

## Essential Oils in Broiler Nutrition

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**Abstract:** Based on literature data it can be concluded essential oils originating from plants have anti-microbial activity and have toxic effects in poultry only when administered at very high doses. Antioxidant activity and hypocholesterolemic effects have been reported in chickens. In various studies, but not all, a growth enhancing effect of essential oils has been found. The characteristic flavor of essential oils might play role in poultry performance, but this needs to be confirmed. Essential oils may stimulate the digestion process. It appears that individual compounds of an essential oil have a wide range of activities and may act in an additive, synergistic and antagonistic fashion. The effect of essential oils in poultry may not only be confined to the microflora, but may extend to animal metabolism. Knowing the activity and effects of individual compounds is useful to formulate mixtures of compounds so as to enhance efficacy. In conclusion, dietary essential oils may be used as alternatives to antibiotics, but whether their effects on growth performance are a consequence of anti-microbial activity needs to be studied further.

**Key words:** Essential oils, anti-microbial activity, nutrition, growth performance, broilers

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### Introduction

There is no doubt that dietary antibiotics have played a fundamental role in animal production as growth and health promoter. However, the current trend is to look for alternatives to antibiotics for in-feed use because of public concerns as to their residues and subsequent occurrence of antibiotic-resistant bacteria. Dietary antibiotics presumably act on the intestinal microflora, leading to improved animal performance. Most supplements claimed to be alternatives to antibiotics have effects on the microflora either directly or indirectly (Taylor, 2001). Thus, the microflora in chickens should not be ignored in relation to birds' performance. However, unlike ruminant and non-ruminant herbivores, chickens have little nutritional benefit from the microflora (Moran, 1982). The microflora, on the other hand, can adversely affect the host if it is not properly controlled. It is well-known that non-starch polysaccharides present in cereals stimulate growth of microflora (Smits and Annison, 1997), leading to low growth performance. Gut microflora can hydrolyse conjugated bile salts (Feighner and Dashkevicz, 1987) which limits fat digestion (Krogdahl, 1985). It is clear that controlling the microflora could positively influence birds' performance and that feed supplements with anti-microbial activity are potential alternatives to antibiotics.

Various authors have extensively reviewed and compared various compounds regarded as alternatives to antibiotics in animal production (Langhout, 2000; Mellor, 2000a,b; Wenk, 2000; Taylor, 2001). Essential oils are already marketed for use in animal production and are claimed to be "digestive enhancers" (Williams and Losa, 2001). This prompted us to collect published

information on essential oils and assess the possible application in poultry nutrition.

**Definition of essential oils and general introduction:** An essential oil is a mixture of fragrant, volatile compounds, named after the aromatic characteristics of plant materials from which they can be isolated (Oyen and Dung, 1999). The term 'essential' was adapted from the theory of 'quinta essentia' proposed by Paracelsus who believed that this quintessence was the effective element in a medical preparation (Oyen and Dung, 1999). Because the term, 'essential oil' is a poorly defined concept from medieval pharmacy, the term 'volatile oil' has been proposed (Hay and Waterman, 1993). However, the name of 'essential oil' will be used preferentially in this review.

Essential oils are very complex mixtures of compounds and their chemical compositions and concentrations of individual compounds are variable. For example, the concentrations of two predominant components of thyme essential oils, i.e. thymol and carvacrol have been reported to range from as low as 3% to as high as 60% of total essential oils (Lawrence and Reynolds, 1984). Cinnamaldehyde, a main principle of cinnamon essential oil, amounts to approximately 60 to 75% of the total oil (Duke, 1986). Because of the large variation in composition, the biological effects (Schilcher, 1985; Janssen *et al.*, 1987; Deans and Waterman, 1993), if any, of essential oils may differ. This diversity of essential oils urged us to select four pure principles, i.e. thymol, cinnamaldehyde, beta-ionone and carvacrol, for evaluating their possible role as alternatives to antibiotics in poultry production. The chemical properties

and biological activities of the four compounds are summarized in Table 1.

**Classification of essential oils:** Essential oils basically consist of two classes of compounds, the terpenes and phenylpropenes. Depending on the number of 5-carbon building blocks (isoprene units), terpenes can be sub-divided into mono-, sesqui-, and di-terpenes in which the number of isoprene units are 2, 3 and 4, respectively. Further derivatives of terpenes are typified by the presence or absence of a ring structure, double bond, addition of oxygen or stereochemistry. It is estimated that there are more than 1000 monoterpenes and 3000 sesquiterpenes. Phenylpropenes consist of a 6-carbon aromatic ring with a 3-carbon side chain (C<sub>6</sub>-C<sub>3</sub> compounds). Only approximately 50 phenylpropenes have been described.

**Synthesis of essential oils:** Terpenes and phenylpropenes are synthesized by the mevalonic and shikimic pathway, respectively. The 6-carbon mevalonic acid, which is formed by condensation of three acetate units and by HMG-CoA reductase, is converted to 5-carbon isopentenyl pyrophosphate (IPP) and then to dimethyl allyl pyrophosphate (DMAPP), which are the activated 5-carbon units of isoprene. IPP and DMAPP are then combined in a 1:1 molar ratio to generate 10-carbon geranyl pyrophosphate (GPP) which is the precursor of monoterpenes. The addition of IPP to GPP produces the 15-carbon sesquiterpene compound, farnesyl pyrophosphate (FPP). Thymol and carvacrol are derived from GPP and classified as monoterpenoids or isoprenoids. On the other hand,  $\beta$ -ionone is derived from FPP and thus classified as either sesquiterpene or isoprenoid.

The shikimic acid pathway produces the aromatic amino acid phenylalanine, the products of which are cinnamic acid and *p*-coumaric acid with trans configuration (Seigler, 1998). Among the important phenyl propene compounds are eugenol, trans-cinnamaldehyde, safrole and also the pungent principles, capsaicin and piperine. These are classified as phenylpropenoids. The synthetic pathways and the related compounds are reviewed in more detail elsewhere (Friedrich, 1976; Waterman, 1993; Seigler, 1998; Loza-Tavera, 1999).

