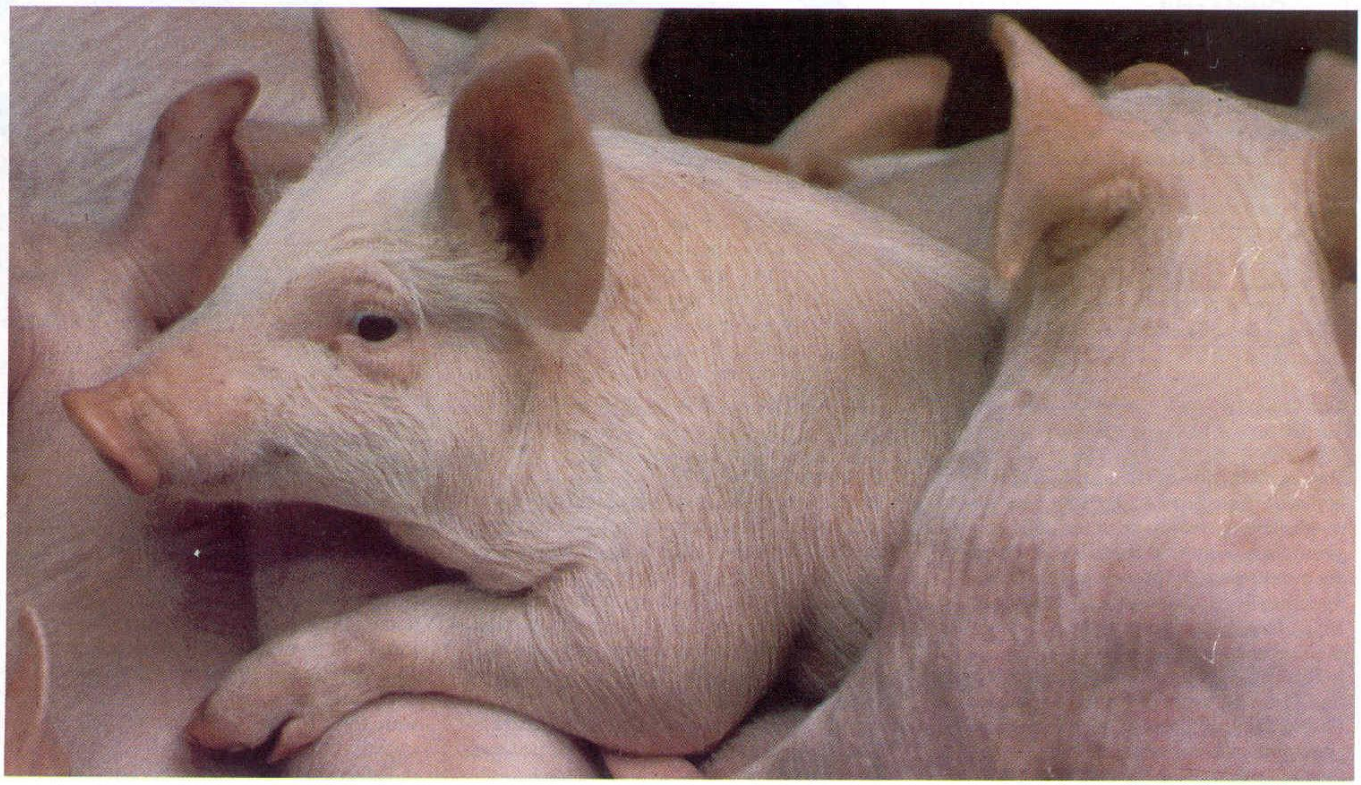
# Short and medium chain fatty acids make a comeback



*In vitro work confirms the antimicrobial properties of medium chain fatty acids. Their differential mode of action compared to the better known short chain fatty acids might explain the fact that piglet trials have repeatedly shown synergistic effects of combinations of the two. MCFA appear to be a potent enhancer of the effects of SCFA, even at low inclusion levels.*

**By Hubèrt van Hees and Bert van Gils**

The antimicrobial activity of short (SCFA) and medium chain fatty acids (MCFA) has already been known for many decades. Short chain fatty acids like sorbic-, benzoic and propionic acid are used by food technologists as food preservatives (See Kabara, 1993 for an overview). Animal nutritionists also appreciate these and other SCFA as (alternative) growth promotors, feed preservatives and gut stabilisers. The potential of MCFA for these applications has been less thoroughly investigated until recently. Vast developments in the applications of antibiotics and chemo-therapeutics during the 1950s and 1960s stopped any fur-

