# Effects of dietary essential oils on growth performance and cholesterol metabolism in chickens

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## Abstract

Dietary antibiotics at low, subtherapeutic levels have been shown to improve growth performance in farm animals. However, there is a trend to look for alternatives to dietary antibiotics, due to occurrence of antibiotic-resistance bacteria. The present review explored the essential oils as the possible alternative to dietary antibiotics. The antimicrobial activities of essential oils originating from plants have been well documented while their toxicological effects are seen only at very high doses. Hypocholesterolemic effect has been reported in chickens. Essential oils may stimulate the digestion process. In conclusion, dietary essential oils may be used as alternatives to antibiotics, but whether their effects on growth performance are a consequence of antimicrobial activity needs to be studied further.

*(Key words: Essential oils, Growth performance, Cholesterol metabolism, Broiler chicken)*

## Introduction

There are no doubts that dietary antibiotics have played a fundamental role in animal production as growth and health promoter. However, it is the current trend to look for alternatives to antibiotics due to public concerns as to their in-feed use and the resulting residues, and subsequent occurrence of antibiotic-resistant bacteria. Dietary antibiotics presumably act on 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). 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 lower growth performance. Gut microflora can hydrolyze conjugated bile salts(Feighner and Dashkevicz, 1987) which limits fat digestion(Krogdahl, 1985). It is thus clear that controlling the microflora could positively influence birds performance and that feed supplements with antimicrobial activity are potential alternatives to antibiotics.

Various authors have reviewed and compared various compounds regarded as alternatives to antibiotics in animal production(Langhout, 2000 ; Mellor, 2000a,b ; Wenk, 2000 ; Taylor, 2001). Among them, essential oils are already marketed for use in animal production and are claimed to be digestive enhancers(Williams and Losa, 2001). However, information on essential oils is lacking. This urged us to collect published information on essential oils and assess the possible application in poultry nutrition.

## Definition of essential oils

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 quintaessentia 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 is proposed(Hay and Waterman, 1993). However, the name of essential oil will be preferentially used in this review.

Essential oils are very complex mixtures of compounds and their chemical compositions and concentrations 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; Jassen et al., 1987 ; Deans and Waterman, 1993), if any, of essential oils may differ.

## In-vitro antimicrobial activities of essential oils

Essential oils have long been recognized because of their antimicrobial 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 antimicrobial activities of essential oils derived from cinnamon, thyme and oregano have been published(Deans and Ritchie, 1987 ; Paster et al., 1990 ; Smith-Palmer et al., 1998 ; Hammer et al., 1999) which supports their possible use as antimicrobial agents. It is reasonable to expect that the main components of essential oils displaying in vitro antimicrobial activity are responsible for this activity. The essential oils and their pure components displaying antimicrobial activities are shown in **Table 1**. Antimicrobial activity of individual compounds against selected microorganisms is presented also(**Table 2**). The minimum inhibitory concentrations(MIC) of the pure compounds differ and vary between experiments.

It is considered beneficial to keep the effective antimicrobial 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 antimicrobial activity in spite of low dosages. This synergism was highlighted in studies of Didry et al.(1994) and Montes-Belmont and Carvajal(1998).

**Table 1. Essential oils and their main components exhibiting antimicrobial activities**

| Microorganisms | Carvacrol | Cinnamaldehyde | Thymol |
|---|---|---|---|
| Escherichia coli | 450 | 396 | 450 |
| Escherichia coli | 225 | NT | 225 |
| Staphylococcus aureus | 450 | NT | 225 |
| Candida albicans | 150 | NT | 150 |
| Candida albicans | 113 | NT | 113 |
| Candida albicans | 200 | 200 | NT |
| Pseudomonas aeruginosa | 500 | NT | 500 |
| Pseudomonas aeruginosa | >900 | NT | >900 |
| Salmonella typhimurium | 150 | 396 | 150 |
| Salmonella typhimurium | 225 | NT | 56 |
| Streptococcus mutans | 125 | 250 | 250 |
| Streptococcus mitis | 125 | 125 | 125 |

**Table 2. Minimum inhibitory concentration(MIC, ppm) of carvacrol, cinnamaldehyde and thymol**

| Scientific name | Common name | Part | Antimicrobial components |
|---|---|---|---|
| Boronia megastima Nees ex Bartl. | Boronia | Flower | β-ionone |
| Zea mays L. | Corn | Leaf | β-ionone |
| Cinnamomum verum J. Presl | Cinnamon | Bark | Cinnamaldehyde |
| Origanum vulgare spp. hirtum(Link)(Ietsw.) | Oregano | Shoot | Carvacrol |
| Syzygium aromaticum(L.) Merr. & Perry | Cloves | Flower | Eugenol |
| Thymus vulgaris L. | Thyme | Plant | Thymol |

NT: not tested.

