In vitro effects of essential oils on potential pathogens and beneficial members of the normal microbiota

A.C. Ouwehand[1], K. Tiihonen[1], H. Kettunen[1], S. Peuranen[1], H. Schulze[2], N. Rautonen[1]

[1]Danisco Finland, Kantvik, Finland
[2]Danisco Animal Nutrition, Leiden, The Netherlands

ABSTRACT: The use of antimicrobial growth promoters has been banned in the EU. This has created an interest in alternative strategies to prevent an imbalance in the intestinal microbiota and the potential development of intestinal disorders in livestock. Essential oils (EOs) have been known to exhibit antimicrobial activity against specific microbial species and could therefore be considered one such alternative in controlling the intestinal microbial population. Under anaerobic conditions, the tested *Clostridium perfringens* strains were found to be sensitive ($P < 0.05$) to carvacrol, cinnamaldehyde, citral, limonene, thymol, particularly at the higher concentration tested (500 mg/l) and to oregano oil, rosemary oil and thyme oil. *Streptococcus epidermis* was sensitive ($P < 0.05$) to most EO's tested, also mainly at the higher concentration. The tested *Salmonella* serovars were found to be sensitive ($P < 0.05$) only to high (500 mg/l) concentrations of the tested EOs. *Escherichia coli* was sensitive ($P < 0.05$) to most of the tested EOs, also at lower concentrations (5 and 50 mg/l). *Bifidobacterium longum*, *Bifidobacterium breve* and *Lactobacillus reuteri* were less sensitive ($P < 0.05$) to most of the tested EOs, while *Bifidobacterium animalis* ssp. *lactis* and *L. fermentum* were relatively sensitive also at lower concentrations (5 and 50 mg/l), although growth reduction by EOs of these bacterial species was less then with the antimicrobial growth promoter avilamycin. With the exception of *Salmonella* and *E. coli*, all tested microbes were sensitive to avilamycin. Selected EOs seem to have the advantage of inhibiting the growth of potential pathogens while only moderately influencing beneficial members of the intestinal microbiota. This difference in sensitivity may strengthen the microbiota and contribute to improved animal health.

Keywords: antimicrobial; *Bifidobacterium*; *Lactobacillus*; *Clostridium perfringens*; *Escherichia coli*; *Salmonella*

The widespread use of antibiotics in human medicine and animal husbandry is thought to contribute to the development of antibiotic resistance (Hamer and Gill, 2002; Wegener, 2003). In order to limit the spread and development of antibiotic resistance the use of antimicrobial growth promoters has been banned in the European Union since 2006. Because of this, there is considerable interest in alternatives for the control of potential pathogens in the animal gastrointestinal tract. A number of approaches have been suggested, such as probiotics (Nousiainen et al., 2004), and essential oils (EOs; Hammer et al., 1999a) as feed additives. Probiotics, as living microorganisms, pose a significant challenge to feed manufacturers in terms of their viability/survival during feed processing. EOs, on the other hand, can be handled and included in the feed similarly to other feed ingredients, although their volatility may pose a challenge.

The antimicrobial activity of EOs has long been recognised and they have been extensively tested *in vitro* against a wide range of pathogenic bacteria and fungi (Kalemba and Kunicka, 2003). Animal trials have also demonstrated the promising effects of EOs against the colonisation and proliferation of *Clostridium perfringens* (Mitsch et al., 2004; Di Pasqua et al., 2007). The mechanism by which the essential oils exert their antimicrobial activity is poorly understood but the main target appears to be the cell membrane of bacterial cells (Burt,

2004; Di Pasqua et al., 2007). Because of this, Gram-negative microbes are in general more resistant to the antimicrobial activity of EOs due to the presence of an outer membrane (Kalemba and Kunicka, 2003; Burt, 2004). Many beneficial members of the intestinal microbiota are Gram-positive, such as *Bifidobacterium* spp. and *Lactobacillus* spp. Thus, the relatively non-specific mode of action of EOs could lead to an undesirable reduction in their numbers. While some researchers have reported a relative resistance of lactobacilli against some selected EOs (Hammer et al., 1999b), this issue has not received further attention nor have the effects of the EOs on bifidobacteria been investigated.

