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In vitro characterisation of the antimicrobial activity of selected essential oil components and binary combinations against the pig gut flora

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ABSTRACT

The antimicrobial activity of selected essential oil (EO) components against the major culturable components of the pig gut flora has been characterised by means of an *in vitro* incubation model simulating the fermentation in different sections of the pig gastrointestinal tract (GIT). In a first study 7 components were screened for their antimicrobial properties. Dose–response equations were established for the 4 components with the highest potential in a second study. Binary combinations were tested as well, and the interaction effects were evaluated following the isobole method. The results of both studies indicated that carvacrol, thymol, eugenol and *trans*-cinnamaldehyde give opportunities to modulate the flora and fermentation pattern of the GIT of pigs. Eucalyptol, terpinen-4-ol and *trans*-anethole were found not to have interesting effects on the growth of the pig gut flora. The minimum concentration for carvacrol, thymol, eugenol and *trans*-cinnamaldehyde in jejunal simulations to reduce the number of total anaerobic bacteria compared to control with a probability of 99.7% was 255, 258, 223 and 56 mg/L respectively. This strong activity of *trans*-cinnamaldehyde was due to its progressively increasing effect against coliform bacteria; a dose of 104 mg/L gave a reduction of 1 $\log_{10}$ CFU/mL *vs.* 371, 400 and 565 mg/L for carvacrol, thymol and eugenol respectively. However, *trans*-cinnamaldehyde showed clearly less inhibitory

Abbreviations: GIT, gastrointestinal tract; EO, essential oil(s); SCFA, short chain fatty acids; MIC, minimum inhibitory concentration; CFU, colony forming units; MEC, minimum effect concentration.

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activity towards lactobacilli than carvacrol and thymol. Therefore, the use of *trans*-cinnamaldehyde (for example 100 mg/L) and to a lesser extent eugenol could result in a shift in the microbial ecology in favour of lactic acid producing bacteria and reducing the number of coliform bacteria. Carvacrol and thymol showed very similar and non-selective antimicrobial properties. Their effect was more pronounced in acidic media and demonstrated a rapidly increasing bactericidal effect from a certain concentration on (400–500 mg/L in jejunal simulations). The inhibition of the production of total short chain fatty acids (SCFA) in jejunal simulations by these 4 candidates was related to their effect against coliform bacteria, however they did not alter the lactic acid and ammonia concentrations. Few combinations demonstrated synergism; most mixtures showed zero interaction or antagonism. Carvacrol + thymol (ratio $\geq$1) was synergistic against total anaerobic bacteria in jejunal simulations, however this effect was rather small. In caecal simulations, carvacrol, thymol and *trans*-cinnamaldehyde were equally effective while eugenol had an effect only on coliforms. These data on the *in vitro* antimicrobial activities of EO components give support for a better control of the gastrointestinal bacterial community and the design of alternative growth promoters. Their *in vivo* potential is also discussed.

1. Introduction

For reasons of public concern and legislation (regulation EC/1831/2003 banned the use of in-feed antibiotics in the EU as from January 2006), there is a need for reliable alternatives for the in-feed antibiotics. For decades, the use of sub-therapeutic or nutritional levels of in-feed antibiotics in intensive pig production demonstrated a consistent ability to improve growth rates and feed conversion ratios (Cromwell, 2002). Their mode of action is not fully understood but there is evidence that nutritional antibiotics work in part by decreasing the overall numbers and/or specific bacterial species or their metabolic activities in the gastrointestinal tract (GIT) (see reviews by Visek, 1978; Anderson et al., 1999; Corpet, 2000; Gaskins et al., 2002; Dibner and Richards, 2005; Page, 2006). Dierick et al. (2002a) stated that growth promotion by in-feed antibiotics is related and proportional to the inhibition of the total microbial load and microbial metabolism in the stomach and the jejunum. A reduction in the general bacterial growth in small intestine and pathogen proliferation should be major targets to improve animal performance and/or health (Apajalahti and Kettunen, 2006).

Herbs, spices, plant extracts and derived products have been proposed and reviewed as alternatives for in-feed antibiotics in pig raising (Doyle, 2001; Kamel, 2001; Turner et al., 2001; Rodehutscord and Kluth, 2002; Lis-Balchin, 2003; Wenk, 2003; Lee et al., 2004; Westendarp, 2005). However, different modes of action (*e.g.* antioxidant, antimicrobial, coccidiostatic, anti-inflammatory, immunomodulating effects and enhancement of endogenous secretions) are described depending on the product considered. Here, research has been carried out concerning the antimicrobial activities of some specific plant-derived pure chemicals. A large quantity of chemicals of herbal origin are well known for their antimicrobial properties (for review see Cowan, 1999; Hulin et al., 1998; Nychas and Skandamis, 2003; Burt, 2004) with the essential oils (EO) and their components being a very interesting and promising group.

Most of the observations of antimicrobial properties of EO and their components found in literature are based on standardised broth dilution or disc and agar well diffusion assays. However, problems associated with the assessment of the antimicrobial activity by these diffusion methods are that the method in itself is highly dependent on water solubility and the ability of test components to diffuse through agar (Southwell et al., 1993) and on the volatility of the oils. Turbidimetry (measurements of optical density) used in broth dilution assays detects only the upper part of growth curves (>6 $\log_{10}$ CFU/mL), is influenced by the size of the bacterial cells, and requires calibration in