Essential oils and their use in animal feeds for monogastric animals – Effects on feed quality, gut microbiota, growth performance and food safety: a review

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ABSTRACT: Essential oils (EOs) are important aromatic components of herbs and spices and their biological activities have been known and utilised since ancient times in perfumery, food preservation, flavouring, and medicine. Some of their biological activities include antibacterial, antifungal, anti-oxidant and anti-inflammatory effects amongst others. EOs have received attention in recent years as potential 'natural' alternatives for replacing antibiotic growth promoters (AGPs) in animal diets due to their positive impact on growth performance, gut microbiota and welfare. The purpose of this paper is to provide an overview of our own published and unpublished data on the antibacterial, antifungal and insecticidal activity of thymol and cinnamaldehyde (TC blend), and to describe the effects of this specific EO blend on gut microbiota, growth performance and welfare, carcass characteristics and food safety. The possible modes of action of EOs are discussed and areas for future research are proposed.

Keywords: essential oils; thymol; cinnamaldehyde; antibacterial; gut microbiota; growth performance

Abbreviations

AGPs = antibiotic growth promoters, **AME** = apparent metabolisable energy, **BCFA** = branched chain fatty acid, **EFSA** = European Food Safety Authority, **ENZ** = wheat-formulated xylanase, **EOs** = essential oils, $\mathbf{F_1}$ = first generation population development, $\mathbf{Log_{10}}$ = base –10 logarithm of a number, **MIC** = minimum inhibitory concentration, **OD** = optical density, **SCFA** = short chain fatty acid, **TC** = blend of thymol and cinnamaldehyde

Contents

1. Introduction

Antibiotic growth promoters (AGPs) for animal diets have been banned in the European Union since 2006, and recent guidelines published by the Food Drug Association are exerting pressure for a ban in the US in the near future. To meet market demands for alternatives to AGPs in animal production, Danisco started in 2004 an *in vitro* screening program of various EOs in order to establish their potential antibacterial activity. Several EO blends were selected based on their antibacterial activity and they were tested *in vivo*. The objective was to develop a blend of EO compounds with synergistic properties that support growth performance of monogastric animals fed AGP-free diets.

EOs are volatile, aromatic compounds synthesised by plants for antibacterial, antifungal and antiviral purposes, and to deter herbivorous insects and animals. They are important aromatic components of herbs and spices and contribute to natural preservation of foods. The antibacterial properties of EOs are well documented and recognised (Kalemba and Kunicka 2003) both *in vitro* (Dorman and Deans 2000; Ouwehand et al. 2010) and *in vivo* (Mitsch et al. 2004; Tiihonen et al. 2010).

In vitro studies also suggest that combination of individual EOs has a greater antibacterial effect than individual EOs alone, indicating a synergy between single activities (Burt 2004; Lee et al. 2004b). Due to this synergistic activity, a blend of two carefully balanced nature-identical EO compounds, thymol and cinnamaldehyde (TC blend), was selected due to their combined antibacterial activity against potentially harmful bacteria and because they spare some of the beneficial intestinal bacteria.

EOs comprise a diverse group of components with different structures and chemical properties; for example, cinnamaldehyde is an aliphatic aldehyde, whereas carvacrol and thymol are phenolic compounds. Due to differences in their chemical structure, both groups differ in their mechanism of antibacterial action. In principle, both compounds are able to disturb the integrity and function of bacterial cell membranes (Di Pasqua et al. 2007). It is therefore not likely that the antibacterial activity of EOs can be attributed to one specific mechanism, but rather to the interaction between the EO chemical structure and a variety of targets in the bacterial cell. This makes it difficult to predict how susceptible to EOs a bacterial species is.

Monoterpene phenols, such as thymol and carvacrol, interact with the cell membrane by hydrogen bonding, rendering the membranes and mitochondria more permeable and disintegrating the outer cell membrane. They can inhibit the growth of *Escherichia coli* O157:H7, *Staphylococcus aureus*, *Salmonella enterica* serovar Typhimurium, *Pseudomonas fluorescens*, and *Brochothrix thermosphacta* (Di Pasqua et al. 2007, 2010).

