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**The use of organic acids to combat Salmonella in poultry : a mechanistic explanation of the efficacy**

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**REVIEW ARTICLE**

The use of organic acids to combat *salmonella* in poultry: a mechanistic explanation of the efficacy

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Haesebrouck<sup>1</sup>, F. and Ducatelle<sup>1</sup>, R.

The use of organic acids to combat *salmonella* in poultry: a mechanistic explanation of the efficacy

**ABSTRACT**

*Salmonella* is a human pathogen that is commonly found in poultry products. It is possible to decrease chicken carcass and egg contaminations by adding organic acids to the feed or drinking water at appropriate times. Medium-chain fatty acids are more antibacterial against *Salmonella* than short-chain fatty acids. The antibacterial effect of these acids is species-specific. Bacteria that are unable to decrease intracellular pH accumulate organic acid anions in accordance with the pH gradient across their cell membranes. The short-chain fatty acid butyrate specifically down-regulates expression of invasion genes in *Salmonella* spp. at low doses. Also medium-chain fatty acids and propionate decrease the ability of *Salmonella* spp. to invade epithelial cells, in contrast to acetic acid. Because not all bacteria are affected in a similar fashion by organic acids, it may be possible to use probiotic and prebiotic bacteria to achieve beneficial effects. If diets can be designed to stimulate organic acid production in the caecum, it may be possible to control *Salmonella* spp. via even easier and more cost effective measures, compared with addition of acids to feed or drinking water.

## Introduction

With the perception that antibiotics should no longer be used as animal growth promoters, there has been widespread interest in natural methods of inhibiting detrimental bacteria. Man has used fermentations as a method of food preservation for more than 6000 years (Ohmomo *et al.*, 2002), but now it appears that fermentation acids also have value as feed or drinking water additives. Commercial preparations appear to enhance digestibility and diet palatability, thus improving feed conversion and growth of animals, including pigs and poultry. Some acid mixtures prevent mould growth on feed, and claims of increased egg production have been made. Pathogen control has also been reported, but the peer-reviewed scientific literature has few definitive studies. Until recently the use of short-chain fatty acids, medium-chain fatty acids and other organic acids was largely based on their antimicrobial activity outside the intestinal tract. This review: (1) describes the current use of organic acids to control *Salmonella* in poultry, (2) attempts to provide a rationale for assessing potential pathogen control, (3) summarizes *in vivo* experiments and field observations, and (4) discusses future applications of using acids, bacteria or dietary changes to control *Salmonella*.

## Bacterial Metabolism Of Organic Acids

Bacteria can use organic acids as both carbon and energy sources. In *Escherichia coli*, the hydrophobic long chain fatty acids (LCFA,  $\geq C_{12}$ ) are transported across the cell membrane by carrier mechanisms, in which the fadL (outer membrane proteins) and the fadD proteins (inner membrane) are involved. FadL carries LCFA to the periplasmic space and fadD is an acyl-CoA synthetase (Dirusso and Black, 2004; Van Den Berg, 2005). Once the acyl-CoA molecules are formed inside the cell, degradation occurs through the  $\beta$ -oxidation pathway, yielding multiple acetyl-CoA molecules (Clark and Cronan, 1996). Degradation of LCFA having odd number of carbon atoms also yields propionyl-CoA as an end product. Whether medium chain fatty acids (MCFA, C<sub>6</sub> to C<sub>10</sub>) can be transported by carrier proteins or are able to diffuse freely across the cell membrane in undissociated form is less clear, but also the fadD protein and the  $\beta$ -oxidation

