is necessary to understand the mechanism of action involved in improving performance and their antimicrobial activity. Most of the beneficial effects of AGPs are linked to the reduced incidence of subclinical infections (George et al., 1982; Snyder and Wostmann, 1987; Brennan et al., 2003). Furthermore, stability of the microbial ecology by AGPs has been reported to alleviate problems associated with microbial production of toxins including the thinning of the intestinal wall, suppression of inflammation, and the reduction of bioamines and toxins produced by bacteria (Feighner and Daskevicz, 1987; Knarreborg et al., 2004).

Studies using in vitro methods have demonstrated antimicrobial activity that some plants and plant extracts have against pathogenic bacteria including oregano (*carvacrol*), thyme (*thymol*), clove (*eugenol*), mustard (*allysithiocyanate*), cinnamon (*cinnamaldehyde*), and garlic (*allicin*) (Table 1; Kollanoor et al., 2010, 2012). Yet, most of the microstatic and microbicidal effects reported are still unclear and discrepancies

Table 1. Examples of phytobiotics used as feed additives and their antimicrobial effect.

| Feed additive | Inclusion Rate | Antimicrobial effect | Reference |
|----------------------------|--------------------------------|--|--------------------------------|
| Capsaicin | 18 ppm | Reduction of <i>S. enteritidis</i> in ceca | (Télez et al., 1993) |
| Capsaicin | 150 to 300 ppm | Reduction of <i>E. coli</i> and <i>C. perfringens</i> | (Jamroz et al., 2003) |
| Cinnamic aldehyde | | | |
| Thyme oil | 1 g/kg 3 g/kg 5 g/kg | Reduction of coliform counts | (Cross et al., 2003) |
| Blend A | 100 ppm | Reduction of <i>C. perfringens</i> colonization in the gut | (Mitsch et al., 2004) |
| CRINA®poultry Eugenol | | | |
| Blend B + Curcumin | | | |
| Thymol/Carvacrol Piperin | | | |
| XTRACT™: | 100 mg/Kg | Reduction of <i>E. coli</i> | (Jamroz et al., 2005) |
| Carvacrol | | Light reduction of <i>Cl. perfringens</i> | |
| Trans-cinnamaldehyde | | | |
| Capsaicin | 5 ppm | Prophylactic effect against <i>S. enteritidis</i> | (Orndorff et al., 2005) |
| Capsaicin | 20 ppm | | |
| Oleoresin | | | |
| Extracts from: | | Increase lactic acid bacteria | (Vidanarachchi et al., 2006) |
| Cabbage tree | 5g/kg | Reduction of <i>C. perfringens</i> in ileum and ceca | |
| Golden wattle tree | 10g/Kg | | |
| Seaweed | | | |