The aim of the current study was therefore to investigate the effect EOs on the growth of selected pathogenic bacteria, bifidobacteria and lactobacilli. This would give information on the influence of these oils on beneficial members of the intestinal microbiota. It would also indicate whether a combination with probiotics might be possible. For comparison, the influence of a commercial antimicrobial growth promoter avilamycin (Butaye et al., 2003) on the studied microbes was determined.

MATERIAL AND METHODS

In the current study, 13 EOs or their natural extracts were tested (Table 1). The oils were dissolved in DMSO or ethanol to give stock solutions of 10 000 mg/l and stored in tightly closed glass bottles at –20°C until use. EOs were tested at levels of 5, 50 and 500 mg/l, with the exception of rosemary oil which was not tested at 500 mg/l as the format (powder) caused too high a turbidity. The same levels of DMSO or ethanol in culture broths were used as controls. Avilamycin was included at a level of 5 mg/l as a positive control for the comparison of EO antimicrobial activity.

As target bacterial strains, three serovars of *Salmonella enterica*, three strains of *Clostridium perfringens*, *Streptococcus epidermis*, *Lactobacillus reuteri*, *L. fermentum*, *Bifidobacterium animalis* ssp. *lactis*, *B. longum* and *B. breve* were used. All strains were maintained as stocks frozen at –80°C. The bacteria were pre-cultured at 37°C under anaerobic conditions in the media indicated in Table 2.

After pre-culturing, bacteria were inoculated at a level of 1% into fresh broth containing one EO dissolved in either DMSO or ethanol as outlined above. The bacteria were incubated anaerobically (80% N_2, 10% CO_2 and 10% H_2) at 37°C. Growth was monitored by measuring the absorbance at 600 nm every 30 min for 24 h using a Bioscreen (Growth Curves Ltd, Naantali, Finland). Results are expressed as the area under the curve (OD_{600} × min) and are the mean of three independent experiments ± standard error of mean.

Table 1. Essential oils and avilomycin tested in the current study, their origin and solvent used

Essential oil	Origin	Solvent used
Anethole	nature identical	ethanol
Benzaldehyde	nature identical	ethanol
Carvacrol	nature identical	ethanol
Cinnamaldehyde	nature identical	ethanol
Citral	nature identical	ethanol
Cresol	nature identical	ethanol
Eugenol	nature identical	ethanol
Guaiacol	nature identical	ethanol
Limonene	nature identical	ethanol
Oregano oil	natural extract	ethanol
Rosemary oil	natural extract	DMSO
Thymol	nature identical	ethanol
Thyme oil	natural extract	ethanol
Avilamycin	–	DMSO

Differences in bacterial growth were compared with a two-tailed unpaired *t*-test in comparison to the EO-free control; *P*-values below 0.05 were taken as significant.

RESULTS

Of the tested Gram positive potential pathogenic strains, *C. perfringens* strains exhibited a varying sensitivity to the tested EOs; in particular cinnamaldehyde and citral were observed to inhibit growth the most, also at 5 mg/l. Furthermore, all three natural EO extracts tested inhibited the growth of the tested *C. perfringens* strains (Table 3). Only rosemary oil exhibited a similar growth inhibitory effect as the commercial antibiotic avilamycin.