Phenylpropanes, such as cinnamaldehyde bind with proteins through their carbonyl group, preventing the action of important cell enzymes such as amino decarboxylases (Burt 2004). Cinnamaldehyde has been shown to inhibit the growth of *Clostridium perfringens* and *Bacteroides fragillis*, but it exhibits weak or no inhibitory activity against *Bifidobacterium longum* or *Lactobacillus acidophilus* (Lee and Ahn 1998).

The pH of the matrix in which the EOs are present affects their hydrophobicity which will influence their interaction with the bacterial cell membrane, and therefore, affect the antibacterial action of EOs across the different segments of the gut. Using an *in vitro* model for pigs, (Michiels et al. 2009) showed that thymol and carvacrol have the ability to inhibit lactobacilli in the stomach more so than in the small intestine. The inhibitory effect of thymol against lactobacilli is beneficial in the proximal gut to reduce the risk of acidosis, and the stimulatory effect of cinnamaldehyde on lactobacilli is beneficial in the distal gut to prevent the proliferation of undesirable microorganisms.

The impact of pH on the biological activity of EOs and their ability of affecting the bacterial growth of some undesirable bacterial species makes them ideal candidates as gut bacterial modulators. As a result, the inclusion of EOs in animal diets could potentially minimise the occurrence of intestinal diseases caused by undesirable bacteria, and could favour the growth of beneficial gut microbiota supporting growth performance.

2. Antibacterial and antifungal activities of thymol and cinnamaldehyde

In vitro, pure cultures of various microbes have been exposed to increasing amounts of EOs under anaerobic conditions (Ouwehand et al. 2010). The tested *C. perfringens* strains were found to be sensitive to carvacrol, cinnamaldehyde, citral, limonene and thymol, particularly at higher concentrations (500 mg/l) and to oregano oil, rosemary oil and thyme oil. *Salmonella* serovars that were tested, were found to be sensitive only to high (500 mg/l) concentrations of the selected EOs, while *E. coli* was sensitive to most of the tested EOs at lower concentrations (5 and 50 mg/l). *Bifidobacterium longum*, *B. breve* and *L. reuteri* were less sensitive to most of the tested EOs, while *B. animalis* ssp. *lactis* and *L. fermentum* were relatively sensitive at lower concentrations (5 and 50 mg/l). With the exception of *Salmonella* and *E. coli*, all tested microbes were sensitive to the AGP avilamycin (Ouwehand et al. 2010). These findings indicate that one of the advantages that EOs may have over AGPs is that they have a better selectivity profile in that they spare the beneficial intestinal bacteria.

From these initial pure cultures and single EO tests, it was concluded that a blend of thymol and cinnamaldehyde (TC) would have the best potential to be of benefit to commercial animal production. Subsequent tests showed that this specific blend of EOs (TC) exerts a growth inhibition of at least 50% towards the Gram-negative bacterial species (Figure 1) with *E. coli* and *Salmonella* strains being particularly affected. The growth inhibition elicted by the TC blend in Gram$^+$ bacterial species was less than 25%. In contrast, growth inhibition of the Gram$^-$ bacterial species was not affected by avilamycin, while the Gram$^+$ bacterial species were highly sensitive to avilamycin.

As well as bacterial species, parasites such as *Eimeria* have also been demonstrated to be affected by EOs. Remmal et al. (2011) observed that in particular artemisia, clove, tea tree and thyme were lethal to *Eimeria* oocysts *in vitro* at concentrations below 0.5 mg/ml. Exposure to these EOs led to lysis of the oocysts. In this respect, it is important to note that the main EO constituent of the latter is thymol. Cinnamon (with the main EO constituent cinnamaldehyde) was also tested but was found to be less lethal.