pathway are used for metabolism (Clark and Cronan, 1996). The LCFA and MCFA can also be used for incorporation in the membrane as phospholipids. Short-chain fatty acids (SCFA,  $\leq$  C4) presumably cross the outer membrane mainly through diffusion in the undissociated form (Clark and Cronan, 1996). Once inside the cell, they can be converted to their CoA thioester forms. Butyric acid is converted to butyryl-CoA by the acetoacetyl-CoA transferase system (AtoAD system), converted to acetoacetyl-CoA by the fadB/E system, and then further breakdown to acetyl-CoA is performed by the atoB gene product (Jenkins and Nunn, 1987a, 1987b; Clark and Cronan, 1996). Thus as an example, butyric acid is converted to 2 molecules of acetyl-CoA. Propionic acid, either taken up from the environment or generated as end-product of degradation of LCFA with odd number of carbon atoms is metabolized in *Salmonella* and *E. coli* in the methylcitrate cycle. Propionyl-CoA reacts with oxaloacetate to form 2-methylcitrate, that is converted through a series of reactions, mediated by the Prp operon, to succinate and pyruvate (Horswill and Escalante-Semerena, 1999). These products can be used in the citric acid cycle. Although it is thought that acetate can diffuse across the cell membrane, an acetate permease (ActP) was detected in *E. coli* (Gimenez *et al.*, 2003). In *E. coli* and *Salmonella*, acetate is converted to acetyl-CoA by either acetyl-CoA synthetase (encoded by *acs* gene) or the sequential action of acetate kinase and phosphotransacetylase (encoded by *ackA* and *pta*, respectively) (Wolfe, 2005). Acetyl-CoA, generated by either the  $\beta$ -oxidation pathway, butyric acid breakdown or by acetate conversion, can be used for oxidation in the citric acid cycle and for replenishing intermediates of the citric acid cycle via the glyoxylate shunt.

### **Mechanism OF ANTIMICROBIAL ACTIVITY OF ORGANIC ACIDS**

Fermentative bacteria produce organic acids when oxygen is not available as terminal electron acceptor, but they differ greatly in the types of acids that they produce. Because the oxidation of one molecule must be coupled to the reduction of another, anaerobic bacteria often produce several acids. The simplest fermentation is conversion of sugar to lactate, and many lactobacilli, streptococci, lactococci and enterococci have a scheme that is virtually homolactic when sugar is plentiful. However, when sugars are scarce, all of these bacteria are capable to switch to a fermentation that produces acetate, formate and ethanol, so ATP production can be enhanced.

Butyric acid producing bacteria typically utilize the hydrogenases of butyrate (or other even longer chain fatty acids) production as a mechanism of reducing equivalent disposal. If the bacterium has a hydrogenase, interspecies hydrogen transfer to a methanogen decreases the need for dehydrogenase activity and acetate production typically is enhanced. Bacteria capable of utilising fatty acids are found in stagnant anaerobic environments, but these bacteria grow very slowly, and fermentative environments are typically acidic. Fermentation acids are inhibitory when the pH is low but some bacteria are much more resistant than others.

Traditionally, microbial growth inhibition by organic acids was explained by the ability of these acids to pass across the cell membrane, dissociate in the more alkaline interior and acidify the cell cytoplasm (Kashket, 1987). Organic acids were compared to synthetic uncouplers that could remain membrane associated, and shuttle protons in a cyclic manner to dissipate the protonmotive force. The problem with this analogy was the fact that organic acid anions are charged and not lipid permeable. Indeed, the accumulation of benzoate anion is typically used to estimate intracellular pH. If benzoate acted like an uncoupler the pH gradient across the cell membrane would be dissipated by the tool being used to measure it! The uncoupling model of fermentation acid toxicity, also failed to address another practical question. Why are some bacteria so much more sensitive than others (Russell and Diez-Gonzalez, 1992)?

For many years it was assumed that bacteria maintained a slightly alkaline intracellular pH, but this assumption was largely based on work with laboratory cultures of *E. coli* (Padan *et al.*, 1981). It is now clear that many fermentative bacteria have the ability to let their intracellular pH decline when the extracellular pH becomes highly acidic. This decline in intracellular pH necessitates a metabolism that can tolerate a lower pH, but the strategy appears to be highly adaptive. When intracellular pH remains high, the pH gradient across the cell membrane can become very large. The protons can be pumped back out of the cell, but the pH gradient causes a logarithmic accumulation of the fermentation acid anions. By letting intracellular pH decrease, the bacterium has a much smaller pH gradient across the cell membrane and it is protected from anion accumulation.

Continuous culture studies with *E. coli* K-12 and O157: H7 indicated that the two strains differed greatly in their sensitivity to acetate at pH 5.9, and the ability of O157: H7 to tolerate more acid than K-12 could be correlated with ability to decrease intracellular pH (Diez-Gonzalez and Russell 1997). These experiments also revealed another important observation about

fermentation acid toxicity. When the intracellular acetate concentration of K-12 increased there was a nearly equal molar increase in intracellular potassium. These results indicated that fermentation acid anion accumulation was at least in part an osmotic stress. Recent work with *Clostridium sporogenes*, a silage and food contaminant, indicated that it accumulated lactate anion at acidic pH values in accordance with the pH gradient across the cell membrane, but lactate anion accumulation caused a secondary effect (Flythe and Russell, in review). When lactate anion increased, the cells lost intracellular glutamate, and its fermentation scheme of amino acid deamination is dependent on glutamate transaminase. The final result was a virtually complete inhibition of ammonia production. The antimicrobial activity of organic acids on other bacterial species has not been correlated with intracellular pH regulation, but bacteria that could be classified as neutrophiles seem to be more sensitive than those that are acid tolerant. For example, the minimal inhibitory concentration (MIC) of acetic acid is 250 times lower for *Bacillus subtilis* than lactobacilli (Hsiao and Siebert, 2002).