### Biological effects of essential oils

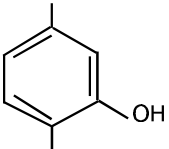
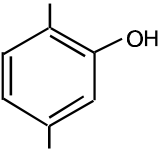
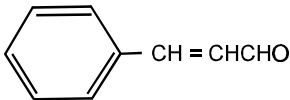
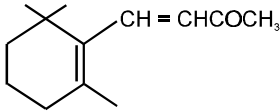
**In vitro anti-microbial activities of essential oils:** Essential oils have long been recognized because of their anti-microbial activity (Deans and Ritchie, 1987; Paster *et al.*, 1990; Reddy *et al.*, 1991; Lis-Blachin *et al.*, 1998; Smith-Palmer *et al.*, 1998; Hammer *et al.*, 1999). Due to this property, essential oils have gained much attention in investigations on their potential as alternatives to antibiotics for therapeutic purposes and applications in the cosmetics and food industry. For

example, Lee and Ahn (1998) found that cinnamaldehyde, derived from the cinnamon essential oil, strongly inhibits *Clostridium perfringens* and *Bacteroides fragilis* and moderately inhibits *Bifidobacterium longum* and *Lactobacillus acidophilus* isolated from human feces. The selective inhibition by cinnamaldehyde of pathogenic, intestinal bacteria may have a pharmacological role in balancing the intestinal microbiota. The wide range of *in-vitro* anti-microbial activities of essential oils derived from cinnamon, thyme and oregano have been published (Deans and Ritchie, 1987; Paster *et al.*, 1990; Biondi *et al.*, 1993; Stiles *et al.*, 1995; Sivropoulou *et al.*, 1996; Nelson, 1997; Adam *et al.*, 1998; Farag *et al.*, 1989b; Manou *et al.*, 1998; Smith-Palmer *et al.*, 1998; Cosentino *et al.*, 1999; Hammer *et al.*, 1999; Ferhout *et al.*, 1999; Dorman and Deans, 2000), supporting their possible use as anti-microbial agents. It is reasonable to suggest that the main components of essential oils that display *in vitro* anti-microbial activity are responsible for the activity of the oils. The essential oils and their pure components displaying anti-microbial activities are shown in Table 2. Anti-microbial activity of individual compounds against selected microorganisms is presented also (Table 3). The minimum inhibitory concentrations (MIC) of the pure compounds differ and vary between experiments. It is considered beneficial to keep the effective anti-microbial concentration of essential oils as low as possible due to their characteristic flavors. This problem can be overcome, as suggested by Moleyar and Narasimham (1992), by using synergistic properties of different oils, thus improving the anti-microbial activity in spite of low dosages. This synergism was highlighted in studies of Didry *et al.* (1994) and Montes-Belmont and Carvajal (1998).

**Anti-microbial mode of action of essential oils:** The exact anti-microbial mechanism of essential oils is poorly understood. However, it has been suggested that their lipophilic property (Conner, 1993) and chemical structure (Farag *et al.*, 1989b,c) could play a role. Helander *et al.* (1998) investigated how two isomeric phenols, carvacrol and thymol, and the phenylpropanoid, cinnamaldehyde, exert their antibacterial effects on *Escherichia coli* O157 and *Salmonella typhimurium*. Both carvacrol and thymol, in a similar fashion, disintegrated the membrane of bacteria, leading to the release of membrane-associated material from the cells to the external medium. On the other hand, cinnamaldehyde failed to affect the membrane, but exhibited antibacterial activity, indicating that two molecules have different mechanisms underlying antibacterial activity. It was thus suggested that terpenoids and phenylpropanoids can penetrate the membrane of the bacteria and reach the inner part of the cell because of their lipophilicity (Helander *et al.*, 1998),

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Table 1: Chemical properties of the essential oil constituents thymol, carvacrol, cinnamaldehyde, and \$-ionone

	Thymol	Carvacrol	Cinnamaldehyde	-\$-ionone
Molecular weight	150 C <sub>10</sub> H <sub>14</sub> O	150 C <sub>10</sub> H <sub>14</sub> O	132 C <sub>9</sub> H <sub>8</sub> O	192 C <sub>13</sub> H <sub>20</sub> O
Synonym	5-methyl-2-(1-methylethyl) phenol	2-methyl-5-(1-methylethyl) phenol	3-phenyl-2-propenal	4-(2,3,3-trimethyl-1-cyclohexen-1-yl)-3-buten-2-one
FEMA-GRAS <sup>1</sup>	3066	2245	2286	2595
FDA <sup>1</sup>	21CFR 172.515	21CFR 172.515	21CFR 182.60 GRAS	21CFR 172.515
Found in	Thyme (Lamiaceae)	Oregano (Lamiaceae)	Cinnamon (Lauraceae)	Boronia (Rutaceae)
Appearance	White crystals	Colorless to pale yellow liquid	Slightly yellow liquid	Liquid colorless
Odor	Pungent, caustic taste	Thymol-odor like	Cinnamon	Violet
Boiling point	233	237	246	266
Density, g/ml	0.969	0.976	1.048	0.945
LD <sub>50</sub> <sup>2</sup>	980 mg/kg, orally rat	810 mg/kg, orally rat	2,220 mg/kg, orally rat	4,590 mg/kg, orally rat
Stability <sup>1</sup>	Good	Good	Fair to poor	Good
Structure				
	H <sub>3</sub> CCHCH <sub>3</sub>	H <sub>3</sub> CCHCH <sub>3</sub>	CH = CHCHO	CH = CHCOCH <sub>3</sub>
Biological activity <sup>3</sup>	Antimicrobial Antiinflammatory Antimelanomic Antioxidant Antiseptic Carminative Flavor	Antimicrobial Antiinflammatory Antimelanomic Antioxidant Antispasmodic Carminative Flavor	Antimicrobial Antiinflammatory Antispasmodic Antiurease Cancer-preventive Flavor Hypoglycemic	Antimicrobial Antimelanomic Antitumor Cytochrome-P450-inducer Hypocholesterolemic