Table 1. Minimum inhibitiory concentrations (MICs) in mg/ml of thymol (99% purity); trans-cinnamaldehyde (98% purity) against fungal target strains

Fungal species	Thymol	Cinnamaldehyde
Saccharomyces cerevisiae	0.208	0.038
Zygosaccharomyces bailii	0.297	< 0.026
Rhodotorula mucilaginosa	0.177	0.038
Rhodotorula glutinis	0.095	< 0.026
Pichia anomala	0.374	0.047
Kluyveromyces marxianus	0.386	0.038
Candida parapsilosis	0.319	0.053
Candida tropicalis	0.465	0.086
Debaryomyces hansenii	0.172	< 0.026
Penicillium commune	0.076	< 0.026
Aspergillus versicolor	0.214	< 0.026
Aspergillus parasiticus	0.212	0.035

The minimum inhibition concentrations (MIC) values were determined by performing a 1.5-dilution series of the essential oils (EOs) with concentrations between 0.026–1 mg/ml. The increase in optical density (OD) after incubation (at 25 °C for 48 h for yeast strains and 60 h for mould strains) was compared to the growth control to identify a bacteriostatic (MIC) or no effect of the tested substance. The bacteriostatic effect (MIC) was defined as the OD620 being less than or equal to 20% of the growth control after incubation
< (value) this means that full inhibition was seen at the lowest concentration tested

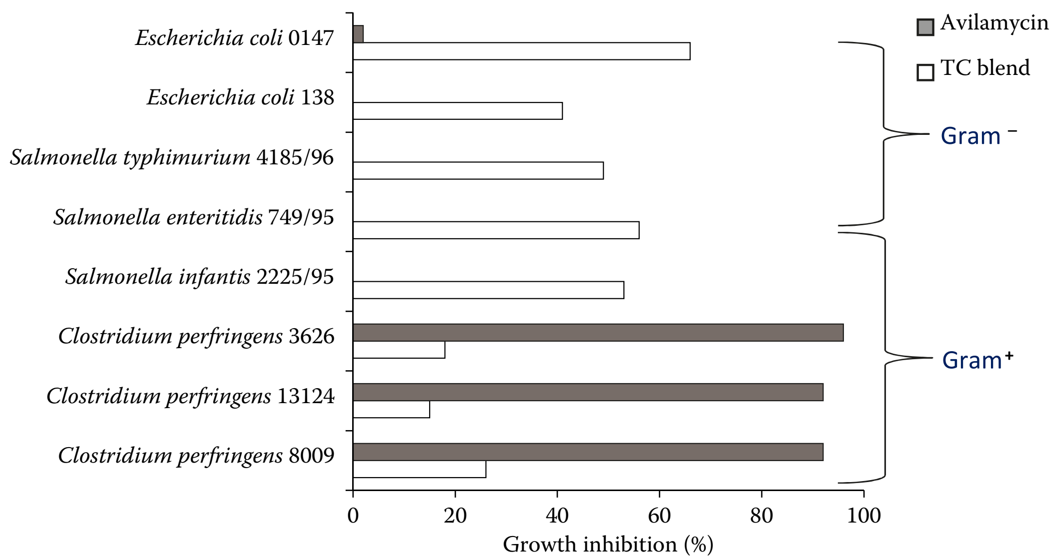

Figure 1. *In vitro* growth inhibition (%) of Gram$^+$ and Gram$^-$ bacteria in pure culture by the blend of thymol and cinnamaldehyde (TC blend) or avilamycin, compared with untreated control

Our *in vitro* research with thymol and in particular cinnamaldehyde has shown that these EOs are also able to inhibit the growth and proliferation of moulds and yeasts. Table 1 shows that thymol and cinnamaldehyde exhibited good antifungal activity when tested on spoilage-related fungal strains. Our findings are in agreement with the reported antifungal activity of purified EOs (Hsu et al. 2007; Klaric et al. 2007; Lopez et al. 2007; Tullio et al. 2007; Cheng et al. 2008). All fungal strains investigated in our study were sensitive to the assayed components. Cinnamaldehyde demonstrated the lowest minimum inhibitory concentrations (MICs), with MICs generally below 0.10 mg/ml and an average MIC against the indicator yeast and mould of 0.039 mg/ml. Thymol inhibited the microorganisms on average at a concentration of 0.249 mg/ml. These results show the potential of both thymol and cinnamaldehyde in preventing food spoilage, and their possible use as additives in food packaging to extend the shelf life of food products.

