THE EFFECT OF AN ORGANIC ACID BLEND, CINNAMALDEHYDE AND A PERMEABILISING SUBSTANCE ON THE INHIBITION OF BACTERIAL GROWTH *IN VITRO* AND GROWTH PERFORMANCE OF WEANING PIGS

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Abstract. The effect of an organic acid blend (**AB**; formic, propionic and acetic acid), cinnamaldehyde (**CA**) and a permeabilising substance (**PS**) was tested *in vitro* on the inhibition of 4 strains of pathogenic bacteria and *in vivo* on growth performance of weaning pigs. The strains for the *in vitro* trial were cultivated and incubated over night at 37°C. The main culture incubated for 4 hours before being used for a microplate assay and the inhibitory effect of the AB and its combination with CA and a PS was calculated. For the *in vivo* trial, 60 pigs weaned at 28 days, weighing 8.72 kg (s.d. +/- 1.15 kg) were used in a 56 day experiment. Pigs were assigned to two different treatments: a standard diet or the standard diet supplemented with the AB, CA and the PS. Synergistic effects on the inhibition of *Salmonella* Enteritidis, *Salmonella* Typhimurium, *E. coli* O55:K59 (B5):H and *E. coli* O128:H2 were found when adding CA to the AB. The AB alone inhibited the growth of *Salmonella* Enteritidis, *Salmonella* Typhimurium, *E. coli* O55:K59 (B5):H and *E. coli* O128:H2 by 53.5, 59.3, 55.2 and 33.3%, respectively, while the addition of CA to the AB resulted in an inhibition of 99.0, 99.8 and 100.0% of *Salmonella* Enteritidis, *Salmonella* Typhimurium and the two *E. coli* strains. Synergistic effects on the inhibition of the four test strains were also found when adding the PS to the AB and the CA. After adjusting the growth medium, the AB combined with the CA inhibited growth of *Salmonella* Enteritidis, *Salmonella* Typhimurium, *E. coli* O55:K59 (B5):H and *E. coli* O128:H2 only by 6.9, 3.9, 29.5 and 2.3%, respectively, whilst the addition of the PS resulted in an inhibition of 86.2, 100.0, 70.5 and 100.0%. In the *in vivo* trial, the group fed the diet containing the AB, CA and the PS had only a slightly higher average daily feed intake (**ADFI**) compared to the control group (1028 vs. 982g; P>0.05), while the average daily weight gain (**ADG**) was significantly (P<0.05) higher in the experimental group (517 vs. 481g) resulting in an improved feed conversion ratio (**FCR**; 1.99 vs. 2.04) and a significantly improved final body weight (37.7 vs. 35.6 kg) of pigs fed the experimental diet. Results of the two experiments indicate that the addition of CA and PS to AB inhibits bacterial growth more effectively and significantly improves growth performance of weaning pigs.

Keywords: organic acids, cinnamaldehyde, permeabilising substance, pathogenic bacteria, weaning pigs.

ORGANINIŲ RŪGŠČIŲ MIŠINIO, CINAMONO ALDEHIDO IR PRALAIDŽIOS MEDŽIAGOS POVEIKIS BAKTERIJŲ AUGIMO *IN VITRO* SLOPINIMUI IR ATJUNKYTŲ PARŠELIŲ AUGIMO INTENSYVUMUI