<sup>1</sup> Furia and Bellanca, 1975; <sup>2</sup> Jenner *et al.*, 1964; <sup>3</sup> Agricultural Research Service (ARS), 2002

Table 2 Essential oils and their main components exhibiting antimicrobial activities

Scientific name	Common name	Part	Antimicrobial components	References
<i>Boronia megastima</i> Nees ex Bartl.	Boronia	Flower	-\$-ionone	Gueldner <i>et al.</i> , 1985
<i>Zea mays</i> L.	Corn	Leaf	-\$-ionone	Gueldner <i>et al.</i> , 1985
<i>Cinnamomum verum</i> J. Presl	Cinnamon	Bark	Cinnamaldehyde	Ouattara <i>et al.</i> , 1997
<i>Origanum vulgare</i> spp. <i>hirtum</i> (Link) (letsw.)	Oregano	Shoot	Carvacrol	Sivropoulou <i>et al.</i> , 1996
<i>Syzygium aromaticum</i> (L.) Merr. & Perry	Cloves	Flower	Eugenol	Hammer <i>et al.</i> , 1999
<i>Thymus vulgaris</i> L.	Thyme	Plant	Thymol	Dorman and Deans, 2000

but it has also been proposed that structural properties, such as the presence of the functional groups (Farang *et al.*, 1989b,c), and aromaticity (Bowles and Miller, 1993) are responsible for the antibacterial activity. It is thought that membrane perforation or binding is the principle mode of action (Shapiro and Guggenheim, 1995; Stiles *et al.*, 1995), leading to an increase of permeability and leakage of vital intracellular constituents (Juven *et al.*, 1994), resulting in impairment of bacterial enzyme systems (Farang *et al.*, 1989b,c). The mechanism of antifungal action of cinnamaldehyde has been investigated (Kurita *et al.*, 1979) and it was proposed that it takes place through the reaction with sulfhydryl groups, which are indispensable for the fungal growth, and that the formation of charge transfer complexes with electron donors in the fungus cell could lead to inhibition of cell division and thus interferes with cell metabolism. It was also reported that cinnamaldehyde inhibits the fungal-cell-wall synthesizing enzymes (Bang *et al.*, 2000).

**In vivo studies:** On the basis of their *in vitro* antimicrobial activity, it is logical to consider essential oils application as prophylactic and therapeutic agents in animal production. It would be expected that the intake of essential oils affects the gastrointestinal microflora composition and population. Allen *et al.* (1997) investigated *Artemisia annua* as a potential anticoccidial drug in poultry. Pure components of *A. annua*, i.e., artemisinin, 1,8-cineole and camphor at the levels of 17, 119 and 119 ppm, respectively, were fed to chicks from 1-d-old to 3 weeks of age. At 2 wk of age, half of the chicks were inoculated with *Eimeria acervulina* and *Eimeria tenella*. Some prophylactic action against the coccidia challenge was shown in treated chicks, especially in those fed artemisinin. Evans *et al.*, (2001) investigated whether a mixture of essential oils from clove (1.0%), thyme (0.1%), peppermint (0.1%) and lemon (0.1%) could have effects on coccidia oocyte output and the number of *Clostridium perfringens* in broiler chicks when artificially inoculated. There was no positive control included. Chicks fed the diets containing an essential oil blend showed a reduced oocyte excretion when compared to those fed the non-supplemented diet. However, the number of *Clostridium perfringens* in the intestine did not differ between treatments. A field study conducted by Köhler (1997) with a commercial preparation of essential oils showed a reduction of colony forming units of *Clostridium perfringens* as compared to the positive control diet containing zinc bacitracin at the level of 20 ppm. The commercial preparation was supplied in a powdered form and added to the diet at the level of 50 ppm. This preparation was also reported to slightly lower ileal ATP concentrations (Veldman and Enting, 1996) which is an indicator of microbial activity in broilers (Smits *et al.*,

1997). Similarly, a blend of capsicum, cinnamaldehyde and carvacrol lowered the number of *Escherichia coli* and *Clostridium perfringens* in ceca (Jamroz and Kamel, 2002).

Essential oils have been reported to affect rumen microbial activity, the effects being either positive (McIntosh *et al.*, 2000) or negative (Oh *et al.*, 1967; 1968). Oh *et al.* (1967, 1968) observed an inhibitory effect of essential oils against rumen microorganisms in terms of total gas and volatile fatty acid production. It was hypothesized that the characteristic odor and antibacterial action of essential oils from unpalatable plant species may reduce voluntary feed intake by ruminants. Scholl *et al.* (1977) studied the utilization of sagebrush by mule deer and found that the presence of essential oils was a negative factor for the utilization of sagebrush. McIntosh *et al.* (2000) reported that a commercial preparation of essential oils did not affect the protozoal numbers in the rumen but did increase the bacterial population, possibly leading to increased nitrogen availability to the host.

**In vitro antioxidant effects of essential oils:** The antioxidative properties of the extracts of oregano, dittany, thyme, marjoram, spearmint, lavender and basil have been evaluated when added to lard kept at 75°C (Economou *et al.*, 1991). Oregano extract was found to be most effective in stabilizing lard, followed by thyme, dittany, marjoram and lavender. It was reported that D-cymene-2,3-diol (Schwarz *et al.*, 1996) and thymol and carvacrol (Aeschbach *et al.*, 1994; Aruoma, 1997; Baratta *et al.*, 1998), which are found in thyme, show strong antioxidant properties. Farang *et al.* (1989a) discussed the relationship between the antioxidant property and the chemical composition of the essential oils. It was suggested that the high antioxidant activity of thymol is due to the presence of phenolic OH groups which act as hydrogen donors to the peroxy radicals produced during the first step in lipid oxidation, thus retarding the hydroxy peroxide formation. Teissedre and Waterhouse (2000) reported a high correlation ( $r = 0.75$ ) between the total phenol content of essential oils and human low-density-lipoprotein oxidation *in vitro*.