| Thyme | Herbs at 10g/kg | No effect on the intestinal microflora populations | (Cross et al., 2007) |
| Oregano | EOs at 1g/kg | | |
| Marjoram | | | |
| Rosemary | | | |
| Yarrow | | | |
| CRINA® poultry | 25mg/Kg 50mg/Kg | Reduce coliforms in ileum and ceca | (Jang et al., 2007) |
| Capsaicin (Chili pepper) | 36 ppm | Reduce <i>S. enteritidis</i> organ colonization | (Vicente et al., 2007) |
| Hops (lupulone) | 62.5 ppm 125 ppm 250 ppm | Inhibition of <i>C. perfringens</i> in the gut | (Siragusa et al., 2008) |
| Biomin P.E.P 125 poultry | 125g/tn | Notable reduction of <i>E. coli</i> in ceca | (Perič et al., 2010) |
| Thymol | 15 g/tn | Reduction of <i>E. coli</i> and <i>Clostridium</i> | (Tiihonen et al., 2010) |
| Cinnamaldehyde | 5 g/tn | Increase <i>Bifidobacterium</i> and <i>Lactobacillus</i> | |
| Thymol | 150 to 200g/tn | Control necrotic enteritis | (Timbermont et al., 2010) |
| Cinnamaldehyde | 150g/tn | (<i>C. perfringens</i>) | |
| Eucalyptus EO | | | |
| Green Tea extract | 0.1g/kg 0.2g/kg | Decrease caecal coliform bacteria | (Erener et al., 2011) |
| Grape Pomace Concent (GPC) | 60g/kg | GSE increase <i>Lactobacillus</i> in ileum | (Viveros et al., 2011) |
| Grape Seed Extract (GSE) | 7.2g/kg | GPC/GSE increase <i>E. coli</i> , <i>Lactobacillus</i> , <i>Enterococcus</i> and <i>Clostridium</i> in ceca | |
| Enviva EO 101 | 100g/tn | Combined with xylanase reduced <i>S. Heidelberg</i> in ceca | (Amerah et al., 2012) |
| Pennyroyal | 0.025% 0.50% | Reduce <i>E. coli</i> Increase lactic acid bacteria | (Erhan et al., 2012) |
| Trans-cinnamaldehyde | 0.75% | Reduction of <i>Salmonella</i> colonization in cecum | (Kollanoor-Johny et al., 2012) |
| Eugenol | 1% | | |
| Carvacrol | 0.05% | Reduction of <i>S. Heidelberg</i> in the crop | (Alali et al., 2013) |
| Thymol | | | |
| Eucalyptol | | | |
| Lemon | | | |
| Capsicum oleoresin | 4mg/kg | Protective immunity against Necrotic Enteritis (<i>C. perfringens</i>) | (Hyen Lee et al., 2013) |
| Turmeric oleoresin | | | |
| Trans-cinnamaldehyde | 500 mg/kg | Reduction of <i>Br. intermedia</i> in the ceca | (Verlinden et al., 2013) |
| Thymol | 0.25% | Reduction of <i>Campylobacter</i> in ceca contents | (Ali et al., 2014) |
| Carvacrol | 0.5% 1% 2% | | |
| Thymol+Carvacrol | 0.5% | | |
| Ground Yerba Mate | 0.5% 1% | No reduction of <i>S. Enteritidis</i> in caecum | (González-Gil et al., 2014) |