S. epidermis was sensitive to all tested EOs. However, the reduction in growth was only very limited in most cases; carvacrol, cinnamaldehyde, citral, eugenol and thymol were the EOs that af-

Table 2. Target strains used, their origin and growth medium

Genus/species	Strain	Growth medium	Origin
Salmonella enterica serovar. *infantis*	2225/95	trypticase soy broth (Becton Dickinson, France)	EVIRA[a]
Salmonella enterica serovar. *enteritidis*	749/95		EVIRA
Salmonella enterica serovar. *typhimurium*	418597		EVIRA
Escherichia coli (K88$^+$)	138		porcine diarrhoea
	0147		porcine diarrhoea
Streptococcus epidermis	37527		CCUG[d]
Clostridium perfringens	8009	Reinforced Clostrial Medium (LabM, UK)	ATCC[b]
	13124		
	3626		
Lactobacillus reuteri	23272	de Man, Rogosa, Sharpe medium (LabM, UK)	ATCC
Lactobacillus fermentum	14931		ATCC
Bifidobacterium animalis ssp. *lactis*	420	DSMZ[c] medium 58	Danisco, Niebull
Bifidobacterium longum	20219		DSMZ
Bifidobacterium breve	20213		DSMZ

[a]Finnish Food Safety Authority
[b]American Type Culture Collection
[c]German National Resource Centre for Biological Material
[d]Culture Collection University Gothenburg

fected the growth of *S. epidermis* most, as were oregano and thyme oil and in particular rosemary oil. The latter reduced *S. epidermis* growth to a level similar to avilamycin (Table 3).

All three serovars of *S. enterica* exhibited a low sensitivity to the tested EOs. Only at the highest level tested (500 mg/l) was a significant reduction in growth observed. The most effective EOs at reducing growth of *S. enterica* were carvacrol, cinnamaldehyde, citral and thymol. Of the three tested natural extracts, oregano and thyme oil significantly reduced the growth of *S. enterica*. The reduction in growth caused by 5 mg/l avilamycin was in the same range as the reduction observed for some of the EOs (Table 4).

Of the two tested *E. coli* strains, in particular *E. coli* 0147 was relatively sensitive to most of the tested EOs, even at lower concentrations of EOs. Carvacrol, cinnamaldehyde, eugenol, guaiacol and thymol were very most effective in reducing growth of *E. coli*, as were oregano, thyme and rosemary oil. Neither *E. coli* strains exhibited any significant sensitivity to avilamycin (Table 4).

Bifidobacterium breve was particularly resistant against the tested EOs, exhibiting only sensitivity to cinnamaldehyde, citral and thyme and rosemary oil. Furthermore, *B. breve* was actually stimulated by 50 mg/l anethole, benzaldehyde and eugenol. *B. longum* was sensitive to all tested EOs, but only at the highest concentration tested. *B. animalis* ssp. *lactis* was sensitive to most tested EOs. Al three tested *Bifidobacterium* strains were highly sensitive to avilamycin (Table 5).

L. fermentum was sensitive to many of the tested EOs also at lower concentrations, although the reductions in growth were relatively small. Avilamycin, however, caused a significant reduction in growth. Interestingly, carvacrol, cinnamaldehyde, thymol, limonene, oregano oil and thyme oil were found to stimulate the growth of *L. fermentum.*

In contrast, *L. reuteri* appeared to be very resistant to the tested EOs, being only sensitive to the highest concentrations of carvacrol and thymol (Table 5). Furthermore, *L. reuteri* was resistant to avilamycin.

DISCUSSION

EOs have long been known to possess antimicrobial activity (Burt, 2004) and may be responsible for the preserving action of certain herbs and spices.

Table 3. Gram positive potential pathogenic target strains. Growth is expressed as area under 24 h growth curve (OD_{600} × min), mean of three independent experiments ± standard error of mean