3. Stability of thymol and cinnamaldehyde in feed

The volatile compounds thymol and cinnamaldehyde (TC) are microencapsulated in a maltodextrin carrier to reduce evaporation and to increase the shelf life of the TC blend in feed. The encapsulation of thymol and cinnamaldehyde could also potentially ensure a more efficient delivery to the target site in the gastrointestinal tract allowing them to exert their antimicrobial and digestive-stimulating activities, and contributing to a better bioefficacy *in vivo*. The stability of this blend was assessed by adding the microencapsulated TC blend to poultry feed to give a concentration of 5 mg and 15 mg/kg of thymol and cinnamaldehyde, respectively. The test feeds were then stored at two different temperatures, 22 °C and 35 °C. Concentrations of thymol and cinnamaldehyde, from each of the test feeds, were analysed using gas chromatography (Tiihonen et al. 2010) at the start of the trial and after two, three and six months of storage.

The results are shown in Figure 2 where the recovery of the compounds is expressed as percentages (%) of the analysed amounts at the beginning of the study. After six months of storage both thymol and cinnamaldehyde showed an average recovery of above 93%. The recoveries of the compounds were over 100% at some time points and the fluctuations in the levels across different time points are due to the variability of the analytical method and small concentrations in the feed matrix. Furthermore, the analysed levels of thymol and cinnamaldehyde across the different time points were not significantly different ($P > 0.05$) from the starting levels. This study shows that the TC blend is stable in feed during storage even at elevated temperatures, for up to six months.

4. Insecticidal properties of thymol and cinnamaldehyde

Infestation of wheat-based feed additives in warehouses is a problem particularly in hot and humid regions. Infested samples of a coarse milled wheat-based product have been found to contain *Tribolium castaneum* (rust-red flour beetle), *T. confusum* (confused flour beetle), *Rhyzopertha*

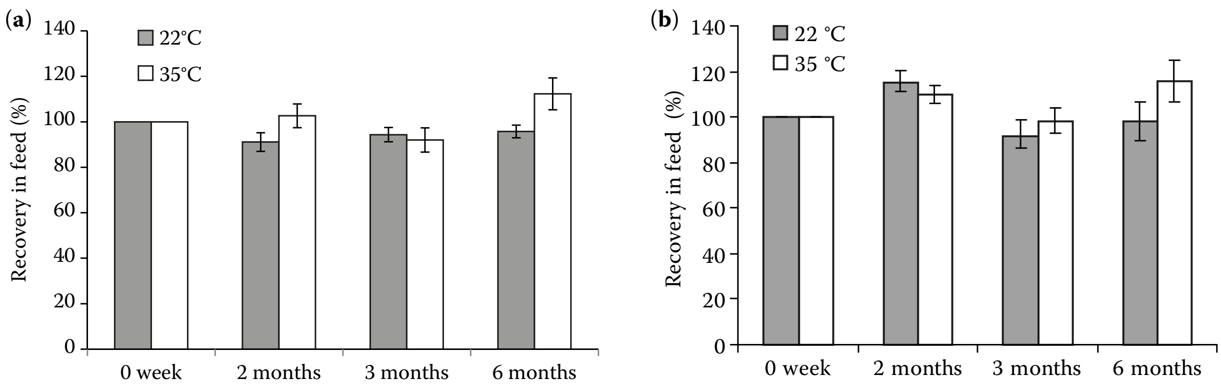

Figure 2. Recovery (%) of thymol (**a**) and cinnamaldehyde (**b**)in feed compared with the amount analysed at the beginning of the study (mean ± standard error of mean) after two, three and six months storage at two different temperatures at 22 °C ($n = 7$) and 35 °C ($n = 14$)