regarding their spectrum of activity, potency, and applications are in debate (Delaquis et al., 2002).

Among the foodborne pathogens that can be transmitted through the consumption of poultry products, *Salmonella enterica* and *Campylobacter jejuni* are the most common infectious agents (Heres et al., 2004; White et al., 1997). Reducing the colonization of poultry by *Salmonella* Enteritidis and *C. jejuni*

in the chicken intestinal tract remains a large challenge. The target of many phytobiotic studies has been to reduce zoonotic pathogens (Table 2), but the information available about the effects and the physiological impact of these active compounds on animal performance is still scarce. It is obvious that, although these compounds may be active against pathogens, they would not be acceptable if production

Table 2. Examples of studies evaluating any effect on performance after including phytobiotics to the feed of chickens.

| Feed additive | Inclusion Rate | Performance effect | Reference |
|--|--------------------------|--|---------------------------|
| Oregano EO | 50 to 100 mg/Kg | No effect on growth | (Botsoglou et al., 2002) |
| Japanese Green tea | 1.0% | Decrease BW and FI with higher dose | (Kaneko et al., 2001) |
| | 2.5% | | |
| | 5.0% | | |
| Japanese Green tea | 0.5% | Decrease BW and FI with higher dose Improve FCR | (Biswas and Wakita, 2001) |
| | 0.75% | | |
| | 1.0% | | |
| | 1.5% | | |
| Carvacrol | 300 mg/Kg | Improvement of daily gain by 8.1% | |
| Improvement in FCR by 7.7% | (Jamroz and Kamel, 2002) | | |
| Thyme EO | 1g/kg | Reduction of FI and WG with high inclusion levels | (Cross et al., 2003) |
| | 3g/kg | | |
| Thymol (thy) | 5g/kg | No improvement in performance | (Lee et al., 2003) |
| Cinnamaldehyde (cn) | 100 ppm (Thy) | | |
| CRINA® poultry | 100 ppm (Cn) | | |
| Herbromix™ | 50 ppm (CRINA) | Improve BWG by 2.060 to 2.063 Improve FCR by 1.96 to 1.97 | (Alçiçek et al., 2004) |
| | 36 mg/kg | | |
| | 48mg/kg | Improve growth performance from 7 to 21 days (age) | (Guo et al., 2004) |
| Chinese herbal formula (mix of 14 herbs) | 0.25g/kg | | |
| | 0.5g/kg | | |
| | 1g/kg | No improvement in performance | (Hernández et al., 2004) |
| | 2g/kg | | |
| EO extract : | | | |
| Oregano | 200 ppm | No improvement in performance | (Hernández et al., 2004) |
| Cinnamon | | | |
| Pepper | | | |
| Labiatae extract : | | | |
| Sage | 5,000 ppm | | |
| Thyme | | | |
| Rosemary | | | |
| Grape Seed Extract (GSE) | 2g/kg | At up to 10g/kg reduced FI No reduction on FE | (Hughes et al., 2005) |
| | 5g/kg | | |
| | 10g/kg | | |
| Concentrate of Tannins | 30g/kg | | |
| Capsaicin | 100 mg/kg | Improvement of BW by 1 to 2% | (Jamroz et al., 2005) |
| Cinnamaldehyde | | | |
| Carvacrol | | | |
| Nor-Spice® Thyme | 1g/kg | No significant effect on BW, FI, and FCR | (Sarica et al., 2005) |
| Nor-Spice S Garlic | 1g/kg | | |
| RepaXol™ | 100 to 150gr/tn | At 150gr/tn improved FCR | (Zhang et al., 2005) |
| Avigro™ | 0.5gr/tn | | |
| Blend EOs: | | | |
| Oregano oil | 24mg/kg | No significant effect on BW | (Çabuk et al., 2006) |
| Laurel leaf oil | 48 mg/kg | | |
| Sage leaf oil | | | |
| Myrtle leaf oil | | | |
| Fennel seed oil | | | |
| Citrus peel oil | | | |
| Hops (<i>Humulus lupulus</i>) | 0.5 lbs/tn | Improve BW by 2.837 Kg Improve FCR by 1.669 | (Cornelison et al., 2006) |
| | 1.0 lbs/tn | | |
| | 1.5 lbs/tn | | |
| | 2.0 lbs/tn | | |
| RepaXol™ | 100 g/tn | Improve BW by 2,665 g Improve FCR by 1.574 | (Lippens et al., 2006) |
| Thyme | Herbs at 10g/kg | Thyme oil and yarrow herb had positive effect on BW, FCR, AFC, AG Feed intake decrease by 10% | (Cross et al., 2007) |
| Oregano | EOs at 1g/kg | | |
| Marjoram | | | |
| Rosemary | | | |
| Yarrow | | | |
| Blend EOs: | | | |
| Oregano | 200 ppm | Improve FCR and BW | (Garcia et al., 2007) |
| Cinnamon | | | |
| Pepper | 200 ppm | | |
| Grape Pomace | 5g/kg | No effect on growth performance | (Goñi et al., 2007) |
| | 15g/kg | | |
| | 30g/kg | | |
| CRINA®poultry | 25 mg/kg | No effect on BW/total gain | (Jang et al., 2007) |
| | 50 mg/kg | | |
| Thyme | 100 ppm | Improve BW and FCR | (Al-Kassie, 2009) |