**Figure 1 - Inhibition of pathogenic *E.coli***

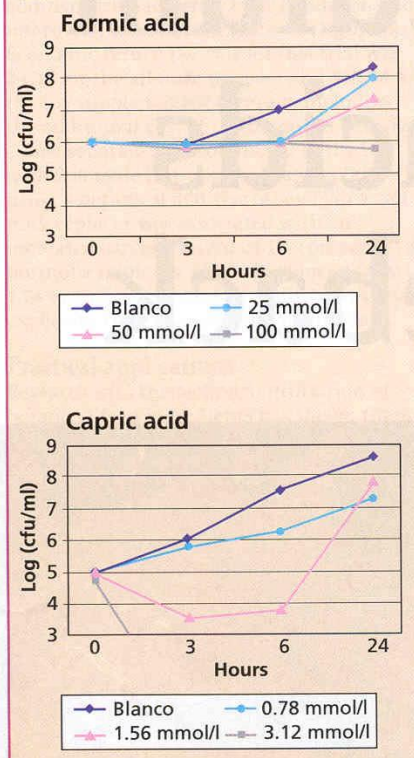
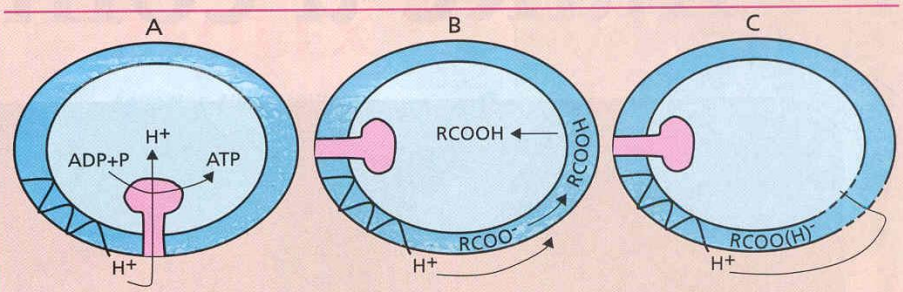



**Table 1 - In vitro effect of test substances on metabolic profile and gas production of a standardised gut flora**

| Additive | Lactic acid | Formic acid | Acetic acid | Propionic acid | Butyric acid | Gas formation |
|---|---|---|---|---|---|---|
| Olaquindox | +++ | - | - | - | - | - |
| Avilamycin | 0 | + | + | -- | - | + |
| C6 | 0 | 0 | 0 | 0 | 0 | 0 |
| C8 | ++ | 0 | 0 | - | - | - |
| C10 | ++ | 0 | 0 | - | - | - |

**Figure 2 - The respiratory chain and electron-transport phosphorylation across the cytoplasmic membrane of bacteria with an without medium chain fatty acids (MCFA ≈ RCOO-H)**




**Normal situation**
$NADH_2$ oxidation and proton extrusion followed by regeneration of ATP as a consequence of the backflow of protons

**With RCOO-H (I)**
The base of the MCFA accepts $H^+$, producing the undissociated acid, which diffuses across the membrane

**With RCOO-H (II)**
MCFA destabilises the membrane which permits the $H^+$ to diffuse across the membrane

ther exploitation of the antimicrobial properties of these fatty acids. However, the ban on the use of certain antibiotics as growth promoters in animal feeds has given rise to renewed research interest in this area. Specifically combinations of SCFA and MCFA have been shown to be of interest as feed supplements.

Fatty acids with a chain length between 6 and 11 or 12 carbon atoms are regarded as MCFA. They are present in so-called lauric vegetable oils, coconut and palm kernel oil. Because of their longer chain lengths they have a more lipophilic character compared to short chain fatty acids (SCFA). Hence they have distinct different absorption mechanism but also a distinct antimicrobial action. Already some years ago a Belgium research team presented *in vitro* results on SCFA and MCFA in pigs (Decuypere *et al.*, 1994). By culturing the intestinal flora of the pig, with or without organic acids, they found that SCFA selectively inhibited *E. coli* bacteria. Caproic (hexanoic) acid also depressed the growth of *Lactobacillus* and *Streptococcus* spp., while capric (decanoic) and lauric (C12) acids only depressed *Lactobacillus* and *Streptococcus* spp.