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Santrauka. Organinių rūgščių mišinio (AB; skruzdžių, propiono ir acto rūgštis), cinamono aldehido (CA) ir medžiagos (PS), didinančios gramneigiamos bakterijos išorinės membranos pralaidumą, poveikis buvo analizuojamas *in vitro* slopinant keturių bakterijų štamų augimą ir *in vivo* stebint atjunkytų paršelių augimo intensyvumą. *In vitro* analizei parinkti štamai per naktį buvo auginami ir inkubuojami 37°C temperatūroje. Prieš analizuojant bandinį mikroplokštelėje, pagrindinė kultūra buvo 4 val. inkubuojama. Apskaičiuotas AB ir jos derinio su CA ir PS slopinantis poveikis. *In vivo* bandymas truko 56 dienas. Buvo panaudoti šešiasdešimt 28 dienų atjunkyti paršeliai, kurie svėrė 8,72 kg (s. d. +/- 1,15 kg). Paršeliams buvo taikomos dviejų rūšių dietos – standartinė bei standartinė, papildyta AB, CA ir PS. Taikant AB kartu su CA, pastebėtas sinergetinis slopinamasis poveikis bakterijoms *Salmonella enteritidis*, *Salmonella typhimurium*, *E. coli* O55:K59 (B5):H ir *E. coli* O128:H2. Taikant vien tik AB, buvo slopinamas *Salmonella typhimurium*, *E. coli* O55:K59 (B5):H ir *E. coli* O128:H2 augimas atitinkamai 53,5; 59,3; 55,2 ir 33,3 proc. Tuo tarpu panaudojus CA kartu su AB, *Salmonella enteritidis*, *Salmonella typhimurium* ir dviejų *E. coli* štamų augimas buvo prislopintas 99,0; 99,8 ir 100,0 proc. Sinergetinis poveikis slopinant keturių tirtų štamų augimą taip pat pastebėtas taikant PS kartu su AB ir CA. Pakoregavus augimo terpę, AB kartu su CA nuslopino *Salmonella enteritidis*, *Salmonella typhimurium*, *E. coli* O55:K59 (B5):H ir *E. coli* O128:H2 augimą atitinkamai 6,9; 3,9; 29,5 ir 2,3 proc., o pridėjus PS, slopinimas siekė 86,2; 100,0; 70,5 ir 100,0 proc. Bandymo *in vivo* metu paršeliai, kuriems buvo taikoma AB, CA ir PS dieta, vidutiniškai pašarų per dieną suėdė (ADFI) tik šiek tiek daugiau, nei kontrolinės grupės (1028 vs. 982 g; p>0,05). Tuo tarpu eksperimentinių paršelių vidutinis dienos prieaugis (ADG) buvo ženkliai (p<0,05) didesnis (517 vs. 481g),

dėl to ir galutinis svoris buvo didesnis (37,7 vs. 35,6 kg). Vadinasi, bandomosios grupės paršeliai maisto medžiagas (FCR; 1,99 vs. 2,04) pasisavino geriau. Abiejų bandymų rezultatai rodo, kad CA ir PS priedai su AB efektyviau slopina bakterijų augimą ir skatina atjunkytų paršelių augimo intensyvumą.

Raktažodžiai: organinės rūgštys, cinamaldehidas, pralaidi medžiaga, patogeninės bakterijos, atjunkyti paršeliai.

Introduction. Antibiotic growth promoters (**AGPs**) have been used in animal production for many decades. It was indicated already around 1950 that AGPs improve production efficiency (Moore *et al.*, 1946, Jukes *et al.*, 1950). However, not much later first reports of resistance in food animals were published (Starr and Reynolds, 1951; Barnes, 1958; Elliott and Barnes, 1959) and first concerns raised about the development of antibiotic resistance in human pathogens (Swann, 1969). Nowadays, the transfer of antibiotic resistant genes from animals to humans is evident (Greko, 2001). This led to the ban of antibiotic growth promoters in animal production within the European Union in 2006. However, it is necessary to fight the bacterial challenge as the presence of bacteria comes at a cost. They compete with the host for nutrients, may secrete toxic compounds and initiate immune, inflammatory responses, respectively in the gastro-intestinal tract (Dibner and Richards, 2005). Not only the competition for nutrients and the loss of net energy to the gut microbiota leads to reduction in growth performance (Dibner and Richards, 2005), but also the alteration of appetite and metabolism during infections caused by pathogenic bacteria results in loss of body weight and reduced growth rate (Balaji *et al.*, 1988). As organic acids show antimicrobial effects, they were put into the centre of attention in order to replace AGPs (Vondruskova *et al.*, 2010). The antimicrobial mode of action is two-fold: First, the use of organic acids leads to the reduction in pH inhibiting the growth of pathogenic microorganisms. Second, organic acids in their non-dissociated form can penetrate through the bacterial cell wall and destroy some vital cell functions (Hansen *et al.*, 2007). Phytochemicals are non-nutritive plant chemicals that have protective and disease preventive properties. Phytochemicals protect the plant itself but are also known to have a disease preventive action in humans (Kumar *et al.*, 2009). Therefore it might be hypothesised, that a disease preventive action might also be seen in animal species. Cinnamaldehyde (**CA**) is characterised as being a phytochemical (Michiels *et al.*, 2007) and was shown to have antimicrobial effects, as it targets the FtsZ protein, which plays an important role in the cell division of pathogenic bacteria. The CA binds to the FtsZ, inhibits its assembly and perturbs the formation of the Z-ring thus inhibiting the process of cell division (Domadia *et al.*, 2007). Therefore, it is not surprising that research found a strong antibacterial effect of cinnamaldehyde on bacteria at low inclusion levels (Michiels *et al.*, 2007). However, even though natural replacements for AGPs are known, fighting Gram-negative bacteria is still a challenge. Gram-negative bacteria possess an additional outer membrane, a barrier, which prevents toxic compounds entering the cell and destroying vital functions (Cánovas *et al.*, 2005). This outer membrane can be disturbed by permeabilising substances (**PS**; Vaara, 1992), which make the bacterial cell more susceptible to toxic compounds (Alakomi, 2007). The hypothesis of the current studies was that (1) if different antimicrobial substances are combined synergistic effects on the inhibition of different bacteria might be found. Furthermore, (2) as PS are able to weaken the outer membrane of Gram-negative bacteria making them more susceptible to hydrophobic substances which are capable of destroying vital cellular functions, the effects of a combination of organic acids and CA might be enhanced by the addition of a PS as they most probably can penetrate the bacterial cell more easily. If (3) the effects on the inhibition of bacteria by a combination of organic acids, CA and a PS can be found *in vitro*, this may also be seen *in vivo* resulting in improved growth performance as bacterial load within the gastro-intestinal tract is decreased and the host does not have to compete with the bacteria for nutrients at such a high extent and less immune responses due to the presence of pathogenic bacteria, which negatively impact growth performance, are required.