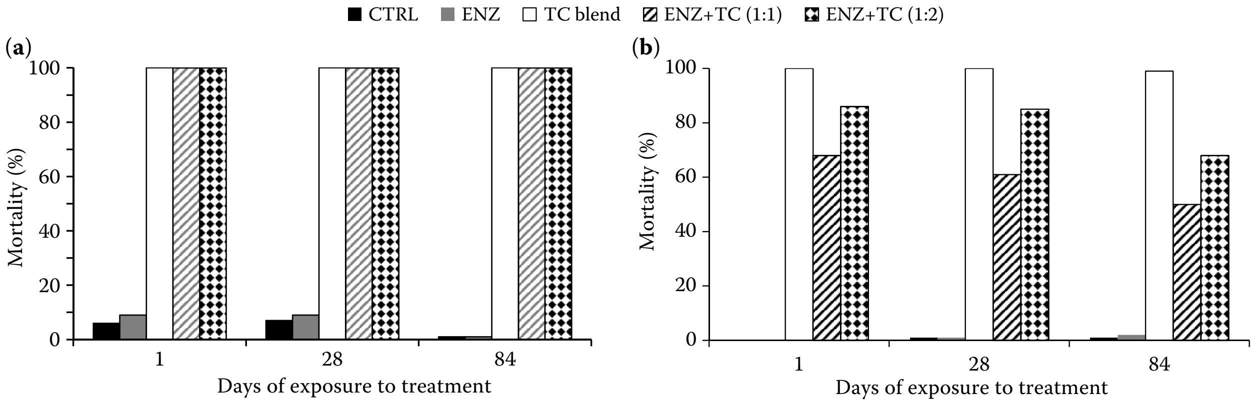

Figure 3. Mortality (%) of *Oryzaephilus surinamensis* (**a**) and *Tribolium castaneum* (**b**) adults when exposed to wheat-formulated xylanase (ENZ), a blend of thymol and cinnamaldehyde (TC), and combinations of enzyme and thymol + cinnamaldehyde (ENZ + TC: 1:1, 1:2)

dominica (lesser grain borer), *Oryzaephilus surinamensis* (saw-toothed grain beetle), and *Cryptolestes ferrugineus* (rust-red grain beetle), capable of developing and reproducing within the product. All these insect species are found in tropical and temperate areas and are pests of cereals and cereal products. The range of species found and the damage sustained by the product, suggest that preventative strategies need to be developed urgently.

A study to test the infestability of a wheat-formulated xylanase (ENZ) with two flour beetles was conducted to assess the efficacy of the TC blend in killing adult beetles and inhibiting first generation population development (F_1).

Complete mortality was recorded in adults of *O. surinamensis* exposed to the TC blend and both combinations of ENZ and TC blend (ENZ + TC: 1:1, 2:1) after 84 days exposure to treatment (Figure 3a). No development of F_1 insects took place in any of the combinations indicating that there was complete inhibition of population development. The mortality of adult *T. castaneum* was near 100% for the TC blend throughout exposure time. However, mortality of adult *T. castaneum* decreased ($P < 0.001$) with time from 86% to 68% in the 2:1 combination of ENZ + TC, and from 68% to 50% in the 1:1 combination of ENZ + TC (Figure 3b). Only one F_1 insect of *T. castaneum* was found in the 1:1 combination of ENZ + TC after 84 days exposure to treatment.

In a second study testing the repellent effects of the product combinations, with choice or no choice of alternative food, there was a lower ($P < 0.05$) proportion of *O. surinamensis* insects in the treatments containing the TC blend compared with those containing ENZ after 84 days exposure (results not shown). The proportion of insects from *T. castaneum* was lower ($P < 0.05$) in the TC-treatments compared with the ENZ-treatments at any time point (results not shown).

These results indicate that the TC blend, when added to a wheat-formulated xylanase, has adulticide activity, inhibits the proliferation and actively prevents infestability due to its repellent effect against the two flour beetles tested.

Our findings are supported by literature which suggests that when EO compounds are added to mixtures of grains they can exert insecticidal and repellent effect against flour beetles. *Origanum acutidens* oils (carvacrol, thymol and *p*-cymene), when added to grain mixtures, have shown insecticidal effects against *Sitophilus granaries* and *T. confusum,* causing 68% and 37% mortality, respectively, in adult insects (Kordali et al. 2008).