Despite their potent antimicrobial activity, MCFA have a low toxicity to mammals and humans in general (Traul *et al.*, 2000).

## *In vitro* test results

*In vitro* tests are used to predict the effect of a candidate feed product on the intestinal flora. The product should be able to modulate the intestinal flora in such a way that it supports animal health and increases efficiency of nutrient use. This means that, on the one hand candidate products should suppress potential pathogenic strains of for example *E. coli*, Salmonella and *Clostridium* spp. On the other hand they should stimulate micro-organisms which are considered positive for intestinal health, like *Lactobacillus* spp. and possibly bifidobacteria.

MCFA were tested in an *in vitro* gas production model (modified from Williams, 1998). In this test (*Table 1*), a standardised complex microflora is used to measure the effects of molecules on microflora metabolism. This test mimics the complex interactions between micro-organisms in a gut biotope. After revitalising the micro-organisms they are incubated under anaerobic conditions with a broth containing sufficient energy, protein and micro-elements. Test substances are added to the broth simulating normal feeding conditions at fixed temperature and pH. During incubation, the gas production of the microflora is measured continuously, and at the end of incubation the medium is analysed for short chain fatty acids profile and ammonia content. It can be seen that traditional growth promoters, like olaquindox, have a large influence on gas production and end-point concentration of various volatile fatty acids (lactic, butyric etc.). It seems logical therefore that, given the altered metabolic profile, the composition of the flora (quantities of different species) had also changed. MCFA were found to change this metabolic profile in a similar way.

The gas production model is not sufficient to relate microflora metabolism to inhibition of individual species, and for this purpose other models were used. Firstly, the minimum inhibitory concentration (MIC) of several SCFA and MCFA was determined relative to avilamycin. The MIC is defined as the additive concentration at which no population growth of the different bacterial species takes place. MCFA appeared to be strongly inhibitory for several bacterial species, including *Clostridium perfringens*.

To study in more detail the inhibition of intestinal flora species over time, an adapted MIC model was been developed in collaboration with ID-Lelystad in the Netherlands. The model measures the population growth in time of pathogenic and commensal bacterial species. Even at very low concentra-

**Figure 3 - *E. coli* challenge with weaned piglets. % improvement of supplemented v. control diets**

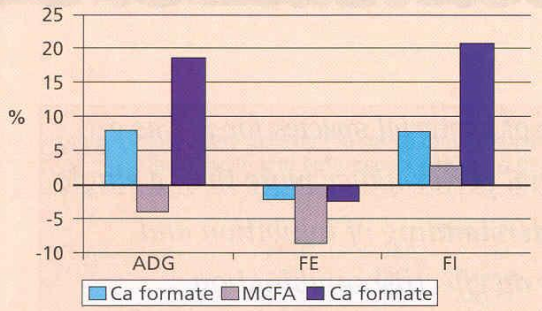
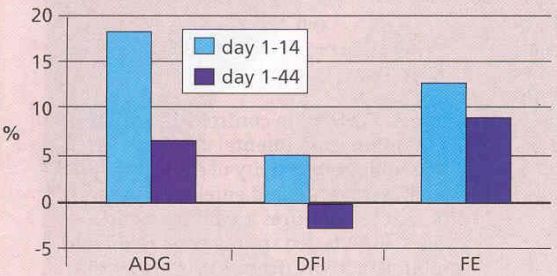



**Figure 4 - The effect of MCFA on the performance of weaned piglets. Improvement (%) by MCFA v. control diet with 2% organic acid blend**




tions some MCFA are able to inhibit the growth of pathogenic bacteria. *Figure 1* clearly demonstrates this in the case of pathogenic *E. coli*. Capric acid appears to be much more effective than formic acid to inhibit the development of *E. coli* in this *in vitro* model.

## Assumed mode of action of medium chain fatty acids

According to current knowledge MCFA antimicrobial activity seems to be based on at least two modes of action. First MCFA, like SCFA, act in the classical way of weak-acid preservatives. They diffuse in a non-dissociated state through the microbial plasma membrane into the cytoplasm. Here they encounter a pH near neutral and consequently dissociate releasing hydrogen ions. The consecutive fall in pH has to be counteracted by intercellular buffer systems in combination with an active discharge of protons from the cell lumen. This takes place at the expense of energy that is channelled away from growth processes. If the cell fails to maintain its pH than vital components like enzymes, DNA and membranes will be damaged (Northolt *et al.*, 1999).