**Antioxidant property of essential oils as based on animal studies:** Youdim and Deans (1999a,b and 2000) investigated the effect of thyme oil and its major compound, thymol, as dietary antioxidant supplements on age-related changes in polyunsaturated fatty acids in various organs. Thyme oil and thymol were fed to rats on a basis of 42.5 mg/kg of body weight daily until 28 months of age. Rats fed the supplements maintained higher levels of polyunsaturated fatty acids, especially C20:4n-6 and C22:6n-3 in the phospholipid fractions of liver, brain, kidney and heart, than did those fed the control diet. It would appear that the supplements act as

Table 3: Minimum inhibitory concentration (MIC, ppm) of carvacrol, cinnamaldehyde and thymol

Microorganisms	Carvacrol	Cinnamaldehyde	Thymol	References
<i>Escherichia coli</i>	450	396	450	Helander <i>et al.</i> , 1998
<i>Escherichia coli</i>	225	NT	225	Cosentino <i>et al.</i> , 1999
<i>Staphylococcus aureus</i>	450	NT	225	Cosentino <i>et al.</i> , 1999
<i>Candida albicans</i>	150	NT	150	Ali-Shtayeh <i>et al.</i> , 1997
<i>Candida albicans</i>	113	NT	113	Cosentino <i>et al.</i> , 1999
<i>Candida albicans</i>	200	200	NT	Ferhout <i>et al.</i> , 1999
<i>Pseudomonas aeruginosa</i>	500	NT	500	Ali-Shtayeh <i>et al.</i> , 1997
<i>Pseudomonas aeruginosa</i>	>900	NT	>900	Cosentino <i>et al.</i> , 1999
<i>Salmonella typhimurium</i>	150	396	150	Helander <i>et al.</i> , 1998
<i>Salmonella typhimurium</i>	225	NT	56	Cosentino <i>et al.</i> , 1999
<i>Streptococcus mutans</i>	125	250	250	Didry <i>et al.</i> , 1994
<i>Streptococcus mitis</i>	125	125	125	Didry <i>et al.</i> , 1994

NT: not tested

Table 4: Effects of dietary essential oils on digestive enzyme activities in pancreatic extracts prepared from female broiler chickens that were fed diets without or with additives<sup>1</sup>

	Control	Thymol	Cinnamaldehyde
21 days of age			
Amylase <sup>2</sup>	22 ± 1.7	23 ± 4.3	21 ± 2.9
Lipase <sup>3</sup>	8.7 ± 1.0	11.2 ± 1.4	9.1 ± 2.0
Trypsin <sup>4</sup>	1.07 ± 0.28	1.26 ± 0.31	1.10 ± 0.10
Chymotrypsin <sup>5</sup>	1.00 ± 0.23	1.14 ± 0.25	1.01 ± 0.17
40 days of age			
Amylase <sup>2</sup>	39 ± 1.9	38 ± 3.1	37 ± 1.9
Lipase <sup>3</sup>	33 ± 6.5	36 ± 7.5	32 ± 9.2
Trypsin <sup>4</sup>	0.96 ± 0.14	1.00 ± 0.23	1.02 ± 0.15
Chymotrypsin <sup>5</sup>	0.97 ± 0.14	1.13 ± 0.11	1.02 ± 0.09

<sup>1</sup> Means (SD, n=4) are expressed as unit/mg of protein.

<sup>2</sup> One unit was defined as hydrolysis of 1 mg of maltose per minute at pH 6.9 and 37 °C.

<sup>3</sup> 1 µmol of free fatty acid released per minute at pH 8.5.

<sup>4</sup> 1 µmol of p-toluenesulfonyl-L-arginine methyl ester hydrolyzed per minute at pH 8.1 and 37 °C.

<sup>5</sup> 1 µmol of benzoyl-L-tyrosine ethyl ester hydrolyzed per minute at pH 7.8 and 37 °C.

effective free radicals scavengers and influence the *in vivo* antioxidant defense systems such as superoxide dismutase, glutathione peroxidase and vitamin E. There is evidence that the antioxidant components in thyme can be transferred to egg yolk as based on a lower concentration of malonaldehyde in the yolk (Botsoglou *et al.*, 1997). Thymol and *D*-cymene-2,3-diol, which are responsible for the antioxidant properties of thyme, have been determined in eggs collected during 24-day-feeding periods (Krause and Ternes, 1999). 1.12 and 1.68% thyme extracts, which correspond with 50 mg *D*-cymene-2,3-diol and 224 mg thymol, and 75 mg *D*-cymene-2,3-diol and 336 mg thymol per 100 g of diet, respectively, were included in the layers' diet. Approximately, 0.004 and 0.006% of the ingested *D*-cymene-2,3-diol and thymol were transferred to egg yolk after 12 days of feeding. The compounds disappeared from egg yolk soon after supplementation was ceased. An antioxidant effect of essential oils in broiler chickens has been reported as well (Lopez-Bote *et al.*, 1998; Botsoglou *et al.*, 2002). Botsoglou *et al.*, 2002 reported

that oregano essential oils exerted antioxidant property in meats and abdominal fat, pointing at the incorporation of the protective antioxidant components of the essential oil into the membrane. The authors further found that the antioxidant effect was dose dependent. It is thus concluded that thymol and carvacrol can act as antioxidant in egg and meat of chickens when introduced into the diets. However, as discussed by Botsoglou *et al.* (2002), a reliable laboratory method to determine essential oils in biological systems is necessary to support the view.

