How to support the antibacterial impact of organic acids

by Nataliya Roth, Biomin Holding GmbH, Industriestrasse 21, 3130 Herzogenburg, Austria.

Gram-negative pathogens continue to cause major public health problems worldwide and continue to be the leading causes of illness and death.

Combating the pathogen bacteria in animals and in the animal's feed is an important task of animal producers. Organic acids have been used for this purpose for decades.

Organic acids are well known to decrease the pathogen numbers and modulate the intestinal microbiome of pigs. In their undissociated form, they can penetrate the bacterial cell and stop bacterial growth.

The use of organic acids decreases the total bacterial count and Gram-negative bacterial count significantly in pigs. Using organic acids instead of single acids may be more beneficial as a result of a broader spectrum of activity.

Combining organic acids

Is it possible to improve the efficacy of organic acids combination? Optimising the activity of organic acids with phytochemicals is a possibility to strengthen the activity of organic acids against pathogens. Combining organic acids with plant derivatives may be beneficial due to the effect on different parts of the gastro-intestinal tract.

Organic acids exert their activity in the feed and upper gastro-intestinal tract and plant derivatives to a greater extent in the distal part of the intestinal tract.

	Neg. control	Pos. control	ACPS
BW (kg)	42.5[a]	43.2[a]	48.1[b]
ADG (kg)	0.58[a]	0.58[a]	0.67[b]
ADFI (g)	1.09[a]	1.11[a]	1.27[b]
FCR	1.82	1.78	1.80

[ab] Means in the same row with no common superscripts are significantly different ($P < 0.05$)

Table 1. Performance characteristics of piglets.

The phytochemical cinnamaldehyde targets the FtsZ protein, which plays an important role in the cell division of pathogenic bacteria.

Cinnamaldehyde inhibits not only the formation of FtsZ into filaments, but also the essential processes involved in the Z-ring formation, its function and thus the cell division. This results in a reduction in the bacterial load within the gastro-intestinal tract.

Control of Gram-negative bacteria is hampered by their relatively good resistance to many external agents.

Cells of Gram-negative bacteria are surrounded by an additional outer membrane (OM), which provides the bacterium with a hydrophilic surface and functions as a permeability barrier for many external agents.

Although the OM of Gram-negative bacteria protects cells from many external agents, it is possible to specifically weaken it by various agents, collectively called permeabilisers. Permeabilisers themselves may not be bacteriocidal but they can potentiate the activity of other compounds, thus acting synergistically. The aim of the trial was to examine the ability of a permeabiliser to increase susceptibility of E. coli and Salmonella typhimurium to organic acids and cinnamaldehyde.

In vitro trial

Increase of susceptibility of Salmonella typhimurium and Escherichia coli to a mixture of organic acids (OA): formic, acetic and propionic acid and cinnamaldehyde (CA) with addition of a permeabiliser (PS) was examined and described in the trial below. The OA and CA mixture caused a decrease in the growth of Salmonella typhimurium and E. coli in comparison to the growth control with no inhibiting substances.

PS alone showed only a small decrease of the growth of Salmonella typhimurium and E. coli, while the combination of OA, CA and PS (ACPS) showed a stronger decrease of Salmonella typhimurium and E. coli in comparison to the OA and CA mixture.

PS efficiently increased sensitivity of Salmonella typhimurium and E. coli cells to OA and CA mixture (Figs. 1 and 2).

Continued on page 9

Fig. 1. Growth inhibition of Salmonella typhimurium.

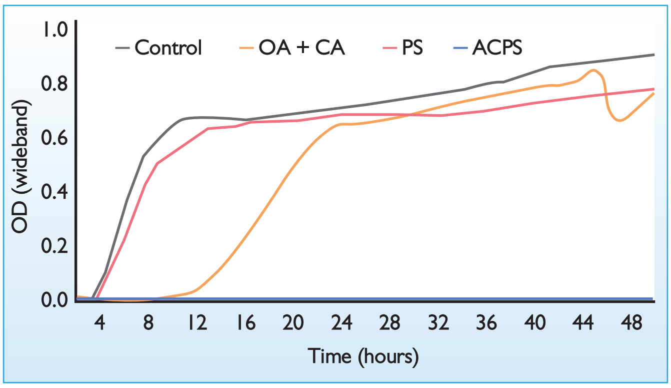
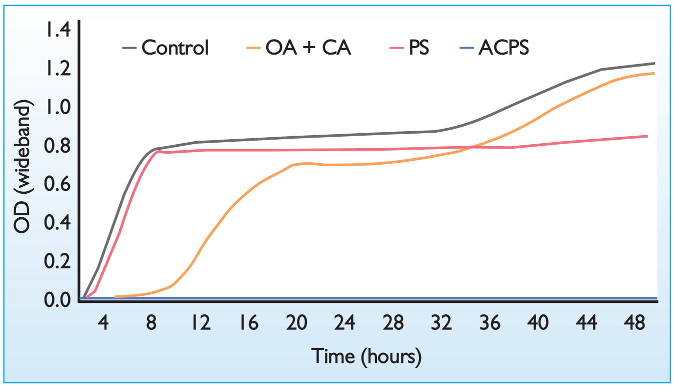


Fig. 2. Growth inhibition of E. coli.