Material and methods. *In vitro experiment*

The effects of an acid blend (**AB**; formic, propionic and acetic acid), CA and PS (Biotronic® Top3, Biomin Holding GmbH, Austria) on the growth inhibition of pathogenic bacteria was tested *in vitro* via a microplate assay. The tested pathogens were *Salmonella* Enteritidis (DSM 9898), *Salmonella* Typhimurium (DSM 554), *E. coli* O55:K59(B5):H (DSM 4779) and *E. coli* O128:H2 (DSM 8703). The medium Trypticase Soy Broth (**TSB**, Oxoid) was prepared following the manufacturer's instructions. Afterwards, the medium was autoclaved at 121 °C for 15 minutes to ensure sterility. For both experiments, each strain was cultivated from a cryo culture using 100 µl defrosted bacterial suspension and 10 ml of the medium and incubated over night at 37 °C. The next day, the main culture was created using 100 µl of the overnight culture and 10 ml medium. The main culture was again incubated for 4 hours without gassing to ensure complete viability. The optical density (**OD**) of the main cultures was measured at 690 nm using a photometer with a tungsten lamp. When blank, the medium was used. For the microplate assay, the main cultures were diluted until they showed an OD of 0.1 ± 0.015, and afterwards applied on the microplates. The two *Salmonella* cultures were diluted 1:10 and the two *E. coli* cultures 1:15, respectively. The first experiment with the AB and CA was carried out in order to test if the addition of CA to the AB can enhance the growth inhibition effect of organic acids on selected bacteria. The AB was diluted with medium to a concentration of 0.405%, representing the working solution. The CA was diluted with 50% ethanol to a concentration of 6%, representing the stock solution, and further diluted with medium to 0.0357% in the