Another study testing the toxicity of cinnamaldehyde and methylchavicol against the infestation of *O. surinamensis*, *S. granaries*, *S. oryzae*, and *Callosobruchus chinensis* in grain mixtures showed high efficacy of these oils as contact insecticides and repellents (Ojimelukwe and Adler 2001).

5. Modulation of gut microbiota and their fermentation metabolites by thymol and cinnamaldehyde

As demonstrated by the *in vitro* research reviewed in section 2 of this paper, the TC blend has antibacterial properties by inhibiting the growth of some bacterial species that could be potentially harmful in animal production. The results obtained *in vitro* were confirmed *in vivo* where similar benefits on gut microbiota can also be achieved in broilers and piglets. Our *in vivo* research has shown a synergy between the single activities of thymol and cinnamaldehyde in promoting a healthy gut microflora and inhibiting potentially harmful bacteria.

In a broiler study where birds were fed wheat-based diets, the TC blend was shown to inhibit the growth of *Salmonella* and *E. coli* in caecal samples, without affecting the growth of beneficial bacteria (Tiihonen et al. 2010). The beneficial changes in microbiota were manifested by an increase in the *Lactobacillus* to *E. coli* ratio and a greater relative proportion of bifidobacteria and propionibacteria. The beneficial shifts in microbiota were associated with improved feed intake which resulted in better performance. Moreover, profiling of the composition of the total microbiota demonstrated that the effects of the TC blend on specific bacteria are large enough to cause a beneficial change in the overall bacterial profile. Three other broiler studies have shown that supplementation of feed with the TC blend reduced *C. perfringens* in the caecum of 41 day old broilers (Figure 4) on average by approximately one $\log_{10}$ (about 90% reduction compared to the control; $P = 0.16$). The results described above with the blend of thymol and cinnamaldehyde are in agreement with that of Jamroz et al. (2005) where a positive impact on gut microbiota and growth performance was seen with capsaicin, carvacrol and cinnamaldehyde.

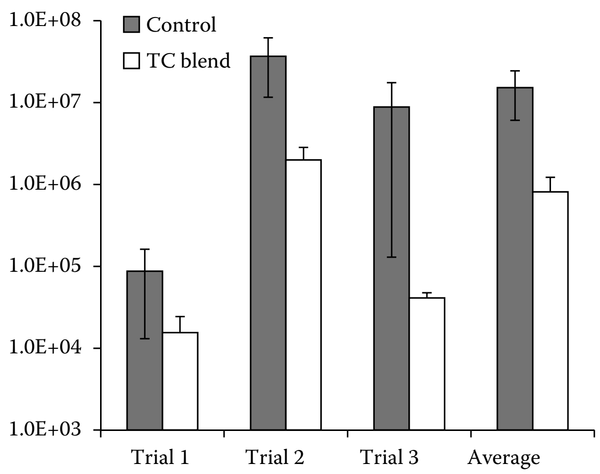

Figure 4. Reduction (log10 ccells/g digesta) of *Clostridium perfringens* in caecal digesta by the TC blend (thymol and cinnamaldehyde) compared with a control diet in three broiler studies (mean ± standard error of mean)

Recent work from Li et al. (2012) with weaner pigs suggests that diets treated with increasing doses of the TC blend have similar effects on the gut microbiota of piglets as that described above for broilers. In their work they reported that in faecal samples of TC-treated piglets, counts were lower for *E. coli* and greater for lactobacilli when compared with untreated piglets. Moreover, there was a considerable reduction in the incidence of diarrhoea in the first week post-weaning in TC-treated piglets.

The composition of the bacterial community and the metabolites produced by the gut microbiota has an effect on health and the subsequent nutritional status of the host. Activity of the intestinal microbiota can be indicated by their fermentation products, such as the short chain fatty acid (SCFA), branched chain fatty acid (BCFA) and biogenic amine concentrations. These bacterial fermentation products are derived either from carbohydrates (i.e. SCFAs) or proteins (i.e. BCFAs, biogenic amines) reflecting digestion and fermentation processes in the gastrointestinal tract. SCFAs provide a valuable source of additional energy for the host from undigestible fibres.