Table 2. *Continued.*

| Feed additive | Inclusion Rate | Performance effect | Reference |
|------------------------------|--|--|----------------------------|
| Cinnamon | 200ppm | Improve BW and FCR | (Al-Kassie, 2009) |
| Grape Seed Extract (GSE) | 0.6g kg ⁻¹ 1.8g kg ⁻¹ 3.6g kg ⁻¹ | No effects on growth performance | (Brenes and Roura, 2010) |
| Moringa oleifera leaf | 5% | Performance decrease at inclusion levels above 5% | (Olugbemi et al., 2010) |
| Biomim P.E.P 125 poultry | 10% 125g/tn | BW improvement 2210 ± 253 gr | (Perić et al., 2010) |
| Thymol (Thy) | 15gr/tn (thy) | Increase BW by 4 to 5% | (Tiihonen et al., 2010) |
| Cinnamaldehyde (Cn) | 5 gr/tn (cn) | | |
| Green Tea extract | 0.1g/kg 0.2g/kg | Increase BW and FE | (Erener et al., 2011) |
| Ginger | 250 g/100kg 500g/100kg 750g/100kg | No effects on performance | (Mohammed and Yusuf, 2011) |
| Grape Pomace Con (GPC) | 60g/kg | GSE decreased weight gain | (Viveros et al., 2011) |
| Grape Seed Extract (GSE) | 7.2g/kg | | |
| Rosemary leaf | 5.7; 8.6; 11.5g/kg | Rosemary EOs improve LWG and FE | (Yesilbag et al., 2011) |
| Rosemary EOs | 100; 150; 200mg/kg | | |
| Thyme extract | 0.2% 0.4% 0.6% | No significant effect on BW/FCR | (Pourmahmoud et al., 2013) |
| Enviva EO 101 | 100 g/tn | Improve BW by 1,924 gr Improve FCR by 1.90 | (Amerah et al., 2012) |
| <i>Moringa oleifera</i> leaf | 25% 50% 75% 100% | Improve feed intake At higher inclusion levels decrease final weight/WG | (Gadzimarayi et al., 2012) |
| Rosemary EO | 50 to 100mg/kg | Improved BW and FE | (Mathlouthi et al., 2012) |
| Oregano EO | 50 to 100 mg/kg | | |
| EO mixture | 1,000 mg/kg | | |
| Copaiba EO | 0.30 mL kg ⁻¹ 0.45 mL kg ⁻¹ 0.60 mL kg ⁻¹ | Decrease on performance at high inclusion levels | (Aguilar et al., 2013) |
| Grape Seed Extract (GSE) | 0.025g/kg 0.25g/kg 2.5g/kg 5g/kg | Reduction in BW gain up to 2.5g/kg | (Chamorro et al., 2013) |
| Thymol+Carvacrol | 60 mg/kg 100 mg/kg 200 mg/kg | Increase ADG (g) by 71.4% Increase G:F (g/Kg) by 601 | (Hashemipour et al., 2013) |
| Tecnaroma Herbal Mix PL | 100 g/tn 200 g/tn 300 g/tn 400 g/tn 500 g/tn | Improve BW by 3.418 to 3.427 Kg Improve F:G ratio by 1.64 to 1.68 | (Khattack et al., 2014) |
| Marjoram leaf | 0.5% 1.0% 1.5% | Improve LBW, BWG, FCR, and FI | (Ali, 2014) |

BW = Body Weight; FCR = Feed Conversion Ratio; AFC = Average Feed Conversion; AG = Average gain; FE = Feed efficiency; LWG = Live weight gain; ADG = Average daily gain; G:F = Weight gain/Feed conversion; FI = Feed Intake.

performance were decreased. In chickens, the primary colonization site of *Salmonella* Enteritidis and *C. jejuni* is the cecum, which can result in horizontal transmission, contamination off egg-shells with feces, and carcass contamination during processing (Stern, 2008; Gantois et al., 2009). Because the cecum is at the posterior end of the gastrointestinal tract, the phytobiotics must retain their activity during transit through the entire gastrointestinal system. Some studies conclude that the antimicrobial property is either reduced or eliminated while moving through the gastrointestinal tract (Kohlert et al., 2002; Meunier et al., 2006). Given the location of *Salmonella* and *Campylobacter*,

retention of the antimicrobial is essential for efficacy (Arsi et al., 2014).