working solution. All wells in the microplates were filled with 300 µl of the respective solutions according to the plate layout, using 100 µl accordingly diluted bacterial suspension of the main culture, except for the medium control. 100 µl of one (then, 100 µl medium were added to reach 300 ml final volume) or two of the test solutions were added, diluting the AB to a final acid concentration of 0.135% in the medium and CA to 0.0125%. In the second experiment, it was tested if the addition of the PS can further enhance the growth inhibition effect of the AB combined with CA. As in the previous experiment, in some cases already a 100% inhibition of the bacteria was reached. Another antimicrobial mixture containing less acid and only small amounts of CA (0.2% of the mixture) was used for the second experiment. The PS was added at a concentration of 8% of the mixture of organic acids and CA. Both mixtures were diluted with medium to create the working solution with an acid concentration of 0.3%, 0.0017% CA and 0.069% PS. The wells of the microplates were filled up to 200 µl, using 100 µl accordingly diluted bacterial suspension of the main culture except for the medium control. The blend of organic acids plus CA alone and in combination with the PS was tested by mixing 100 µl of the working solution with the bacterial suspension in the microplate wells, resulting in final concentrations of 0.15% AB, 0.0009% CA and 0.034% PS in the medium. In both experiments, the microplates were incubated at 37 °C without gassing. The OD of the microplates was measured both before and after 24 hours of incubation at a wavelength of 620 nm with a plate reader. The change of the optical density in the test wells was compared to the change in the wells containing the growth control. As antibiotic positive control, ampicillin was used for all strains. Every microplate assay was carried out three times with four wells for each test substance, providing twelve sources of data per strain and test. The numbers given in the results are the averaged values of these twelve data for each strain, listed with their respective standard deviation.

Feeding trial

The study followed proper ethical standards according to the Austrian Agency for Health and Food Safety. The experiment was conducted to investigate the effects of an AB, CA and a PS on growth performance of weaning pigs. Pigs were crossbred pigs [Landrace x Large White dams mated to Pietrain boars] and weaned with 8.72 ± 1.15 kg live weight at 28 ± 2 days of age. In total, 60 pigs were used and divided into 6 groups (mixed sex) of 10 piglets per group and kept for 56 days. Pigs were randomly assigned to two treatments with three replicates pens in each on the basis of body weight. Pigs were housed in partially slatted and concrete floor pens sized 230 x 150 m (floor space of 0.35 m^2 per pig), provided with cup drinkers to allow *ad libitum* access to water and a computer-operated feeding system (Spotmix, Schauer, Austria). Pigs were fed once daily at 0900 h. Room temperature was maintained at 29.0°C during the first week, at 27.5 °C during the second week and lowered by 2 °C during the following weeks down to 22.0 °C. Two diets were fed in sequence during the experiment, with diet 1 fed from weaning to day 14 (to about 10 kg live weight) and diet 2 fed from day 15 post-weaning until the end of the experiment at day 56 post-weaning (Table 1). The starter diet was formulated to contain 13.70 MJ/kg metabolisable energy (**ME**), 17.27% crude protein (**CP**) and 1.37% Lysine (**Lys**). The grower diet was formulated to contain 12.47 MJ/kg ME, 17.96% CP and 1.14% Lys (Table 1).

Table 1. **Ingredients and chemical composition of experimental diets** (g/kg)

	Starter diet	Grower diet
Barley	400	140
Wheat	100	358
Corn	220	-
Whole plant corn silage	-	250
Soya Hi-Pro	-	190
Soya full fat	82.4	-
Potato protein	62.0	-
Dextrose	33.3	-
Lactose	24.9	-
Whey powder	24.9	-
Pumpkin seed	23.4	-
Mono calcium phosphate	11.7	9.0
Limestone	9.3	13.0
Salt	4.5	4.0
Rapeseed oil	2.8	3.0
Magnesium phosphate	0.8	2.0
Malt sprouts	-	30
Sugar beet molasses	-	1.0
Chemical composition		
Dry matter	880	880
Crude protein	171	188
Lysine	13.5	12.0
Digestible energy (MJ ME/kg)	13.5	13.0

All diets met or exceeded the nutrient requirements as recommended by NRC (1998). Each pig was weighed at weaning, at day 14, day 42 and at the end of the experiment at day 56. Feed intake was recorded weekly, and feed conversion ratio (**FCR**) was calculated by dividing the average daily gain (**ADG**) for each group by average daily feed intake (**ADFI**). Data was calculated for Period 1 (from weaning to day 14 post-weaning), Period 2 (from day 14 post-weaning to day 56 post-weaning) and throughout both feeding periods. The pen was considered as the experimental unit in the analysis of all variables. Performance data was analysed by analysis of variance (ANOVA) using SPSS 15.0. The model included the effect of treatment on ADG, ADFI, FCR and final body weight. Initial body weight was used as a covariate. Data were analysed for the period from day 0 to 14 post-weaning, from day 15 to 56 post-weaning and for both periods combined. Data are expressed as least square means ± standard error, and the Duncan's multiple range test was used to separate least square means differences. Effects were considered as significant if $P<0.05$.