In our research with broilers (Cao et al. 2010; Tiihonen et al. 2010), the TC blend increased the proportion of SCFA butyrate in the caecum which is known to provide energy to colonic mucosa (Roediger 1980) and thus has potentially important implications for intestinal immunity (for a review see (Scheppach et al. 2001; Hamer et al. 2008)). In our earlier work with blends of thymol and cinnamaldehyde (Kettunen et al. 2006), we were able to demonstrate that the intestinal immunocompetence in young broiler chicks was improved; this was manifested by an increase in the immunoglobulin A of the caecum and ileum of chicks. In more recent work with young pigs, Li et al. (2012) showed that the TC blend improved the immune status of the piglets after weaning as shown by increased levels of immunoglobulins and blood immune metabolites. In the broiler study by Cao et al. (2010), birds on the control diet were affected by relatively high mortality and post-mortem examination revealed that the main disease affecting the birds was pullorum which is caused by *Salmonella*. In this study, mortality was greatly reduced in TC-treated birds in the first 21 days, which suggests that the TC blend exerted antibacterial activity against the harmful bacteria causing the disease. Moreover, the TC blend increased the proportion of acetate and butyrate and decreased the proportion of propionate indicating a change in the metabolism of the intestinal microbiota (Cao et al. 2010). Tiihonen et al. (2010) showed that the TC blend decreased the proportion of acetic acid, isovaleric acid (BCFA) and tyramine but increased caecal spermine proportions. The physiological roles of BCFA and biogenic amines in the gastrointestinal tract are not fully understood. However, Sousadias and Smith (1995) reported increased weight gain and feed efficiency when low concentrations of biogenic amine spermine were fed to broilers.

Salmonellosis is the second most frequently reported human zoonotic disease in the EU (EFSA 2011). It is therefore important for the poultry industry to reduce the incidence of *Salmonella*-contaminated broiler carcasses. In a broiler study where birds were challenged with *Salmonella enterica* serovar Heidelberg, Amerah et al. (2012) showed that supplementation of wheat-based diets with the TC blend reduced the incidence of horizontal transmission of *Salmonella* infection between birds and improved growth performance compared with the control birds. The TC blend not only reduced the percentage of *Salmonella*-positive caecal samples of 42 day old birds by 77%, but also elicited a reduction in the number of *Salmonella*-positive drag swab samples from the litter. These results indicate that the TC blend could be part of a *Salmonella* control programme, which can potentially reduce the incidence of *Salmonella* in broiler carcasses and in the broiler house with a positive impact on food safety.

6. Effects of thymol and cinnamaldehyde on growth performance, welfare and carcass characteristics

As EOs are also flavours, they are expected to stimulate appetite, which is particularly crucial for young animals to thrive. There are several hypotheses for the mechanisms that are involved in the stimulation of appetite, namely the aroma typical of EOs, improvement of feed digestibility, and increased fermentation of undigested fibrous feed particles in the most distal part of the gut due to a shift in the profile of gut microbiota.

Our research has shown that the inclusion of the TC blend increased broiler body weight and reduced mortality over a period of 42 days in broilers fed wheat-based diets (Tiihonen et al. 2010). The improvement on broiler performance in this study was attributed to an increased feed intake and a beneficial shift in the gut microbiota (see section 5) of TC-treated birds. Similar results in weaner pigs were recently reported by Li et al. (2012), which showed that the TC blend increased feed intake and body weight gain. Moreover, the addition of TC blend in the feed increased Lactobacillus counts and reduced E. coli counts in faeces. Also, the inclusion of TC blend in piglet feed increased lymphocyte transformation (SI) and phagocytosis rates and resulted in an increase in the levels of immunoglobulins (IgA, IgM) and complement components (C3 and C4) in serum.