The anion model of organic acid toxicity explains why bacteria differ in their sensitivity to organic acids, but it does not provide information on the antibacterial effect of one acid versus another. The MIC for acetic, butyric, lactic and caprylic acid in *E. coli* are less than 4 g/l, but this same bacterium is approximately 10-times more resistant to malic, tartaric and citric acid (Hsiao and Siebert, 2002). This observation indicates that factors such as chain length, side chain composition, pKa values, hydrophobicity could affect the antimicrobial activity.

### **Effects Of Short-Chain Fatty Acids On *Salmonella***

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**Antimicrobial activity against *Salmonella*.** Less is known about the effect organic acids on *Salmonella*, but it should be noted that *E. coli* and *Salmonella* are both enteric bacteria and seem to have a similar physiology. Medium chain fatty acids (C6 to C12; caproic, caprylic, capric and lauric acid) appear to be much more effective against *Salmonella* than the short-chain fatty acids (formic, acetic, propionic and butyric acid), but it is important to differentiate bactericidal and bacteriostatic effects. As little as 25 mM C6 to C10 acids were bacteriostatic to a *Salmonella* Enteritidis, but the same strain tolerated 100mM of short-chain fatty acids (Van Immerseel *et al.*, 2003; 2004). Sprong *et al.* (2001), reported that caprylic and capric acids were bactericidal, but



C14:0, C18:1 and C18:2 acids were not. When *Salmonella* Enteritidis and Typhimurium were incubated with low concentrations of monocaprin (5 mM) that had been combined with an emulsifier, the bacteria did not survive (Thormar *et al.*, 2006). In general, these data indicate that medium chain fatty acids have the greatest antibacterial activity against *Salmonella*, but large scale studies are lacking.

**Effect of short-chain fatty acids on virulence of *Salmonella*.** *Salmonella* is an opportunistic intracellular pathogen that has an elaborate set of virulence genes. These genes enable the bacterium to adapt to the environment and move between various micro-niches within a host. An early step in the pathogenesis of *Salmonella* is the penetration of intestinal epithelium (Lostro and Lee, 2001). This activity is promoted by invasion genes that are located on a pathogenicity island (SPI-1), but several pathogenicity islands are required for full virulence (Hensel *et al.*, 2004). SPI-1 has genes encoding regulatory proteins, structural components of a needle complex and additional effector proteins. Bacterial effector proteins facilitate the entry of *Salmonella* into the cytosol of epithelial cells, by inducing actin rearrangements that lead to uptake of the bacteria. SPI-1 is in turn activated by HilA, and this latter protein is environmentally regulated. When *Salmonella* Typhimurium was pre-incubated in growth media supplemented with various concentrations of butyrate and propionate, epithelial cell invasion was suppressed. However, if the cells were preincubated in media supplemented with acetate invasion was still observed (Durant *et al.*, 1999.; Lawhon *et al.*, 2002; Van Immerseel *et al.*, 2004). Similar results were obtained with *Salmonella* Enteritidis when primary caecal epithelial cells of the chicken were employed (Van Immerseel *et al.*, 2004).

The effects of organic acids on epithelial invasion can be explained by changes in SPI-1 expression. Durant *et al.* (2000) measured *hilA* and *invF* (major activators of SPI-1) expression after exposure to acetate, butyrate and propionate in *S. Typhimurium* (2000). At pH 6, exposure of the bacteria to acetate increased the expression of these genes, but similar effects were not observed with propionate or butyrate. More recently, Lawhon *et al.* (2002) noted that butyrate and propionate, but not acetate led to a decrease in *hilA*, *invF* and *sipC* expression. Acetate, after its conversion to acetyl-phosphate, acts to phosphorylate BarA and subsequently SirA. BarA/SirA is a two component system thought to be involved in environmental sensing, and SirA enhanced transcription of *hilA*, finally resulting in increased invasion (Jones, 2005). DNA microarrays of