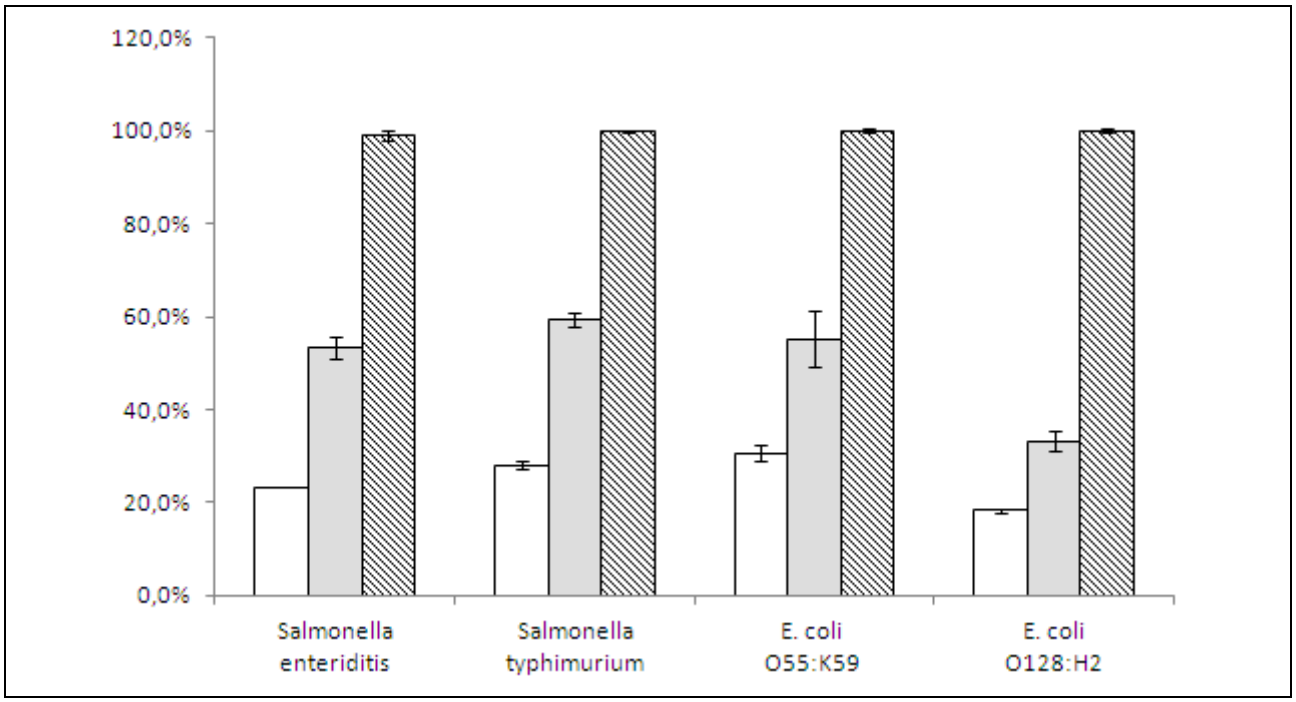

Fig. 1. **Growth inhibition of *Salmonella* Enteritidis, *Salmonella* Typhimurium, *E. coli* O55:K59 (B5):H and *E. coli* O128:H2** by (☐) cinnamaldehyde, (▩) a blend of organic acids (formic, propionic and acetic acid) and (▨) a blend of organic acids combined with cinnamaldehyde (antimicrobial mixture)

Table 2. **Effects of feeding a blend of organic acids, cinnamaldehyde and a permeabilising substance on weight development and growth performance of weaning pigs** (least-square means, s.e.)