Target organism	*C. perfringens* 8009			*C. perfringens* 13124			*C. perfringens* 3626			*S. epidermis* 37527		
Concentration (mg/l)	5	50	500	5	50	500	5	50	500	5	50	500
Control		518 ± 58			1557 ± 7			1242 ± 33			671 ± 11	
Anethole	451 ± 3*	488 ± 6*	500 ± 4	1519 ± 4	1531 ± 2	1622 ± 12*	1091 ± 6*	1107 ± 24	997 ± 6*	589 ± 14*	279 ± 133*	458 ± 58*
Benzaldehyde	448 ± 27	479 ± 2*	521 ± 25	1517 ± 10	1520 ± 3	1592 ± 11	1082 ± 36	1126 ± 5*	1101 ± 7	508 ± 97*	411 ± 29*	421 ± 100*
Carvacrol	526 ± 136	422 ± 142	32 ± 3*	1603 ± 2*	1609 ± 32	655 ± 39*	1106 ± 14*	935 ± 64*	49 ± 1*	605 ± 39	259 ± 139*	30 ± 2*
Cinnamaldehyde	381 ± 13*	148 ± 57*	88 ± 2*	1577 ± 3	1467 ± 13*	199 ± 15*	998 ± 21*	60 ± 2*	93 ± 5*	573 ± 65	309 ± 114*	102 ± 21*
Citral	319 ± 96*	339 ± 124	27 ± 6*	1593 ± 15	1533 ± 8	127 ± 2*	974 ± 40*	322 ± 103*	24 ± 5*	408 ± 50*	418 ± 41*	25 ± 7*
Cresol	441 ± 2*	476 ± 6*	535 ± 12	1518 ± 3*	1542 ± 2	1581 ± 17	1094 ± 17*	1123 ± 2*	1266 ± 0	465 ± 31*	349 ± 17*	541 ± 20*
Eugenol	423 ± 23*	480 ± 10*	537 ± 17	1493 ± 6*	1525 ± 11	1683 ± 5	1080 ± 1*	1142 ± 23	1053 ± 27*	433 ± 38*	308 ± 67*	34 ± 0*
Guaiacol	436 ± 12*	479 ± 10*	526 ± 25	1481 ± 8*	1554 ± 12	1614 ± 25	1103 ± 20*	1181 ± 14	1296 ± 14	368 ± 76*	367 ± 77*	414 ± 38*
Limonene	586 ± 117	234 ± 54*	0 ± 7*	1580 ± 10	1607 ± 2*	1172 ± 84*	1083 ± 76	587 ± 89*	0 ± 3*	470 ± 14*	162 ± 64*	391 ± 86*
Thymol	660 ± 134	733 ± 129	48 ± 4*	1524 ± 21	1617 ± 5*	624 ± 76*	1202 ± 20	1061 ± 107*	45 ± 4*	300 ± 71*	411 ± 47*	29 ± 2*
Oregano oil	634 ± 79	202 ± 60*	13 ± 3*	1560 ± 11	1533 ± 97*	463 ± 98*	1088 ± 53	739 ± 86*	0 ± 4*	382 ± 57*	227 ± 79*	10 ± 5*
Rosemary oil	46 ± 0*	83 ± 20*	–	43 ± 2*	114 ± 10*	–	39 ± 0*	103 ± 31*	–	59 ± 20*	18 ± 2*	–
Thyme oil	389 ± 270	248 ± 125*	0 ± 5*	1580 ± 10	1611 ± 18*	1134 ± 102*	1210 ± 66	686 ± 47*	0 ± 12*	353 ± 42*	303 ± 75*	0 ± 14*
Avilamycin	43 ± 8*	–	–	120 ± 4*	–	–	46 ± 3*	–	–	224 ± 92*	–	–

*$P < 0.05$ as compared to the control

Table 4. Gram negative potential pathogenic target strains. Growth is expressed as area under 24 h growth curve (OD_{600} × min), mean of three independent experiments ± standard error of mean