both *Salmonella* Typhimurium and *Salmonella*. Enteritidis indicated that low doses of butyric acid downregulated SPI-1, but it did not alter metabolic gene expression. HilD, a positive regulator of HilA, was also downregulated in both strains. *S.* Typhimurium and *S.* Enteritidis carrying plasmid-borne *hilA::luxCDABE* and *hilD::luxCDABE* transcriptional fusions confirmed the idea that butyrate down-regulated *hilA* and *hilD* (Gantois *et al.*, 2006). The primary target of butyrate in the bacterial cell is still unknown but butyrate could interfere with HilA dependent regulation of SPI1 by altering the regulation of *hilD* transcription. These data indicate that short-chain fatty acids can regulate the invasive phenotype of *Salmonella*, and it should be mentioned that pre-incubation of *Salmonella* with short-chain fatty acids also increased acid resistance and survival in macrophages (Kwon and Ricke, 1998).

**Effects of short-chain fatty acids in *Salmonella* control *in vivo*.** The use of acidic compounds to control *Salmonella* first appeared in the late 1960s, and mainly focused on decontamination of carcass meal (Khan and Katamy, 1969; Smyser and Snoeyenbos, 1979; Van Staden *et al.*, 1980). Khan and Katamay (1969) evaluated the efficacy of 32 different acid preparations to decontaminate bone meal, and showed that low molecular weight volatile fatty acids were the most promising. Their results were a basis for the development of non-toxic, naturally occurring acidic compounds to control *Salmonella*. More than 35 years later, it is clear that their thoughts were prophetic. These acids have been added to feed, drinking water, and other matrices, in order to prevent *Salmonella* colonization of animal tissue and transmission through the food chain.

Poultry feed is a major source for *Salmonella* introduction to the farm (Williams, 1981). When chickens are given artificially contaminated feed, the gut is colonized and *Salmonella* are shed into the environment (Hinton, 1988). The original concept of incorporating acids into feed was based on the notion that the acids would decontaminate the feed itself and prevent *Salmonella* uptake by the chickens. When Iba and Berchieri (1995) inoculated feed with high doses of a *S.* Typhimurium strain, a commercial mixture of formic and propionic acid decreased the viability more than 1000-fold over seven days. When broiler chicks were given the acid treated feed that had been mixed with either *Salmonella* Enteritidis, Typhimurium or *agona*, the caecal *Salmonella* numbers were 7 log<sub>10</sub> lower than control animals at day 5 (10<sup>2</sup> versus 10<sup>9</sup> cfu/g)(Iba and Berchieri, 1995). Mixtures of formic and propionic acid were also effective when

feed was artificially inoculated with low doses of *S. kedougou*, and the decrease was most obvious after several weeks of storage (Hinton and Linton, 1988).

In a large scale study, (Humphrey and Lanning, 1988) the number of *Salmonella* positive breeder feed samples decreased from 4.1 to 1.1% after the feed was supplemented with 0.5% formic acid. The antibacterial activities of organic acids were dependent on the temperature and moisture. Since the water content of poultry feed is generally low, the action of the acids is not always optimal, and it is not clear if in feed effects are the major reason of protection (Hinton, 1990). In the 1980s, it became clear that the acid concentrations were also increased in the crop, and this antibacterial action could aid in controlling infection caused by horizontal transmission. Indeed, when the acid treated feed is eaten by the chickens, it is both warmed and moistened and the activity of the short chain fatty acids should increase. It appears that supplemental acids are most apt to affect in the crop and gizzard rather than in the intestine. This point is illustrated in a study of Thompson and Hinton (1997), who fed laying hens a feed supplemented with a commercial mixture of formic and propionic acid. In these animals, pH values of the crop, gizzard, jejunum, caecum and colon were not altered relative to control animals, but formic and propionic acid concentrations in crop and gizzard were significantly increased. At the same time, the lactic acid concentration in the crop decreased significantly, suggesting that lactobacilli were either inhibited or killed (Thompson and Hinton, 1997). This is in accordance with results obtained by Hume *et al.* (1993), in which large increases in propionic acid concentration in the crop of 4-day old broilers were detected, when propionic acid was added to poultry feed, despite no observed changes in crop pH. Caecal short chain fatty acid patterns were not affected (Hume *et al.*, 1993).