Because there are several active compounds present in the EOs, elucidating the mechanisms of antimicrobial activity can be difficult (Skandamis et al., 2001; Carson et al., 2002). One antimicrobial property is attributed to the hydrophobic nature of EOs, which disrupts the bacteria cell membrane (Sikkema et al., 1994). Other non-phenolic components including functional groups and aromaticity have been demonstrated to have antimicrobial activity (Farag et al., 1989; Bowles and Miller, 1993). Kollanoor et al. (2012) reported that *Salmonella* motility and invasion of avian intestinal epithelial cells

were substantially inhibited by trans-cinnamaldehyde and eugenol. Evaluation of gene expression revealed that motility and invasion genes, *motA*, *flhC*, *hilA*, *hilD*, and *invF*, were significantly downregulated.

EOs tend to be more effective against gram-positive than gram-negative bacteria (Burt, 2004). The phenolic compounds present in the EOs cannot penetrate the lipopolysaccharide wall of gram-negative cells (Ratledge and Wilkinson, 1988). But the outer membrane of gram-negative bacteria is not totally impermeable and hydrophobic molecules can pass through pores. EOs extracted from basil, sage, hyssop, rosemary, oregano, and marjoram have demonstrated a wide antibacterial spectrum against most gram-positive and gram-negative bacteria evaluated in vitro (Burt, 2004; De Martino et al., 2009). In addition, multiple in vivo experiments have reported antimicrobial activity of phytobiotics as feed additives (Table 1). Cross and co-workers (2007) observed a decrease of cecal coliform populations in birds treated with thyme oil after a colisepticemia infection, suggesting a protective effect after administration of the oil. Nevertheless, the susceptibility of each bacteria species is different and also strain dependent. De Martino and co-workers (2009) reported different susceptibilities of two *Bacillus cereus* strains against the same EOs. Outtara and co-workers (1997) reported that treatment time was a factor as gram-negative cells died after 48 hours while gram-positive cells died within 24 hours.

The beneficial properties of the metabolites present in the plants and EOs are well documented. In chicken nutrition, it has been demonstrated that some phytobiotics select for beneficial bacterial growth leading to enhanced digestion of nutrients, improving body weight and weight gain (Jamroz et al., 2003; Hernández et al., 2004; Lee et al., 2004b,c). Viveros and co-workers (2011) suggested the potential role of grape-derived products including grape seed extract as a feed additive due to the ability of the products to favor the growth of specific groups of beneficial bacteria. Using new molecular techniques, this same study reported an increase of bacteria diversity and *Lactobacillus* populations when grape seed extract was included at 7.2 g/kg in the chicken diet. Yerba Mate extracts supported and increased the growth of *Lactobacillus* and *Pediococcus* and were antimicrobial against *Salmonella* and *Campylobacter* (González-Gil et al. 2014).

Antioxidant Properties

Lipid metabolism and hypocholesterolemic effects have also been reported. Qureshi et al. (1988) reported the role of limonene in cholesterol synthesis. Similarly other authors reported that thymol, carvacrol, and β -ionone have a regulatory effect of non-sterol products (Case et al., 1995; Elson, 1996). Lipid oxidation occurs during meat processing, cooking, and refrigerated storage, which affects the quality of food prod-

ucts because they lose the desirable color, odor, and flavor and shorten shelf-life (Botsoglou et al., 1997; Maraschiello et al., 1998). The antioxidant effect of EOs is attributed to their redox properties, chemical structure, and mainly to the presence of phenolic groups (Brenes and Roura, 2010). Studies testing the effectiveness of certain aromatic plants such as rosemary, oregano, sage, (Economou et al., 1991) and spices including cinnamon (Kamel, 1999) can retard the process of lipid peroxidation in oils and fatty acids. The high content of polyunsaturated fatty acids in pre-cooked and ready-to-eat poultry products make it particularly susceptible to oxidative deterioration (Igene and Pearson, 1979). Herbs including rosemary (Lopez-Bote et al., 1998), tea catechins (Tang et al., 2000), and EOs have been reported to stabilize raw and pre-cooked chicken meat during refrigeration storage (Botsoglou et al., 2003, 2004; Young et al., 2003; Mirshekar et al., 2009). Poultry nutritionists are also interested in the antioxidant properties of certain plants and EOs that might improve meat quality. Grape seed and grape pomace concentrate have been suggested as promising additives due to their antioxidant effects as free radical scavengers (Viveros et al., 2011; Chamorro et al., 2013). However, some of these botanicals can affect the sensory quality of the meat.