Target organism	*S. infantis* 2225/95			*S. enteritidis* 749/95			*S. typhimurium* 4185/96			*E. coli* 138			*E. coli* 0147		
Concetration (mg/l)	5	50	500	5	50	500	5	50	500	5	50	500	5	50	500
Control	870 ± 57			501 ± 21			638 ± 26			923 ± 69			576 ± 14		
Anethole	685 ± 31*	785 ± 10*	735 ± 3*	439 ± 37*	470 ± 44	370 ± 6*	605 ± 17	643 ± 7	618 ± 6	928 ± 95	590 ± 0	771 ± 115	455 ± 81	483 ± 58	565 ± 166
Benzaldehyde	655 ± 38*	842 ± 13	894 ± 8	418 ± 42*	430 ± 43	586 ± 21	499 ± 24*	600 ± 17	619 ± 10	786 ± 46	722 ± 16*	731 ± 127	177 ± 127*	454 ± 18*	324 ± 18*
Carvacrol	805 ± 35	735 ± 55	0 ± 5*	479 ± 81	567 ± 52	0 ± 4*	777 ± 26*	572 ± 32	0 ± 3*	547 ± 157	138 ± 114*	26 ± 2*	304 ± 59*	386 ± 43*	18 ± 2*
Cinnamaldehyde	788 ± 85	711 ± 76	70 ± 4*	401 ± 99	424 ± 23*	48 ± 2*	732 ± 32	616 ± 42	60 ± 5*	606 ± 199	383 ± 184*	113 ± 7*	322 ± 145*	270 ± 84*	95 ± 5*
Citral	815 ± 55	817 ± 51	42 ± 10*	586 ± 23	522 ± 12	176 ± 80*	654 ± 6	732 ± 48	398 ± 20*	525 ± 116	598 ± 157	14 ± 3*	4 ± 9*	473 ± 58	16 ± 9*
Cresol	633 ± 46*	698 ± 17*	711 ± 18*	353 ± 62*	428 ± 23*	444 ± 27*	520 ± 1*	550 ± 10*	550 ± 3*	561 ± 46	604 ± 124	16 ± 3*	186 ± 85*	396 ± 108	23 ± 6*
Eugenol	604 ± 116*	647 ± 53*	432 ± 165*	326 ± 41*	465 ± 35	295 ± 20*	566 ± 16*	565 ± 3*	567 ± 14*	633 ± 203	651 ± 64*	41 ± 2*	134 ± 108*	36 ± 10*	19 ± 1*
Guaiacol	383 ± 111*	731 ± 20*	748 ± 27*	396 ± 26*	385 ± 54*	533 ± 20	490 ± 41*	577 ± 3*	584 ± 4*	603 ± 197	500 ± 28*	445 ± 92*	146 ± 83*	259 ± 109*	91 ± 44*
Limonene	726 ± 64	710 ± 96	425 ± 65*	529 ± 23	448 ± 45	208 ± 21*	663 ± 33	712 ± 59	407 ± 20*	278 ± 88	845 ± 35	621 ± 65*	253 ± 20*	316 ± 94	368 ± 32
Thymol	767 ± 25	763 ± 61	20 ± 5*	343 ± 29*	525 ± 74	13 ± 7*	672 ± 45	755 ± 42	0 ± 1*	316 ± 167*	300 ± 188*	33 ± 0*	69 ± 43*	422 ± 45*	23 ± 1*
Oregano oil	746 ± 46	692 ± 102	0 ± 5*	512 ± 81	494 ± 62	0 ± 7*	670 ± 46	717 ± 49	0 ± 11*	570 ± 137	430 ± 38*	13 ± 4*	167 ± 127*	334 ± 113	10 ± 8*
Rosemary oil	646 ± 9*	634 ± 13*	–	471 ± 51	559 ± 55	–	597 ± 13	556 ± 34*	–	351 ± 108*	631 ± 114*	–	144 ± 53*	276 ± 27*	–
Thyme oil	875 ± 39	771 ± 61	0 ± 8*	454 ± 16	499 ± 18	0 ± 5*	635 ± 11	727 ± 40	0 ± 47*	550 ± 10*	765 ± 137	3 ± 7*	113 ± 107*	284 ± 75*	0 ± 24*
Avilamycin	601 ± 161*	–	–	576 ± 9	–	–	466 ± 7*	–	–	1039 ± 8	–	–	566 ± 11	–	–