Later in the 1980s, many studies examined the effects of supplemental acids on *Salmonella* colonization of chicken tissues. Action of formic and propionic acids were variable. In a small-scale field trial, formic acid controlled shedding and caecal colonization by *Salmonella* serovars in naturally infected the animals. Indeed, 50% of all control animals had *Salmonella* positive cloacal swabs and caecal content samples, but *Salmonella* could not be detected in animals that consumed significant concentrations of formic acid (Hinton *et al.*, 1985). In 3 year study, the cumulative number of infections of newly hatched chicks with *Salmonella* decreased after breeder stocks were given formic acid treated feed (Humphrey and Lanning, 1988). Breeders that received acidified feed had fewer numbers of *Salmonella* in the breeder litter (4.3

versus 1.4%), hatchery waste (15.3 versus 1.2%) and insert paper samples (4.6 versus 1.4%). These decreases were evident from the moment the breeders received acidified feed and illustrate the effects of vertical transmission (Humphrey and Lanning, 1988). The most striking proof of the efficacy of formic and propionic acid as feed additives to control *Salmonella* was given by Hinton and Linton (1988). In three independent experiments, no artificial infections or feed inoculations with *Salmonella* were performed. Formic acid supplemented feed, given from the day of hatch, decreased the number of positive faeces and caecal content samples dramatically. The control groups had 25, 27 and 60% *Salmonella* positive faecal samples, but the treatment groups were 3, 0 and 0% (Hinton and Linton, 1988). When the formic acid treated feed was given at later age (16 or 32 days), no differences were detected between control and treated groups. This illustrates that preventing initial colonization of *Salmonella* is most important. Once an infection is established, it is very difficult to counteract by using acid treated feed, at least in the same production round.

When a formic and propionic acid mixture (0.5 to 0.68% w/w) was added to broiler feed that was artificially contaminated with very low numbers of *S. Kedougou* (less than 50 cfu/g feed), only 1 of 30 groups (10 chickens each) became infected, compared with 22 out of 27 control groups (Hinton and Linton, 1988). These experiments were a basis for numerous publications on this topic. Izat *et al.* (1990a) found a significant effect when 0.4% propionic acid was added to broiler feed and the number of *Salmonella* bacteria on post-chill carcasses was assayed. No significant differences in *Salmonella* count in the small intestine could be detected, but the relevance of small intestinal sampling can be questioned because *Salmonella* mainly colonizes the caeca (Desmidt *et al.*, 1997). Izat *et al.* (1990b) concluded that formic acid addition to the feed was not a reliable means of reducing the incidence or level of *Salmonella* in the caeca or processed carcasses. Some reductions in cecal *Salmonella* were detected after calcium formate addition, but no effects were observed in most treatment groups (Izat *et al.*, 1990b). This can be attributed to the infection protocol. They infected the animals on day 2, 7, 14, 21 and 28 by adding *Salmonella* to the drinking water (approximately  $10^5$  per ml) rather than the feed. McHan and Shotts (1992) fed chickens a diet supplemented with 1% of a formic and propionic acid mixture and infected the animals at 2 days post-hatch with  $10^6$  cfu *Salmonella* Typhimurium. They found significant reductions in caecal *Salmonella* colonization ( $>2.5$  log units at 14 days of age, and  $> 3.5$  log units at 21 days of age). Hume *et al.* (1993) concluded that propionic acid

(0.22%) in the feed was ineffective in reducing the number of *Salmonella* in the crop and caeca of broilers. Their work was based on 8 trials in which the broilers were inoculated with  $10^4$  cfu *S. Typhimurium* at day 4 of age, but they were sampled 6 days post-infection. The low dosage and the short time between infection and sampling could explain these results. When chickens received feed containing mixtures of formic and propionic acid, mortality after oral challenge with *S. Pullorum* and *Gallinarum* declined (Berchieri and Barrow, 1995; Al-Tarazi and Alshawabkeh, 2003).