Sensory Properties

Palatability of phytobiotics is an important factor to consider. Some herbs and spices have a positive influence on feed intake due to increased palatability and, thus, improve the growth rate (Brenes and Roura, 2010; Lee et al., 2004b,c). However, some phytobiotics have a negative effect on total feed intake due to the strong flavors (Windisch et al., 2008; González-Gil et al. 2014).

Regarding consumption, the smell of phytobiotics is the first step. When oronasal stimulation occurs, the GI starts to prepare for food reception including the increase of digestive secretions, favoring gut motility and the protection of the intestinal epithelium (Katschinski, 2000; Teff, 2000; Hiraoka et al., 2003; Akiba et al., 2002; Platel and Srinivasan, 2004). But the sensitivity of the somatosensing system is different between species. In poultry production the study of the effect of flavors has not received as much attention, because the response to flavors is less notable when compared to other animals like pigs (Moran, 1982). Studies in pigs showed the negative response to some EOs including capsaicin, cinnamaldehyde, carvacrol, or formic acid as they evoke feed refusal when added to food (Green, 1989; Bikker et al., 2003; Eissemann and van Heugten, 2007). Cross and co-workers (2003) observed a feed intake reduction during the first two weeks of age when thymol oil was added to the feed, suggesting that young chickens may be more sensitive to flavor and odor characteristics. Similarly, Ali (2014) reported suppression on feed

intake in chicks when marjoram leaves were added to the diet.

Effects on the Digestion Process

To date, the studies that have evaluated the growth promoting effects of phytobiotics are not conclusive on their beneficial effects in poultry production (Table 2). Some authors have reported different *in vivo* results in poultry performance parameters using the same plants or plants extracts as additives (Jamroz and Kamel, 2002; Guo et al., 2004; Cornelison et al., 2006; Cross et al., 2007; Al-Kassie, 2009; Perić et al., 2010; Tiihonen et al., 2010; Yesilbag et al., 2011; Amerah et al., 2012; Mathlouthi et al., 2012; Hashemipour et al., 2013; Khattack et al., 2014).

The exact mode of action of growth-promoting feed additives is still unknown. However, it is suggested that the interaction of growth promoters with the intestinal microbiota community may result in their optimal stabilization, and, therefore, better use of nutrients to enhance growth (Lin, 2011). Similarly, the use of EOs as supplements in broiler diets may lead to shifts in the microbial population enhancing the growth of lactic acid bacteria including *Lactobacillus* and *Bifidobacterium* and, therefore, decrease the pH of the GIT (Bedford, 2000; Tiihonen et al., 2010). Optimal enzyme activity is dependent on pH and, because EOs can reduce the pH, the break down of feed can be enhanced (Kamel, 1999).

There is also evidence of beneficial activity of EOs on other factors of the digestion process. Lee and co-workers in 2003 and Jang et al. (2007) observed that EOs enhance the activity of digestive enzymes such as trypsin and amylase and also bile salts. Furthermore, it has been reported that EOs improve liver function and increase the concentration of the pancreatic digestive enzymes (Al-Kassie, 2009). Similarly, curcumin, capsaicin, and piperine have been reported to stimulate digestive enzyme activities of the pancreas (Platel and Srinivasan, 2004). Lee et al. (2004a,b) demonstrated the efficiency of cinnamaldehyde EOs to improve fat digestion in broilers.

By enhancing digestion, some phytobiotics do not change final body weight but can improve feed conversion ratios (Windisch et al., 2008). To achieve improvements in feed conversion, it is important to select the proper plants with the desired active components at an optimized dietary dose. It is suggested that the effectiveness of phytobiotics as feed additives rely on the EOs' components and the synergistic effects of the active molecules in the plants or extracts (Jamroz et al., 2003; Mitsch et al., 2004; Tiihonen et al., 2010; Mathlouthi et al., 2012; Hashemipour et al., 2013; Hyen Lee et al., 2013).