Continued from page 7

In vivo trial

A trial was carried out at the Research Center of Hunan Agricultural University, Changsha, Hunan, China, using 96 weaning pigs. Pigs (body weight 10.5kg; 35 days) were assigned to three treatments.

The negative control group diet contained no feed additives, whereas the positive control group was supplemented with antibiotics (100g/t colistin and 100g/t chlortetracycline) and the diet of the trial group supplemented with a blend (ACPS) of organic acids, cinnamaldehyde and permeabiliser in a form of a commercial product Biotronic Top3 (Biomin Holding GmbH) at the inclusion rate of 1.0kg/t feed.

The duration of the trial was 56 days. The weight and feed intake of the pigs was recorded at each diet change and feed conversion was calculated. Mortality and clinical symptoms for diarrhoea or other medical incidences were observed daily.

After 56 days pigs were killed and the pH of digesta in the stomach and jejunum was measured. The content of the ileum was collected and analysed for E. coli, salmonella, lactobacilli and bifidobacteria populations. Histological samples of jejunum were analysed for villus length and crypt depth.

Growth performance was improved due to the supplementation of the diets with a blend of organic acids, cinnamaldehyde and permeabiliser (Table 1).

Body weight (BW) at trial day 56 was similar in positive and negative control groups, but 13% higher in the trial group when compared to the negative control group and 11% higher when compared to the positive control group.

Average daily gain (ADG) was improved in the ACPS group compared to the negative and positive control groups. Also, average daily feed intake (ADFI) increased in the ACPS group compared to the negative and positive control groups. Feed conversion ratio (FCR) was similar in all three groups.

The pH in the stomach of pigs fed the negative and positive control diet was similar (4.28 vs. 4.30), while pH in the stomach of pigs fed the diet supplemented with ACPS was significantly ($P<0.05$) lower compared to other groups. Also pH in the jejunum of pigs fed the diet supplemented with ACPS was lower compared to the pH in the jejunum of pigs fed the negative and positive control diet.

However, differences were not significant ($P>0.05$). Microbiological analysis showed that the E. coli counts in the ileum were lowest in the group fed the ACPS (Table 2).

E. coli counts found in the ileum of pigs fed the negative control diet were higher compared to E. coli counts found in the ileum of pigs fed the positive control and ACPS diet.

Salmonella counts in the ileum of pigs fed the negative control diet were higher compared to salmonella counts found in the ileum of pigs fed the positive control and the ACPS diet. Increased villus height indicates the enlarged surface in the jejunum leading to better absorption of nutrients. Villus height of jejunum was significantly greater in the positive control group and the group fed ACPS in comparison to the negative control (Table 3). The difference of crypt depth was not significant between groups.

	Neg. control	Pos. control	ACPS
E. coli	6.30^{a}	5.97^{b}	5.87^{b}
Coliform	8.28^{a}	7.98^{b}	8.15^{b}
Salmonella spp.	8.22^{a}	7.94^{b}	8.05^{ab}
Lacto-bacillus spp.	6.29^{a}	6.17^{b}	6.51^{b}
Bifidobac-terium spp.	6.89^{a}	6.88^{a}	7.13^{b}

^{ab} Means in the same row with no common superscripts are significantly different ($P<0.05$)

Table 2. Analyses of intestinal microbiota (logCFU/g).

Improving the antimicrobial activity of organic acids against salmonella and E. coli with the mean of CA and PS was shown in the current study in vivo and in vitro trials. The in vitro trials indicate that the effect of OA and CA on the inhibition of E. coli and Salmonella typhimurium can be synergistically improved by the inclusion of PS. The in vivo trial showed that feeding an OA, CA and a PS increases growth performance.

The addition of ACPS reduced pH of digesta collected from stomach and jejunum. Microbial analysis showed that E. coli and salmonella in the ileum of pigs were combated as successfully by ACPS as it was by the addition of colistin and chlortetracycline.

However, the addition of colistin and chlortetracycline had negative effects on lactobacilli and bifidobacteria, while the addition of ACPS increased the amount of beneficial bacteria in the ileum of pigs. Villus height was increased in the positive control group as well as ACPS group in comparison to the negative control.

These results indicated that the compound natural growth promoters improved growth performance in piglets by changing the intestinal micro-ecological environment. ■

Table 3. The effect of different diets on villus height and crypt depth of piglets (µm).

	Neg. control	Pos. control	ACPS
Villus height	418.33^{a}	448.63^{b}	456.18^{b}
Crypt depth	192.37^{a}	188.77^{a}	187.15^{a}

^{ab} Means in the same row with no common superscripts are significantly different ($P<0.05$)