		Control group	Experimental group	s.e.	Sig.†
Weight development	Initial weight	8.71	8.76	0.12	
	Weight day 14	10.81	11.10	0.16	
	Weight day 42	25.68	27.24	0.35	*
	Final weight	35.54	37.71	0.42	**
Performance day 0 to 14	Average daily gain (g/day)	156	174	7.01	
	Feed intake (g/day)	292	284	13.6	
	Feed conversion ratio	1.87	1.63	0.06	
Performance day 15 to 42	Average daily gain (g/day)	535	573	9.67	
	Feed intake (g/day)	1 028	1 054	36.7	
	Feed conversion ratio	1.93	1.87	0.05	
Performance day 43 to 56	Average daily gain (g/day)	842	878	12.9	
	Feed intake (g/day)	1 650	1 671	58.8	
	Feed conversion ratio	1.95	1.93	0.01	
Overall performance (day 0 to 56)	Average daily gain (g/day)	483	516	6.7	*
	Feed intake (g/day)	976	1 028	32.0	
	Feed conversion ratio	2.04	1.99	0.05	

† Sig. = Significance; *p<0.05; **p<0.01

Results. *In vitro trial*

Results of the *in vitro* trial on the effects of an AB and the AB in combination with CA on the inhibition of *Salmonella* Enteritidis, *Salmonella* Typhimurium, *E. coli* O55:K59 (B5):H and *E. coli* O128:H2 are shown in Fig. 1. The AB alone inhibited the growth of *Salmonella* Enteritidis, *Salmonella* Typhimurium, *E. coli* O55:K59 (B5):H and *E. coli* O128:H2 by 53.5, 59.3, 55.2 and 33.3%, respectively. The CA alone showed an inhibition of 23.4, 28.1, 30.7 and 18.4% for *Salmonella* Enteritidis, *Salmonella* Typhimurium and the two *E. coli* strains. The addition of CA to the AB resulted in an inhibition of 99.0% in *Salmonella* Enteritidis, 99.8% in *Salmonella* Typhimurium and 100.0% in the two *E. coli* strains. In the second experiment, the mixture of the AB and CA combined in lower levels compared to the previous

experiment inhibited the growth of *Salmonella* Enteritidis, *Salmonella* Typhimurium, *E. coli* O55:K59 (B5):H and *E. coli* O128:H2 by 6.9, 3.9, 29.5 and 2.3%, respectively. The addition of the PS to the antimicrobial mixture resulted in a growth inhibition of *Salmonella* Enteritidis, *Salmonella* Typhimurium, *E. coli* O55:K59 (B5):H and *E. coli* O128:H2 of 86.2, 100.0, 70.5 and 100.0%, respectively.