**Role of essential oil as flavor:** Besides as antimicrobials and antioxidants, essential oils and their pure components are also used as flavor in human foods. Carvacrol can be used in non-alcoholic beverages up to the level of 26 ppm and in baked goods up to 120 ppm (Furia and Bellanca, 1975). Cinnamaldehyde can be used at low as 8 ppm in ice cream products and as high as 4900 ppm in chewing gum (Furia and Bellanca, 1975). Thymol and beta-

Table 5: Effect of dietary essential oils on ileal viscosity and fat digestibility in female broiler chickens fed on diets containing CMC

	Base	CMC	CMC+thymol	CMC+cinnamaldehyde	CMC+Commercial preparation <sup>1</sup>	SEM <sup>2</sup>
Viscosity, mPa.s	1.56 <sup>3</sup>	8.20	8.83	9.17	10.30	2.076
Fat digestion, % of intake	88.9 <sup>a</sup>	86.8 <sup>b</sup>	86.7 <sup>b</sup>	87.2 <sup>ab</sup>	87.5 <sup>ab</sup>	0.432

<sup>1</sup> CRINA® Poultry (CRINA S. A. Akzo Nobel, Gland, Switzerland).

<sup>2</sup> SEM = standard error of means.

<sup>3</sup> Values are means for 3 replicates.

<sup>a,b</sup> Means in the same row not sharing a common superscript are significantly different (P<0.05).

ionone are also used as flavoring agents in foods.

The characteristic flavors of essential oils can be advantageous in standardizing tastes and smells of the diet if the diet ingredients are changed such as during the weaning transition of piglets (Anonymous, 1998). Specific effects of flavors on chickens' performance has not received much attention because poultry may not acutely respond to flavor when compared to pigs (Moran, 1982). There is evidence (Deyoe *et al.*, 1962) that flavors could affect feed intake. On the other hand, the effects of flavors on poultry performance are regarded as negligible (Moran, 1982). The aspect of essential oil as flavor in poultry nutrition needs to be assessed.

**Can essential oils affect the digestion process?:** There are suggestions that dietary essential oils can improve digestion (Anonymous, 1997; Mellor, 2000a,b). It might be reasoned that spices and herbs, from which essential oils are derived, will positively affect food digestion (Pradeep *et al.*, 1991; Pradeep and Geervani, 1994). A number of studies have reported the effect of spices or their active components on bile salt secretion (Bhat *et al.*, 1984, 1985; Bhat and Chandrasekhara, 1987; Sambaiah and Srinivasan, 1991). In addition, the dietary pungent principles, i.e. curcumin, capsaicin, and piperine, have been shown to stimulate digestive enzyme activities of intestinal mucosa and of pancreas (Platel and Srinivasan, 1996 and 2000). It was reported earlier (Harada and Yano, 1975) that cinnamaldehyde increased bile secretion in the rat. It is interesting to note that the pungent principles, capsaicin and piperine and cinnamaldehyde share their synthetic pathways (shikimic pathway). Whether or not dietary thymol and cinnamaldehyde, at the level of 100 ppm, stimulate secretion of pancreatic digestive enzymes, i.e. amylase, lipase, trypsin, and chymotrypsin has been tested in our laboratory in female broiler chickens (Lee *et al.*, 2003a). As shown in Table 4, there are no clear effects of thymol and cinnamaldehyde on the enzyme activities at either 21 or 40 days of age of the chickens. On the other hand, cinnamaldehyde, and eugenol, a main component of clove essential oils, when fed at the dietary concentrations of 1000 and 850 ppm, significantly impaired the absorption of alanine by rat jejunum (Kreydiyyeh *et al.*, 2000). The authors postulated that the two principles inhibit the activity of Na<sup>+</sup>-K<sup>+</sup>-ATPase

located in enterocyte, and consequently impair transport processes in the intestine. In addition, *in vitro* results showed that IC<sub>50</sub> values, i.e. the concentration of the principles that inhibit the activity of intestinal Na<sup>+</sup>-K<sup>+</sup>-ATPase by 50%, were 1.1 and 1.4 mg/mg of protein for cinnamaldehyde and eugenol, respectively. It can be expected that high doses of the two principles, when introduced into the chickens' diet, could inhibit the digestion process. However, the inhibitory concentration in diet has not been established yet. In any event, dietary cinnamaldehyde when compared to thymol seems to participate in the digestion process.

**Effects of essential oils on lipid metabolism:** Craig (1999) reviewed the role of herbs and their essential oils as to their cholesterol lowering properties and in the protection against cancer. Elson *et al.* (1989) reported the hypocholesterolemic effect of lemongrass oil, which rich in geraniol and citral, in human subjects. On the contrary, hardly any effects on plasma lipids other than cholesterol were observed (Cooke *et al.*, 1998).

The pure components of essential oils inhibit hepatic 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase activity (Crowell, 1999) which is a key regulatory enzyme in cholesterol synthesis. As a result, a hypocholesterolemic effect of essential oils can be expected. According to Case *et al.* (1995), a 5% inhibition of HMC-CoA reductase lowered serum cholesterol by 2% in poultry. Qureshi *et al.* (1983) reported a correlation between HMG-CoA reductase activity and either total or LDL cholesterol in chicken, but not with HDL cholesterol. It has been reported (Qureshi *et al.*, 1988) that when cockerels are fed dietary limonene at levels of 25-100 ppm for 26 days, hepatic HMG-CoA reductase activity and serum cholesterol show a dose-dependent decrease whereas hepatic fatty acid synthetase activity was unaffected. A variety of essential oil compounds, such as borneol, cineole, citral, geraniol, menthone, menthol, fenchone, fenchyl alcohol, and  $\delta$ -ionone have been shown to suppress hepatic HMG-CoA reductase activity (Middleton *et al.*, 1979; Clegg *et al.*, 1980; Middleton and Hui, 1982; Fitch *et al.*, 1989; Yu *et al.*, 1994).  $\delta$ -Ionone is a precursor of vitamin A (Naves, 1971), but the relation with its hypocholesterolemic effect is not clear. Hood *et al.*, (1978) tested the hypothesis that dietary essential oils

may inhibit biosynthesis of FPP, a precursor of cholesterol synthesis. Layers were force-fed individually with a capsule containing an essential oil daily for 5 weeks and cholesterol levels in plasma were monitored. Essential oils studied and their levels were  $\alpha$ -terpineol (10, 50, 100, and 200 mg/day), citronellol (100mg/day), linalool (100 mg/day) and geraniol (100mg/day). Contrary to the hypothesis, no significant differences among treatments were observed as to cholesterol levels in plasma. The authors ascribed the non-significant effect of the selected essential oil components to either ineffective inhibition of HMG-CoA reductase or to their fast degradation in liver.