## Antimicrobial mode of action of essential oils

The exact antimicrobial mechanism of essential oils is poorly understood. However, it has been suggested that their lipophilic property(Conner, 1993) and chemical structure(Farag et al., 1989a,b) could play a role. Helander et al.(1998) investigated how two isomeric phenols, carvacrol and thymol, and the phenylpropanoid, cinnamaldehyde, exerts their antibacterial effects on *Escherichia coli* O157 and *Salmonella typhimurium*. Carvacrol and thymol both, 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 did exhibit antibacterial activity, indicating that two molecules have different mechanisms underlying antibacterial activity. The authors, however, suggested that terpenoids and phenylpropanoids can penetrate the membrane of the bacteria and reach the inner part of the cell because of their lipophilicity, but it has also been ascribed to structural properties such as the presence of the functional groups(Farag et al., 1989a,b) and aromaticity(Bowles and Miller, 1993). 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(Farag et al., 1989a,b). 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 fungal cell wall synthesizing enzymes(Bang et al., 2000).

## 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, have been shown to 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 3**, 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 1,000 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 $Na^+$-$K^+$-ATPase located in enterocyte, and consequently impair transport processes in the intestine. In addition, in vitro results showed that IC50 values, i.e. the concentration of the principles that inhibit the activity of intestinal $Na^+$-$K^+$-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 as compared to thymol seems to participate in the digestion process as based on the literature published so far.

**Table 3. Effects of dietary essential oils on digestive activities in pancreatic extracts prepared from female broiler chickens that were fed diets without or with additives[1]**

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

[1]Means(±SD, n=4) are expressed as unit/mg of protein.
[2]One unit was defined as hydrolysis of 1 mg of maltose per minute at pH 6.9 and 37℃
[3]1μmol of free fatty acid released per minute at pH 8.5.
41μmol of p-toluenesulfonyl-L-arginine methyl ester hydrolyzed per minute at pH 8.1 and 37℃
51μmol of benzoyl-L-tyrosine ethyl ester hydrolyzed per minute at pH 7.8 and 37℃

## Effect 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 cital, 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 β-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). β-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 terpineol(10, 50, 100, and 200 mg/day), citronellol(100 mg/day), linalool(100 mg/day) and geraniol(100 mg/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 HMC-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 β-ionone might induce a putative regulatory non-sterol product(s)(Case et al., 1995; Elson, 1996).

## Effect of essential oils on growth performance

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 recent finding(Lee et al., 2003b) shows 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 β-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 β-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.

In conclusion, dietary essential oils may be used as alternatives to antibiotics, but whether their effects on growth performance are a consequence of antimicrobial activity needs to be studied further.

## 국문적요

사료내 낮은 수준의 항생제 첨가는 가축의 성장능력을 향상시키는 목적으로 사용하고 있다. 그러나, 항생제 사용에 따른 내성균의 출현은 결과적으로 항생제 대체제 개발을 촉진하는 계기가 되었다. 본 논문은 식물유래 정유성분의 항생제 대체제로서의 그 가능성을 알아보고자 실시하였다. 식물 정유의 항균효과는 많이 보고되었으며 정유의 독성효과는 사료첨가량 수준에서는 미약한 것으로 사료된다. 또한, 정유급여는 가금의 혈중내 콜레스테롤을 감소시키며, 또한 소화작용에도 관여하여 소화를 촉진시키는 것으로 보여진다. 결론적으로 사료내 정유의 첨가는 가금의 성장을 향상시킬 수 있으며, 식물유래 정유성분은 항생제의 대체제로서 이용될 수 있는 잠재성을 내포한 것으로 사료된다.

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