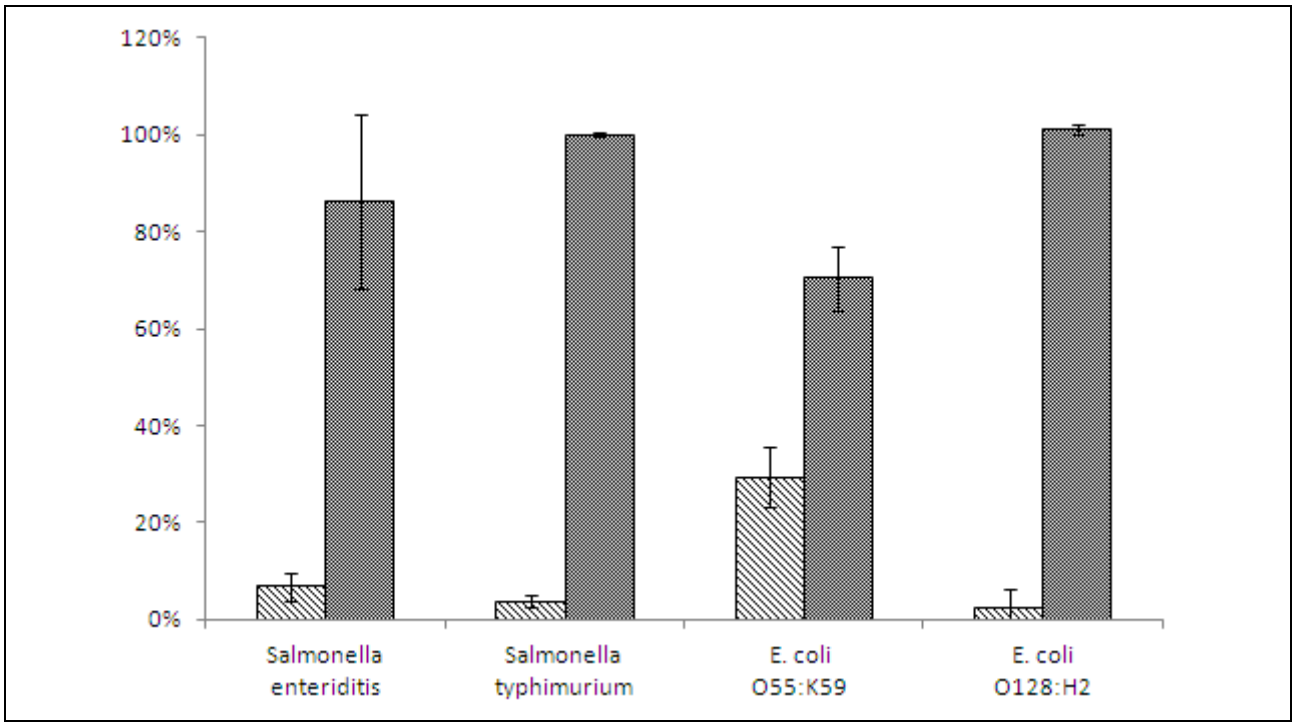

Fig. 2. **Growth inhibition of *Salmonella* Enteritidis, *Salmonella* Typhimurium, *E. coli* O55:K59 (B5):H and *E. coli* O128:H2** by a (▨) blend of formic, propionic and acetic acid combined with cinnamaldehyde (antimicrobial mixture used in lower levels compared to experiment described in Fig. 1) and (▩) the antimicrobial mixture combined with a permeabilising substance

Feeding trial

As shown in Table 2, weight at day 42 ($p<0.01$) and final body weight ($p<0.05$) were significantly higher in pigs fed the experimental diet compared to pigs fed the control diet with no feed additives added (25.7 vs. 27.24 and 37.7 vs. 35.5, respectively).

For the overall period, ADG was significantly improved ($p<0.05$) in the experimental group compared to the control group (517 g vs. 481 g).

Discussion. The addition of CA to the AB had a synergistic effect on the inhibition of the bacterial strains used in the *in vitro* experiment as shown in *Fig. 1*. The CA is a phenylpropene and is mainly used as a flavouring agent (Michiels, 2009). It is known, that CA is highly electronegative due to its aldehyde group which is conjugated to a carbon-carbon double bond. Those electronegative arrangements may interfere in biological processes involving electron transfer and react with vital nitrogen components such as functional groups on proteins and nucleic acids, which may lead to an inhibition of the growth of micro-organisms (Lis-Balchin, 2003). Another possibility would be that they form Schiff's bases with membrane proteins and in this way prevent biosynthesis (Friedman, 1996). In fact it was shown that treatment with CA did not result in significant protein leakage of the cell (Kwon *et al.*, 2003), which indicates that no membrane damage took place. However, Kwon *et al.* (2003) found a strong inhibition of cell separation. The mechanism behind the inhibition of the cell separation was in detail described by Domadia *et al.* (2007). The cell division of bacteria is regulated by FtsZ, a protein which is assembling into the Z-ring at the site of cell division. The CA binds FtsZ, perturbs the cytokinetic Z-ring formation and inhibits its assembly dynamics. This mechanism leads in general to a reduced bacterial load. Due to the strong effects of CA on this mechanism it seems less surprising that synergistic effects were found when combining the AB with CA. It was already shown, that using ABs instead of single acids may be more beneficial as a result of a broader spectrum of activity (Namkung *et al.*, 2003). This was also suggested for combining organic acids and other natural derived compounds and is in agreement with findings in the *in vitro* trial of the current experiment. However, it was suggested that combining organic acids with essential oils also may be beneficial due to its effects in different parts of the gastro-intestinal tract. Organic acids would exert their activity in feed and the upper gastro-intestinal tract and essential oils more in the distal part of the intestinal tract (Langhout, 2000). Therefore, beneficial effects would derive as effects on bacteria could be seen over a wider part of the gastro-intestinal tract. As