**Mode of action:** The hypocholesterolemic effects of essential oils are mediated by down-regulating the regulatory enzyme, HMG-CoA reductase, post-transcriptionally without changing the enzyme mRNA levels (Elson and Qureshi, 1995; Qureshi *et al.*, 1996). Middleton and Hui (1982) proposed that the inhibitory action of essential oils on hepatic HMG-CoA reductase is independent of the diurnal cycle of the enzyme, and of hormones such as insulin, glucocorticoids, triiodothyronine, and glucagon. The complete inhibition of cholesterol synthesis, as proposed by Goldstein and Brown (1990), requires two regulators, i.e. cholesterol derived from LDL and a non-sterol product(s) derived from mevalonate, both of which modulate HMG-CoA reductase activity. It has been reported that thymol, carvacrol and  $\delta$ -ionone might induce a putative regulatory non-sterol product(s) (Case *et al.*, 1995; Elson, 1996).

**Effect of essential oils on growth performance in chickens:** The observed effects of essential oils on growth performance in chickens are either positive (Anonymous, 1997; Bassett, 2000; Langhout, 2000; Kamel, 2001) or non-significant (Vogt and Rauch, 1991; Case *et al.*, 1995; Veldman and Enting, 1996; Botsoglou *et al.*, 2002). The inclusion levels varied from 20 to 200 ppm. When the effect was positive, weight gain and feed intake were increased whereas the feed:gain ratio was lowered when compared to control. On the other hand, Botsoglou *et al.* (2002) reported that when dietary oregano essential oils, at the concentrations of 50 and 100 ppm, were fed to broiler chickens for a period of 38 days hardly any effects on body weight and feed conversion ratio could be demonstrated. The authors explained the lack of effect by pointing out that the birds' performance was already superior, leaving no room for growth enhancing effects of the additives. This statement could be in line with studies of Coates *et al.* (1951) and Hill *et al.* (1952), who demonstrated that well-nourished healthy chicks responded less to antibiotic supplements when they were housed in a carefully cleaned and disinfected place. Caution is

required when interpreting the results of Botsoglou *et al.* (2002) because the experimental diet used contained 75 ppm lasalocid and 0.01% exogenous enzymes which might either mask or diminish an effect of the essential oils. Vogt and Rauch (1991) also failed to observe any effects on growth performance when thyme essential oils were supplemented at one of four levels, 0, 20, 40 and 80 ppm. On the other hand, positive effects of essential oils have been obtained from the numerous field studies (Anonymous, 1997; Bassett, 2000; Langhout, 2000; Kamel, 2001). This may indicate that when experimental conditions and diets are marginal for the birds, a growth-enhancing effects of essential oils will be seen. Indeed, Allen *et al.* (1997) reported that two essential oil components, camphor and 1,8-cineole at the dietary level of 119 ppm, showed no clear effects on weight gain when birds were reared without coccidia challenge, but led to significant weight gains when the birds were infected with coccidia. This result, and the outcome of field studies (Anonymous, 1997; Bassett, 2000; Langhout, 2000; Kamel, 2001), indicates that the effects of dietary essential oils on growth performance become apparent when chickens are subjected to suboptimal conditions such as a less digestible diet and/or a less clean environment.

Our own results (Lee *et al.*, 2003b) show that dietary carvacrol versus thymol at the concentration of 200 ppm lowered weight gain and feed intake, but improved the feed:gain ratio when birds were fed the respective diet for 4 weeks. We proposed that the effect of dietary carvacrol on feed:gain ratio could relate to increased efficiency of feed utilization and/or altered carcass composition. According to Yu *et al.*, (1994), chicks fed on a diet containing  $\delta$ -ionone at the level of 100 or 250 ppm were heavier by on average 10.6 and 22.3%, respectively, when compared to the controls. The  $\delta$ -ionone-induced increase in body-weight-gain did not reach statistical significance, but the statistical power was low due to large inter-individual variation. It might be suggested that dietary essential oils may act not only on intestinal microflora, but also on nutrient utilization. Moreover, it seems that isomers can have different effects on growth performance.

**Metabolic pathway of components of essential oils:** Kohlert *et al.* (2000) reviewed the various pure components of essential oils that are used in herbal medicine and summarized their absorption, metabolism and excretion. The authors concluded that essential oil constituents are quickly absorbed after oral, pulmonary, or dermal administration and that most are metabolized and either eliminated by the kidneys in the form of glucuronides or exhaled as CO<sub>2</sub>. Their accumulation in the body is unlikely due to rapid clearance and short half lives. Igimi *et al.* (1974) studied the metabolic fate of <sup>14</sup>C-labelled d-limonene in rats. The authors reported that d-

limonene absorbed from the intestine was rapidly excreted without significant deposition in the body. Two hours after administration, the adrenals, liver and kidney showed the highest concentrations of d-limonene and then declined to negligible concentrations at 24 hours after administration. Most radioactivity was recovered in the urine. The metabolites of d-limonene in rabbits also were excreted with urine (Kodama *et al.*, 1974).