Not all botanicals or their derived products are effective. Some reports did not find any statistical differences when supplements were included in the diet even

when using different types, concentration, or combinations of plant extracts (Botsoglou et al., 2002; Cross et al., 2003; Lee et al., 2003; Hernández et al., 2004; Sarica et al., 2005; Çabuk et al., 2006; Jang et al., 2007; Olugbemi et al., 2010; Mohammed and Yusuf, 2011; Pourmahmoud et al., 2013; Aguilar et al., 2013) (Table 1). Initial studies by Dale and co-workers (1980) reported a marked reduction in the average weight gain in chicks when they were fed tannic acid and sorghum. Similarly Botsoglou and co-workers (2002) did not observe any difference in body weight and feed conversion ratio, after adding oregano EOs at the concentrations of 50 and 100 ppm in broiler chicken diets over a 38-day time span. Lee and co-workers (2003) observed that a diet containing highly digestive ingredients can limit the proliferation of bacteria in the intestinal tract because there is a reduction of the substrate left for bacteria present in the posterior portion of the GIT.

Other factors must also be considered including immune regulation, (Freitas and Fonseca, 2001) environmental, and dietary conditions (Hill et al., 1952; Lee et al., 2004b). Hyen Lee and co-workers (2013) demonstrated the activity of capsicum and turmeric oleoresins stimulating the immune response against *C. perfringens* colonization in chickens. Early studies have noted that under extremely clean environments, the enhancing effects of additives are less obvious (Hill et al., 1952).

The efficiency of EOs as growth promoters is dose-dependent (Cross et al., 2003; Zhang et al., 2005; Olugbemi et al., 2010; Gadzimarayi et al., 2012; Aguilar et al., 2013). Viveros and co-workers (2011) reported growth depression when including GSE at 7.2g/kg in the chicken diet, whereas at lower doses the growth performance was not affected. Chamorro and co-workers (2013) confirmed that the use of GSE up to 2.5 g/kg had no adverse effects on chicken growth. In order to increase the effectiveness of the phytobiotics some studies have used a combination of EOs at low concentrations (Langhout, 2000; Lee et al., 2004c; Manzanilla et al., 2004) or with enzymes (Amerah et al., 2012).

The form of herbal supplementation is also important for its bioactivity. Cross and co-workers (2007) observed that yarrow appears to be more effective when fed as an herb than an extracted essential oil, because the precursors of the terpenes and another phytochemical compounds are present in the herb but not in the oil. Also, Yesilbag and co-workers (2011) observed a higher efficiency to improve live body weight and feed efficiency when rosemary EOs were administered, but not the ground leaf form.

APPLICATIONS OF BOTANICALS IN FOOD SYSTEMS

Plant extracts have considerable promise in a wide range of applications in the food industry, and because most of them are considered to have GRAS status (Over et al., 2009), there is no time delay for implementation.

Due to the complex nature of foods, a greater concentration of EOs is needed in order to achieve the desired effect than what is needed during *in vitro* evaluations using pure cultures of bacteria (Shelef, 1983; Smid and Gorris, 1999; Kumudavally et al., 2008). Furthermore, nutrients are available in some food items, which can facilitate bacterial repair of damaged cells (Gill et al., 2002). The intrinsic properties of the food (fat, protein, water content, antioxidants, preservatives, pH, salt, and other additives) and the extrinsic properties (temperature, packaging, and atmospheric composition) can also influence bacterial sensitivity (Shelef, 1983; Tassou et al., 1995). For example, at a low pH the EOs increase the hydrophobicity, enabling them to more easily dissolve in the lipids of the cell membrane of target bacteria (Juven et al., 1994).