phytochemicals such as CA are defined as being chemical substances characterized as organic biomolecules found and isolated from different plant derivative products, such as essential oils (de Souza *et al.*, 2005) this might also be expected when combining organic acids and CA. However, out of the literature there is no evidence that CA can improve the effects of organic acids on the inhibition of bacteria so far. Therefore, the findings of the current experiment give evidence of a possible additional advantage when combining organic acids and substances derived from essential oils. It is known that PS weaken the outer membrane of Gram-negative bacteria. This makes the bacteria more susceptible to hydrophobic antimicrobials. The mode of action of PS regarding the damage of the outer membrane of Gram-negative bacteria is described in detail by Alakomi (2001). However, it can be assumed that if the Gram-negative bacteria are more susceptible to hydrophobic antimicrobials when their outer membrane is damaged by a PS it also might be possible to enhance the antibacterial effects of organic acids and CA. This was proven in the second experiment in which the antimicrobial mixture consisting of the AB combined with CA and the antimicrobial mixture in addition with the PS on the inhibition of bacteria was tested. The synergism found in this experiment clearly indicates that adding a PS enhances the effects of organic acids and CA most probably due to an increase in outer membrane permeability. Organic acids are well known to improve growth performance and modulate intestinal microbiota of pigs (Piva *et al.*, 2002). Initially the organic acid supplementation of pig diets are targeted at weaned piglets, but there is evidence that diet acidification is also beneficial for pigs in later stages of growth in terms of improved apparent ileal protein and amino acid digestibility and increased absorption of minerals (Mroz *et al.*, 1997; Jongbloed and Jongbloed, 1996). In general, organic acids lower the pH in feed and the gastro-intestinal tract, creating unfavourable conditions for potentially harmful bacteria (Freitag, 2007). In their non-dissociated form, they can penetrate the bacterial cell and stop the bacterial growth or may even kill the bacterial cell (Stonerock, 2007). Especially in weaned pigs the usage of organic acids is beneficial not only due to its effect on pH reduction which has beneficial effects on enzyme production which is insufficient in newly weaned pigs (Freitag, 2007). But what is also the case in this stage of production is that pigs at this age have a low immunological status as passive immunity acquired through maternal colostrums is not provided anymore and active immunity only begins to develop (Gaskin and Kelley, 1995). This makes the piglets extremely vulnerable to pathogenic bacteria. However, also in other stages of growth the bacterial challenge needs to be combated. On the one hand, the bacterial microbiota within the gastro-intestinal tract provide real benefits to the host such as nutrition and protection via providing fermentation products and prevention of colonization by pathogens. On the other hand, the microbiota (1) competes with the host animal for nutrients, (2) produces toxic amino acid catabolites, (3) decreases fat digestibility, (4) stimulates a rapid turnover of absorptive epithelial cells, (5) requires an increased rate of mucus secretion by intestinal goblet cells, and (6) stimulates immune system development and inflammatory responses (Dibner and Richards, 2005). This may all lead to decreased growth performance. Therefore, it can be assumed that the combination of organic acids with CA and a PS might be beneficial *in vivo*. The effects of CA and a PS are described above and the results from the *in vitro* trials indicate that the combination of the AB with CA and a PS makes it possible to combat bacteria more effectively. Thus, an improvement in growth performance of pigs fed an AB, CA and a PS can be expected. This is as more nutrients for the host are available and less energy will be lost to the microbiota. This is also reflected in the results of the current experiment. In the overall period, feed intake did not differ significantly between the control and the experimental group, but ADG was significantly higher in the experimental group ($p<0.05$). The fact that with similar feed intake a higher ADG was achieved leads to the conclusion that more nutrients were available for the host due to a reduced bacterial load, resulting in improved growth performance.

Conclusion. In conclusion, results of the *in vitro* trials indicate that the effect of organic acids on the inhibition of bacteria can be synergistically improved by the inclusion of CA. In addition, the inclusion of a PS synergistically improved the inhibition of bacteria by an AB combined with CA. The *in vivo* trial supported the hypothesis that feeding an AB, CA and a PS increases growth performance. However, it cannot be concluded if the inclusion of CA and PS can further enhance the effect of organic acids *in vivo*, as the control group was a negative control group without any additives added to the diet. Therefore, further experiments have to be carried out in order to proove if the addition of CA and a PS to an AB can enhance the effects of organic acids on animal performance. It was assumed, that the improvement in growth performance derives from a reduction in bacterial load within the gastro-intestinal tract. This can only be hypothesised, as also for the proof of this hypothesis further investigations are needed.

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