**Metabolic pathway of thymol and carvacrol:** The metabolic pathways of thymol and carvacrol are well studied in rats. Rats have been given thymol and carvacrol at 1 mmol/kg of body weight and the excretory patterns of the compounds in urinary samples have been measured (Austgulen *et al.*, 1987). Urinary excretion of metabolites was rapid, and negligible amounts were excreted after 24-hrs. No metabolites were found in urine samples after 48 hours. Large quantities of carvacrol, and especially of thymol, were excreted in unchanged form. Extensive oxidation of the methyl and isopropyl groups of both compounds occurred which resulted in the formation of derivatives of benzyl alcohol and 2-phenylpropanol and their corresponding carboxylic acids. On the other hand, the hydroxyl group of the two compounds was not affected. Takada *et al.* (1979) reported that the major portions of thymol (when orally given to rabbits) were excreted within 24 hours and that thymol glucuronide was the main metabolite in urine. However, intact thymol was not excreted in appreciable amounts.

**Metabolic fate of cinnamaldehyde and  $\beta$ -ionone:** Urinary metabolites of cinnamaldehyde have been studied in rats and mice (Peters and Caldwell, 1994). Trans-[3-<sup>14</sup>C]cinnamaldehyde was given to laboratory rodents by different routes of administration (ip injection and oral gavage) and with various dosages (2 to 250 mg/kg of body weight). Generally, cinnamaldehyde was rapidly excreted via urine in the first 24 hrs and less than 2% of the administered doses remained in the carcass after 72 hrs. The major metabolite was hippuric acid, which was supposed to arise from oxidation of the aldehyde to cinnamic acid and further metabolism by way of  $\beta$ -oxidation of the side-chain followed by glycine conjugation to yield hippuric acid. In addition, another metabolic pathway of the compound was conjugation with reduced glutathione, leading to mercapturic acids as end products in the urine. Hoskins (1984) extensively reviewed the metabolic pathways of cinnamaldehyde via oxidation and glutathione conjugation. Yuan *et al.* (1993) studied the bioavailability of cinnamaldehyde when rats were gavaged at doses of 50, 250 and 500 mg/kg of body weight. A low bioavailability of less than 20% was shown when gavaged at 250 and 500 mg/kg of body weight. No blood cinnamaldehyde was detected at the lowest levels while

1.3 and 2.4  $\mu$ g/mL for the doses of 250 and 500 mg/kg, respectively, were observed. The authors suggested that systemic exposure by low concentrations of absorbed cinnamaldehyde would be negligible due to rapid oxidation in the liver and that at higher concentrations of cinnamaldehyde, some escaped oxidation and was conjugated with protein.

The metabolic pathway of  $\beta$ -ionone was studied by Ide and Toki (1970).  $\beta$ -ionone was gavaged to rabbits for 7 days at the level of 1 g per kg body weight and urine was collected during the administration and for 4 days after the last administration. Five metabolites were found to be excreted in the urine: unchanged  $\beta$ -ionone, 3-oxo- $\beta$ -ionone, 3-hydroxy- $\beta$ -ionol, 3-oxo- $\beta$ -ionol, and dihydro-3-oxo- $\beta$ -ionol. The last two compounds were detected as major metabolites. The glucuronides of 3-oxo- $\beta$ -ionol and dihydro-3-oxo- $\beta$ -ionol were also detected. The authors suggested that  $\beta$ -ionone is metabolized by hydroxylation of the ring system at the carbon atom " to the ring double bond and then oxidation of the hydroxyl group to 3-oxo derivatives occurs.

Besides the metabolism by the host, it seems unlikely that the intestinal microflora could metabolize the essential oils. Varel (2002) reported that thymol and carvacrol were not metabolized by microorganisms residing in swine feces.

**Toxicological studies:** The toxicity studies consist of determination of the acute oral effects, subacute studies in which the flavoring agents are mixed in the diet or administered by stomach tube, and chronic feeding studies. Acute oral toxicity studies with carvacrol, cinnamaldehyde, beta-ionone and thymol have been conducted (Jenner *et al.*, 1964). The acute oral LD<sub>50</sub> (mg/kg of body weight) of carvacrol, cinnamaldehyde, beta-ionone, and thymol in the rat are 810, 2220, 4590 and 980, respectively.

Hébert *et al.* (1994) studied the toxicological effects of cinnamaldehyde in rodents. Rats were fed 188 to 3000 mg/kg of body weight/day and mice 474 to 7500 mg/kg of body weight/day. Cinnamaldehyde was added to the rodent's diet. Rats and mice receiving cinnamaldehyde with their feed showed a dose-related decrease in body weight gain, which resulted from a decreased food consumption (food aversion) at the beginning. Rodents typically show an aversion to food with strong odors. Relative weights of liver, kidney and spleen (g of organ/100 g of body weight) were not affected by the various doses in the feed. Toxicological studies with cinnamaldehyde have been extensively reviewed and reported elsewhere in relation to its carcinogenicity, mutagenicity, its characteristic being a potent teratogen in chickens, and being a skin sensitizer (Abramovici and Rachmuth-Roizman, 1983; Hoskins, 1984; National Toxicology Program, 1989; Stamatii *et al.*, 1999; Smith *et al.*, 2000). When rats were fed diets containing thymol

at the level of 1000 and 10000 ppm for 19 weeks, no clear signs of toxicity were observed (Hagan *et al.*, 1967). On the other hand, ionone (a mixture of  $\alpha$ - and  $\beta$ -ionone) administered at the level of 2500 and 10000 ppm for 17 weeks caused slight to moderate swelling of parenchymal cells.

**Tissue residue of essential oils:** Accumulation of essential oils in the body is unlikely due to their fast metabolic conversion and excretion. However, when continuously feeding diets containing essential oils to chickens without withdrawal periods, essential oil constituents can be deposited in various tissues. Botsoglou *et al.* (2002) showed that essential oils can be deposited in a dose-dependent fashion. On the other hand, their impact on sensory quality of poultry meat is regarded as minor (Vogt and Rauch, 1991). Essential oils deposited in poultry tissue can be consumed by humans. Whether this consumption of essential oils with poultry meat will evoke negative effects needs to be assessed. It should however be emphasized that the compounds, thymol, carvacrol, cinnamaldehyde, and  $\beta$ -ionone, are given 'GRAS' status by the Flavor and Extract Manufacturers' Association (FEMA) and the Food and Drug Administration (FDA) (Furia and Bellanca, 1975), implying that their use is safe.