*$P < 0.05$ as compared to the control

Table 5. Lactic acid producing target strains. Growth is expressed as area under 24 h growth curve (OD_{600} × min), mean of three independent experiments ± standard error of mean

Target organism	*B. lactis* 420			*B. longum* 20219			*B. breve* 20213			*L. fermentum* 14931			*L. reuteri* 23272		
Concetration (mg/l)	5	50	500	5	50	500	5	50	500	5	50	500	5	50	500
Control	1014 ± 76			1124 ± 52			602 ± 80			696 ± 15			1329 ± 129		
Anethole	898 ± 36	747 ± 156	999 ± 38	1180 ± 15	1340 ± 192	245 ± 14*	558 ± 306	1091 ± 54*	458 ± 27	590 ± 11*	637 ± 11*	653 ± 1*	1373 ± 33	1359 ± 32	1356 ± 31
Benzaldehyde	776 ± 65*	767 ± 63*	847 ± 10*	1142 ± 11	1242 ± 178	291 ± 18*	603 ± 23	939 ± 86*	745 ± 38	576 ± 13*	623 ± 22*	647 ± 2*	1382 ± 23	1359 ± 212	1403 ± 67
Carvacrol	668 ± 68*	772 ± 47*	820 ± 105	1077 ± 52	1065 ± 12	521 ± 6*	558 ± 76	652 ± 150	888 ± 76	805 ± 23*	884 ± 13*	237 ± 9*	1267 ± 114	1525 ± 27	607 ± 100*
Cinnamaldehyde	559 ± 198*	588 ± 162*	302 ± 45*	1166 ± 15	1008 ± 6	194 ± 1*	545 ± 177	883 ± 129	233 ± 34*	831 ± 51*	964 ± 8*	124 ± 35*	1480 ± 51	1408 ± 26	1450 ± 53
Citral	755 ± 90*	627 ± 177*	207 ± 75*	1145 ± 16	1052 ± 22	138 ± 13*	782 ± 183	808 ± 248	204 ± 71*	852 ± 14*	981 ± 25*	670 ± 76	1470 ± 28	1492 ± 23	1173 ± 185
Cresol	716 ± 104*	761 ± 33*	650 ± 139*	1123 ± 60	1099 ± 28	449 ± 54*	447 ± 55	737 ± 93	587 ± 155	576 ± 24*	622 ± 4*	611 ± 9*	1358 ± 23	1381 ± 65	1373 ± 67
Eugenol	501 ± 198*	742 ± 71*	313 ± 170*	1135 ± 15	1122 ± 9	254 ± 62*	1061 ± 392	976 ± 114*	428 ± 12	577 ± 37*	617 ± 0*	597 ± 4*	1371 ± 29	1376 ± 28	1318 ± 140
Guaiacol	700 ± 148*	547 ± 195*	836 ± 30	1175 ± 27	1137 ± 28	516 ± 53*	617 ± 263	1007 ± 129*	738 ± 302	587 ± 17*	610 ± 10*	630 ± 13*	1361 ± 8	1363 ± 49	1358 ± 152
Limonene	716 ± 129*	493 ± 201*	479 ± 60*	1045 ± 59	1094 ± 25	236 ± 73*	370 ± 22*	502 ± 11	294 ± 114	797 ± 9*	881 ± 24*	764 ± 68	1449 ± 34	1476 ± 43	1150 ± 71
Thymol	756 ± 35*	856 ± 47	1340 ± 73*	1209 ± 27	1128 ± 9	685 ± 109*	534 ± 191	875 ± 277	968 ± 62*	792 ± 28*	895 ± 18*	317 ± 23*	1375 ± 138	1495 ± 11	349 ± 3*
Oregano oil	710 ± 48*	456 ± 58*	827 ± 61	1157 ± 15	1118 ± 35	219 ± 11*	855 ± 140	719 ± 162	596 ± 71	799 ± 54*	832 ± 4*	174 ± 23*	1475 ± 19	1500 ± 13	866 ± 189
Rosemary oil	668 ± 84*	644 ± 35*	–	1084 ± 42	631 ± 121*	–	578 ± 177	267 ± 14*	–	495 ± 25*	360 ± 4*	–	1248 ± 48	978 ± 175	–
Thyme oil	694 ± 21*	597 ± 152*	422 ± 78*	1089 ± 66	1106 ± 35	103 ± 23*	332 ± 41*	297 ± 60*	234 ± 19*	766 ± 27*	816 ± 34*	264 ± 72*	1411 ± 44	1376 ± 130	191 ± 63*
Avilamycin	381 ± 4*	–	–	298 ± 6*	–	–	243 ± 3*	–	–	41 ± 9*	–	–	1099 ± 8	–	–