In the work of Tiihonen et al. (2010) a positive correlation was found between the number of caecal bifidobacteria and amounts of ileal spermine and spermidine at 20 days with growth performance at 42 days. In the same study, the concentration of total biogenic amines in the ileum at 20 days was associated with increased mortality at 42 days.

Where our studies showed the importance of the gut microbiota and their fermentation profiles on the immune status and growth performance of the host (Tiihonen et al. 2010; Li et al. 2012), other authors have suggested that EOs improve growth performance because they stimulate the secretion of digestive enzymes leading to improved nutrient digestion, rate of gut passage or feed intake (Salam et al. 2002; Lee et al. 2003, 2004a,b; Jamroz et al. 2005).

Improved nutrient digestibility after administration of the TC blend has been observed in several of our studies when enzymes were absent or present in the diet. Positive effects of the TC blend were observed on nitrogen ileal digestibility and AME in two studies where broilers were fed corn-based diets (Cao et al. 2010) or wheat-based diets (Amerah et al. 2011) both containing supplemental enzymes. The effects of EOs on nutrient digestion can be associated with the stimulation of digestive enzyme secretion (Lee et al. 2003) and/or antibacterial effects that can lead to better intestinal immunocompetence.

The beneficial impact of EOs in the modulation of gut microbiota is interlinked with health and immunity, and as a consequence will affect growth performance and welfare.

A reduction in bird mortality in response to the TC blend was observed in the study of Cao et al. (2010), where the addition of TC blend to the basal diet decreased mortality by over 6% unit from week

1 to week 3. However, no effect on mortality was noticed over week 4 to 6.

Also, in the study by Tiihonen et al. (2010), the mortality over the study period of six weeks was reduced from 9.7% in the control group to 5.0% in the TC group.

In addition, improved nitrogen digestibility elicited by EOs (Cao et al. 2010; Amerah et al. 2011) can also impact gut microbiota and litter quality, and thereby affect foot pad and gait scores (Leterrier et al. 2008; Sirri and Meluzzi 2012).

Little is known about the impact of EOs on the carcass characteristics and its sensory quality. A broiler study tested the feeding of EO and organic acids in broilers and found no effect on carcass characteristics such as dressing percentage and breast percentage (Zhang et al. 2005). These findings are in agreement with our research with the TC blend. The supplementation of broiler diets with the TC blend showed increased broiler performance compared with control diets ($P < 0.05$), with no effect on dressing percentage, breast meat yield, or sensory quality of the breast meat (Schulze et al. 2008). In contrast, research with rosemary oil in broiler diets by (Yesilbag et al. 2011) has found that performance and carcass yield was improved with treatment, while sensory quality scores and acceptability of the meat by panellists was lowest in the rosemary oil treatments. These contradictory findings on carcass characteristics and sensory quality may be explained by differences in the chemical structure and properties of EOs.

7. Conclusions

The blend of thymol and cinnamaldehyde is proven to have selective antibacterial properties and inhibit the growth of yeast and fungi. The blend is insecticidal and repellent to flour beetles, with the additional benefits of maintaining excellent feed stability. Based on the evidence of our own *in vivo* research, supplementation of diets with thymol and cinnamaldehyde has positive impacts on gut microbiota, growth performance and welfare in monogastric animals. The TC blend showed a positive impact on food safety by lowering the incidence of the horizontal transmission of *Salmonella* infection in the farm. This blend of thymol and cinnamaldehyde is able to effectively target bacteria such as *Salmonella* and *E. coli*, while sparing other bacteria to beneficially modulate the gut microbiota. These characteristics could be a useful alternative to AGPs in animal diets. Our work encourages future research to focus on the correlation between the gut microbiota and their fermentation metabolites and the intestinal immunocompetence of the host.

8. Acknowledgments

Mrs D.A. Collins from the Central Science Laboratory, United Kingdom is greatly acknowledged for carrying out the flour beetles repellent testing. The authors thank Dr Rachel Crowe from Danisco Animal Nutrition, United Kingdom for proof reading the manuscript.

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Received: 2013–07–13
Accepted after revision: 2013–09–30

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