**Microflora, fat digestibility and essential oils:** Schaedler (1973) stated that an 'ideal flora' would allow optimum growth performance. Any alteration of the indigenous flora by diet or environment can be deleterious to the host. Extensive reviews (Jukes, 1955; Visek, 1978; March, 1979; Fuller, 1989; Vanbelle *et al.*, 1990; Ewing and Cole, 1994; Stavric and Kornegay, 1995) on the role of microflora on animal performance support the view of Schaedler (1973). It was thus assumed that dietary essential oils could have growth-enhancing effects due to their actions on the intestinal microflora. This implies that the efficacy of essential oils on animal performance could be affected by the microbial status. In other words, their effects on germ-free chicks are expected to be negligible at best. This statement is backed up by a study of Coates *et al.* (1963) who demonstrated that dietary penicillin had no apparent effect on growth performance by germ-free chicks. There are also indications showing that dietary antibiotics may not play a significant role in growth performance when birds are kept in a clean environment and fed well-balanced diets. On the other hand, when birds are challenged by gut microbial loads, then dietary essential oils can positively affect growth performance. This concept has been tested as to the efficacy of dietary antibiotics in counteracting the negative effects of rye in chickens (MacAulliffe and McGinnis, 1971; Wagner and Thomas, 1978; Patel *et al.*, 1980; Antonoiu and Marquardt, 1982; Honeyfield *et al.*, 1983). It is well known

that feeding rye to chickens increases number of bacteria in the intestine (Wagner and Thomas, 1978; Feighner and Dashkevicz, 1987) so that growth-enhancing property of dietary antibiotics can be seen. The microbial over-population by rye feeding is attributed to its pentosan contents which raise intestinal viscosity. It is suggested that intestinal viscosity, caused by ingestion of soluble fiber, impairs the normal digestion process so that more undigested materials travel to distal parts where they can be used as substrates by the microflora. Increased microbial populations are also apparent in the upper part of the small intestine (Smits *et al.*, 1998) where digestion occurs.

There is evidence (Kussaibati *et al.*, 1982; Smits *et al.*, 1998) that the intestinal microflora has a pronounced impact on fat digestion in chickens because of a lowered bile acid availability. Bile salts are known to be a limiting factor for efficient fat digestion (Krogdahl, 1985). Redinger *et al.* (1973) found that 87% of total bile acid pool is present in the intestine. Birds are well equipped with the efficient re-absorption of bile acids, the absorption be as high as 93% (Hurwitz *et al.*, 1973). This reduces the need of hepatic synthesis of bile acids in order to maintain the bile salts pool in the chicken (Freeman, 1984), and can be an important factor especially in young chicks with limited secretion of bile acids (Kussaibati *et al.*, 1982). Ketels (1994) reported that when the bile concentration is lower than 50 to 60  $\mu\text{mol/g}$  of fat-free matter, the digestibility of tallow will be impaired.

It has been reported that the intestinal microflora can hydrolyse bile salts (Freigher and Dashkevicz, 1987). This flora includes *Clostridium*, *Lactobacillus*, *Peptostreptococcus*, *Bifidobacterium*, *Fusobacterium*, *Eubacterium*, *Streptococcus*, and *Bacterioids* (Feighner and Dashkevicz, 1987). Freigher and Dashkevicz (1988) reported that chickens fed on a diet rich in rye exhibited an 18-fold increase in bacterial cholytaurin hydrolase activity in ileal homogenates when compared their counterparts fed on corn diet. Unconjugated bile acids are less effective in forming micelles (Smits *et al.*, 1998). It is thus expected that feeding soluble fiber to chicken can severely affect fat digestion. Smits *et al.* (1998) suggested that carboxymethyl cellulose (CMC), which is a non-fermentable soluble fiber, can impair re-absorption of bile acids, leading to a lower bile acid availability. The extra fecal loss of bile acids can occur by either direct binding to the soluble fiber (Smits *et al.*, 1998) or by less efficient utilization of unconjugated bile acids (Angelin *et al.*, 1982) as only 40-50% of the deconjugated bile acids are recycled. In this regard, with antimicrobial agents fat digestion may be improved. Antoniou and Marquardt (1982) proposed that fat digestibility can be a good index of the nutritive value of cereals such as barley and rye. Consequently, fat digestion can reflect the efficacy of dietary essential oils

under this circumstance.

Besides the putative, positive antimicrobial effect of essential oils affecting fat digestibility in chickens fed on diets containing soluble fiber, there might be a direct effect of essential oils on either secreting or synthesizing bile acids. The latter holds true for essential oils classified as phenylpropanoids and is highlighted by the effects of capsaicin and piperine on either bile secretion or cholesterol 7 $\alpha$ -hydroxylase activity (Bhat and Chandrasekhara, 1987; Srinivasan and Sambaiah, 1991). Cinnamaldehyde, a phenylpropanoid and a major essential oil component of cinnamon essential oils, shares a common synthetic pathway with capsaicin and piperine and thus affect bile acid metabolism. Indeed, Harada and Yano (1975) reported that cinnamaldehyde increased bile secretion in rats. Whether this effect extends to carvacrol, thymol and  $\alpha$ -ionone awaits further study.

We have recently investigated whether dietary essential oils could counteract the CMC-suppressive effect on fat digestibility in female broiler chickens (Lee *et al.*, 2004). We supplemented either thymol, cinnamaldehyde or a commercial preparation at the level of 100 ppm to a CMC-containing diet and found that cinnamaldehyde and the commercial preparation overcame the CMC effect (Table 5). Unexpectedly, thymol did not show any effect on fat digestibility. The improvement of fat digestibility as induced by cinnamaldehyde was not mediated by a lowering of ileal viscosity as shown in Table 5. At present, a clear explanation for the result is not readily available, but it is certain at this point that two principles, thymol and cinnamaldehyde, have different biological effects in female broiler chickens.

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