*$P < 0.05$ as compared to the control

The feeding of EOs, however, has not always been observed to influence the intestinal microbiota *in vivo* (Cross et al., 2007). We hypothesised that the used EOs may not always have been selected on the basis of their specific antimicrobial activity, and took into account not only the sensitivity of potential pathogenic members of the intestinal microbiota, but also its beneficial members. Therefore, 11 nature identical EOs and three natural extracts were tested *in vitro* for their antimicrobial potential. All tests were performed anaerobically to mimic the conditions in the intestine. For comparison, the now prohibited antimicrobial growth promoter avilamycin was included.

The tested Gram positive potential pathogenic strains were found to be more sensitive to the tested EO's than to the tested Gram negative strains. This is in agreement with earlier observations (Burt, 2004). Although *Salmonella* and *E. coli* are genetically closely related, they appeared to differ markedly in their sensitivity to the tested EOs. In particular, the higher sensitivity of *E. coli* 0147 to EOs was unexpected.

The nature identical EOs which appear to have the highest growth reducing capacity were carvacrol, cinnamaldehyde and thymol. Citral, eugenol and limonene were also active but less so compared to the above three. All three tested natural extracts exhibited a strong antimicrobial activity. This may, in part, be explained by the fact that thymol is one of the main components of oregano and thyme oil (Chorianopoulos et al., 2004). Although the reductions in growth were in general statistically significant, they were small and the biological relevance of this reduction remains therefore to be established.

Likewise, the tested Gram negative pathogens, *Salmonella* and *E. coli*, were not particularly sensitive to avilamycin. Avilamycin is known to be active mainly against Gram positive microbes (Butaye et al., 2003) and this is therefore in agreement with the current findings.

The most interesting finding, however, is the relative resistance of the tested beneficial members of the intestinal microbiota, lactobacilli and bifidobacteria, to EOs while members of these genera were highly sensitive to avilamycin. Although avilamycin has been reported to be active particularly against Gram-positive microbes (Butaye et al., 2003), its activity against lactobacilli and bifidobacteria has not been reported before. *B. longum* and *B. breve* have earlier been observed to be relatively resistant to thymol, eugenol and carvacrol at 300 mg/l (Si et al., 2006). *L. plantarum* has been found to be resistant to thymol, eugenol and carvacrol at 300 mg/l under aerobic conditions. *L. acidophilus*, however, was highly sensitive to thymol and carvacrol under aerobic conditions at 300 mg/l (Si et al., 2006). The present study confirms these observations and expands them to anaerobic conditions as found in the intestine.

The sensitivity of some of the tested Gram positive potential pathogens combined with the relative resistance of the tested bifidobacteria and lactobacilli to EOs under anaerobic conditions may be an advantage for EOs, inhibiting the growth of potential pathogens while sparing the beneficial members of the intestinal microbiota. This would provide the intestinal microbiota with an opportunity to strengthen one of its main functions, colonisation resistance against incoming pathogens (Adlerberth et al., 2000). Thereby the natural resistance of the animal would be increased. It would therefore be worth testing the most promising EOs, such as thymol and cinnamaldehyde, or natural extracts such as rosemary oil alone or in combination under *in vivo* conditions to determine whether they would beneficially influence or maintain the intestinal microbiota and improve animal health and performance.