METABOLISM OF EOs AND FOOD SAFETY IMPLICATIONS

EOs are quickly absorbed after oral, pulmonary, or dermal administration and then metabolized and typically eliminated by the kidneys in the form of glucuronides. Thus, their accumulation in the body is unlikely due to rapid clearance and short half lives (Kohlert et al., 2000). But, more toxicological studies are needed to determinate the acute oral effects and dosage levels of EOs in poultry diets. Acute oral toxicity studies are typically conducted in rats and mice, and some EOs have been evaluated including carvacrol, cinnamaldehyde, beta-ionone, and thymol. Although there are scarce data about the toxicological effects of EOs in chickens, some studies with cinnamaldehyde evaluated its carcinogenicity and mutagenicity reporting teratogenic activity (Abramovici and Rachmuth-Roizman, 1983; Hoskins, 1984; Stamatati et al., 1999; Smith et al., 2000). Furthermore, handling of pure formulations must be done safely because many are potentially irritating and can cause allergic contact dermatitis (Burt, 2004).

Some studies have reported accumulations of phyto-biotic components in tissue and organs. Botsoglou et al. (2002) reported EOs continuously added to chicken diet, without withdrawal periods, lead to the deposition of residues in various tissues in a dose-dependent fashion. Although the impact on the sensory properties of poultry meat is recognized, more toxicological evidence of EO residues deposited in poultry meat is needed. Because many of the EOs, including thymol, carvacrol, cinnamaldehyde, and beta-ionone, are given GRAS status by the FDA (Furia and Bellanca, 1975), it is implied that their use is safe.

DELIVERY SYSTEMS

A large issue with some EO's is the reduction of the antimicrobial activity when they are delivered in feed (Si et al., 2006), mainly associated with the volatility and poor solubility of the active components. Phyto-

otics can be delivered in the feed or water depending on the composition of the product. However, a reduction in efficacy may occur when ground leaves are used as opposed to using extracts (González-Gil et al. 2014). Plant extracts are typically more effective than ground leaves because the active components are concentrated in extracts. However, extracts can be more costly because chemicals are needed for the extraction process and a large quantity of the plant is required to produce a sufficient amount of extract.

New delivery systems are being investigated to target the site within the animal intestines so that phyto-biotics exert their beneficial activities. Previous studies based on the use of emulsifiers such lecithin, extensively used in food and non-food applications, reported enhancement of the antimicrobials properties of carvacrol and eugenol against gram-positive and gram-negative bacterial pathogens (Li, 2011, Master's Thesis, University of Tennessee). Currently, microencapsulation is one of the most studied tools to achieve this goal (Chambers and Gong, 2011) and has been reported to promote the antimicrobial efficacy of extracted compounds against *E. coli* O157:H7 and *L. monocytogenes* (Lippens et al., 2006; Gaysinsky et al., 2005a,b). Microencapsulation delays the absorption of the coated Eos, and, thus, the EOs will retain antimicrobial activity longer. A significantly large part of current literature on the encapsulation of EOs deals with micrometric size capsules, which are used for the protection of the active compounds against environmental factors (e.g., oxygen, light, moisture, and pH). Other advantages attributed to the use of microencapsulated additives are their capacity to reduce any negative palatability or strong odors, and; therefore, feed intake is not reduced (Lambert et al., 2001; Cross et al., 2003; Lippens et al., 2006).

FUTURE AND PROSPECTS OF BOTANICALS IN ORGANIC POULTRY

Some commercial phytobiotic products are available, such as CRINA[®] poultry, XTRACT[™], and Biomin P.E.P 125 poultry, developed as additives in poultry feed and have had some success for controlling coliforms and *C. perfringens* (Mitsch et al., 2004; Jamroz et al., 2005; Perić et al., 2010; Viveros et al., 2011). Other commercial products including Enviva EO 101 have been demonstrated to be effective at reducing *S. Heidelberg* in the ceca when combined with xylanase (Viveros et al., 2011). In general most of the data generated from evaluation of commercial phytobiotics, reported improvement in performance and antimicrobial activity (Alçiçek et al., 2004; Lippens et al., 2006; Perić et al., 2010; Amerah et al., 2012; Khattack et al., 2014).

Many phytobiotics show promising results for applications in organic and conventional poultry production. However, there are data gaps in production performance and residue accumulation, which need to be filled before some phytobiotics are used in

commercial farms. Furthermore, delivery systems and palatability issues should be resolved in order to optimize the efficacy of these compounds.

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