Secondly, some MCFA are thought to have a so-called 'uncoupler-effect'. This effect has been studied extensively in yeast and is schematically depicted in *Figure 2*, showing a detail of the plasma membrane. Uncouplers interact with the membrane by insertion into the hydrophobic interior, increasing the polarity of this region and weakening the hydrophobic barrier to the free exchange of hydrogen ions (Correia *et al.*, 1989).

Some authors state that this causes passive reflux of protons bypassing the ATPase channels. Others regard MCFA as proton carriers (eg. Stevens and Servaas Hofmeyer, 1993). However, this results in lower synthesis of ATP, a high-energy molecule vital for cell functions like active transport of nutrients into the cell. As a result, uptake of amino acids, especially glutamic acid and lysine, is reduced (Kabara, 1993). Uncoupling thus refers to disengaging electron transport from ATP-synthesis (Stratford and Anslow, 1996). These authors also reported decanoic acid (C10) to cause rapid cell death in yeast. This biocidal action was distinct from uncouplers and weak acid preservatives and probably is a result of major loss of cell membrane rupture and loss of cytoplasm.

## *In vivo* effects of MCFA: *E.coli* challenge test

The "uncoupler effect" differentiates MCFA from SCFA. It remains to be demonstrated, however, that MCFA has real added value in pig nutrition, especially since SCFA is widely used as a feed preservative and gut stabiliser. A series of trials has been conducted to support the hypothesis that, because of their specific mode of action, MCFA is synergistic with and enhances the positive effect of SCFA. To this effect, a series of piglet trials was conducted under a variety of environmental conditions. One typical trial consisted of an *E. coli* challenge test. The 96 piglets in this trial were weaned at 24 days of age and subjected to several stress factors. After weaning they were group-housed for a few days and fed standard starter feeds. Three days after weaning, in addition to the normal stress of weaning, the piglets were stressed further by rehousing in individual pens and oral application of a preparation of pathogenic *E. coli* K88ac. Such stress induces *E. coli* proliferation in the gut and diarrhoea in approximately 80% of all piglets. Immediately after relocation into individual pens, the piglets were allotted into 4 groups of 24 piglets, which each received one of four starter feeds: control feed without organic acid supplement; diet containing 0.7 % calcium formate; diet containing 0.1 % MCFA; diet containing a combination of 0.7% calcium formate and 0.1% MCFA.

The performance of the individual piglets was monitored and recorded over a period of 4 weeks. The summary of the results in *Figure 3* demonstrates a clear synergy between formic acid and MCFA. The MCFA at an inclusion level of 1 kg per tonne was not sufficient to elicit a positive effect on piglet performance under the stressful conditions of this challenge test. Formic acid as a single supplement did show an 8% response in daily gain and feed intake, confirming the positive reputation of SCFA in piglet starter feeds. The combination of the two supplements, however, showed a positive response in daily gain and feed intake close to 20% relative to the control group.

## MCFA in the field

A second example of synergy between SCFA and MCFA was shown in a trial conducted in co-operation with the Catholic University of Leuven (Belgium) in a facility which permits well controlled trials under conditions resembling a practical farm situation. A group of 117 piglets was purchased after weaning from a commercial farm, shipped to the experimental unit and housed in 9 pens of 13 piglets each. The piglets received as prophylaxis a topdressing of colistin premix from day 1 to 6 and from day 10 to 15, indicating also the poor health status of the animals.

The piglets received starter feeds according to common commercial standards containing 2% of a blend of organic acids including lactic, formic, citric, fumaric and malic acids. The MCFA was added on top at an inclusion level of 0.1%. Piglet performance was monitored for 44 days. During this period the piglets were grown from 8.9 kg bodyweight to 25 kg, the average daily gain being approximately 370 grams.

*Figure 4* shows the effect of the MCFA supplement relative to the unsupplemented control group. Despite the fact that the control group already received feeds containing 0.5% SCFA, the addition of 1 kg/tonne of MCFA had an additional effect of 19% on average daily gain during the first two weeks of trial and 7% during the entire test period, resulting in 1 kg extra bodyweight at 44 days post weaning. ●

***References are available from the authors on request.***