Acknowledgements

Mrs. Brita Mäki and Mrs. Kirsi Stenström are gratefully acknowledged for skilled technical assistance. The study was supported by Danisco. At the time of the study, all authors were employed by Danisco.

REFERENCES

Adlerberth I, Cerquetti M, Poilane I, Wold A, Collignon A (2000): Mechanisms of colonisation and colonisation resistance of the digestive tract. Microbial Ecology in Health and Disease, 11, 223–239.

Burt S (2004): Essential oils: their antibacterial properties and potential applications in foods – a review. International Journal of Food Microbiology, 94, 223–253.

Butaye P, Devriese LA, Haesebrouck F (2003): Antimicrobial growth promoters used in animal feed: effects of less well known antibiotics on gram-positive bacteria. Clinical Microbiology Reviews, 16, 175–188.

Chorianopoulos N, Kalpoutzakis E, Aligiannis N, Mitaku S, Nychas G-J, Haroutounian SA (2004): Essential oils of *Satureja, Origanum* and *Thymus* species: chemical composition and antibacterial activities against foodborne pathogens. Journal of Agricultural and Food Chemistry, 52, 8261–8267.

Cross DE, McDevitt RM, Hillman K, Acamovic T (2007): The effect of herbs and their associated essential oils on performance, dietary digestibility and gut microflora in chickens from 7 to 28 days of age. British Poultry Science, 48, 496–506.

Di Pasqua R, Betts G, Hoskins N, Edwards M, Ercolini D, Mauriello G (2007): Membrane toxicity of antimicrobial compounds from essential oils. Journal of Agricultural and Food Chemistry, 55, 4863–4870.

Hamer DH, Gill CJ (2002): From the farm to the kitchen table: The negative impact of antimicrobial use in animals on humans. Nutrition Reviews, 60, 261–264.

Hammer KA, Carson CF, Riley TV (1999a): Antimicrobial activity of essential oils and other plant extracts. Journal of Applied Microbiology, 86, 985–990.

Hammer KA, Carson CF, Riley TV (1999b): In vitro susceptibilities of lactobacilli and organisms associated with bacterial vaginosis to Melaleuca alternifolia (tea tree) oil. Antimicrobial Agents and Chemotherapy, 43, 196.

Kalemba D, Kunicka A (2003): Antibacterial and antifungal properties of essential oils. Current Medicinal Chemistry, 10, 813–829.

Mitsch P, Zittlerl-Eglseer K, Koehler B, Gabler C, Losa R, Zimernik I (2004): The effect of two different blends of essential oil components on the proliferation of *Clostridium perfringens* in the intestines of broiler chickens. Poultry Science, 83, 669–675.

Nousiainen J, Javanainen P, Setala J, von Wright A (2004): Lactic acid bacteria as animal probiotics. In: Salminen S, von Wright A, Ouwehand A (eds.): Lactic Acid Bacteria: Microbiological and Functional Aspects. 3rd ed. Marcel Dekker Inc., New York. 547–580.

Si W, Gong J, Tsao R, Zhou T, Yu H, Poppe C, Johnson R, Du Z (2006): Antimicrobial activity of essential oils and structurally related synthetic food additives towards selected pathogenic and beneficial gut bacteria. Journal of Applied Microbiology, 100, 296–305.

Wegener HC (2003): Ending the use of antimicrobial growth promoters is making a difference. ASM News, 69, 443–448.

Received: 2009–10–15
Accepted after corrections: 2010–01–23

Corresponding Author:

Arthur C. Ouwehand, Danisco Finland, Health & Nutrition, Sokeritehtaantie 20, 02460 Kantvik, Finland
Tel. +358 40 5956353, Fax +358 9 2982203, e-mail: arthur.ouwehand@danisco.com