Recently, researchers have attempted to transport the organic acids further down in the gastrointestinal tract by micro-encapsulation, which should prevent absorption of the acids in the upper tract and ensure release further down in the gastro-intestinal tract. Van Immerseel *et al.* (2004c) examined the effect of microbeads containing formic, acetic, propionic and butyric acid on colonization of *S. Enteritidis* in caeca, liver and spleen. Animals were infected (day 5 post-hatch) with  $5 \times 10^3$  cfu *S. Enteritidis* and samples were taken 3 days post-infection. Cecal colonization was significantly increased when acetic acid was added to the feed, but decreased when butyric acid was added. Internal organ colonization was increased if either formic or acetic acid were added to the feed, and this result is consistent with the idea that acids can enhance the virulence of *Salmonella* (see above). When powder and coated butyric acid additives (0.63 g/kg butyric acid) were compared using the same infection protocol, the coated form decreased colonization of the caeca, but the powdered form did not (Van Immerseel *et al.*, 2005). The inability of the powdered form to give a positive response, may have been due to the short time interval between infection and sampling. In an infection study using a seeder model in which 10 broilers were infected at day 5 post-hatch with  $10^5$  cfu *S. Enteritidis* and housed together with 40 non-inoculated broilers, 0.63 g/kg coated butyric acid in the feed significantly reduced shedding of *S. Enteritidis* in broilers until slaughter age. The effect of the acids on other members of the microbial community were not determined (Van Immerseel *et al.*, 2005).

Drinking water is a source for infection, so it is important to keep drinking water free of *Salmonella*. Short-chain fatty acids have also been used as drinking water sanitizers. In a study where 'natural' infections were recorded, Al-Chalaby *et al.* (1985) evaluated a commercial product containing propionic acid. The acids eliminated *Salmonella* in drinking water, while more than 80% of the samples in control groups were positive. This result could not be explained by *Salmonella* carriage because litter, cloacal swab and caecal content samples were not reduced

(Al Chalaby *et al.*, 1985) Acetic, lactic or formic acid (0.5%) in drinking water reduced crop contamination after *Salmonella* Typhimurium challenge ( $10^8$  cfu) when feed had been withdrawn to simulate pre-transport conditions to the slaughter house (Byrd *et al.*, 2001). However, the number of positive caecal samples was not different between the treatment groups. Lactic and formic acid were even more effective than acetic acid. In a commercial farm study where broilers were provided 0.44% lactic acid in the drinking water during a 10h feed withdrawal period, crop contamination and incidence of *Salmonella* in pre-chill carcass rinses were decreased (Byrd *et al.*, 2003). When moult was induced in layers by 9 days of feed removal and the animals were inoculated with *Salmonella* on day 4 of the feed withdrawal, neither acetic nor lactic acid (0.5%) significantly reduced crop or caecal colonization (Byrd *et al.*, 2004). These results seem to indicate that drinking water acidification is not as effective when chickens are moulted or highly stressed (Holt, 2003).

### **Short-Chain Fatty Acids In The Gut: A Key To Control Pathogen Colonization?**

Feed and drinking water sanitation, and the addition of acid to the crop appears to prevent pathogen colonization in the live animals, but the type of acid and its concentration can be very important. *Salmonella* colonization of the caeca and internal organs is not always affected by these treatments, especially if the infection pressure is high. Acids from feed or drinking water are not effective further down in the intestinal tract because *Salmonella* colonization is mainly in the caeca (Desmidt *et al.*, 1997). Because the caecum is the main fermentation site, the concentrations of short-chain fatty acids are already higher there than in other intestinal segments (Engberg *et al.*, 2002). One-day-old broilers had no short chain fatty acids in their caeca, but concentrations were high by 10 days post-hatch (Van Der Wielen *et al.*, 2000). Acetic acid is the predominant short-chain fatty acid in the caeca, with concentrations ranging between 70 and 90  $\mu\text{mol/g}$  caecal content (Engberg *et al.*, 2002, Van Der Wielen *et al.*, 2002). In most studies, the caecal butyrate concentration ranges between 10 and 40  $\mu\text{mol/g}$  in chicken caeca, and the propionate concentration is even less (Engberg *et al.*, 2002, Van Der Wielen *et al.*, 2002). Because short-chain fatty acids can affect invasion and virulence gene expression of *Salmonella* (Lawhon *et al.*, 2002; Van Immerseel *et al.*, 2004; Gantois *et al.*, 2005), the natural quantities of



the short-chain fatty acids could play an important role in *Salmonella* colonization. If short-chain fatty acid production in the caeca could be altered by changes in feed composition, producers would have a very cost-effective and efficient way of controlling *Salmonella*.

It has already been shown in various animal species that *Salmonella* colonization of the gut is decreased when the bifidobacterial population is increased, either by administration of bifidobacteria as probiotic strains, or by addition of certain types of oligosaccharides that stimulate proliferation of these bacteria in the gut (Asahara *et al.*, 2001; Buddington *et al.*, 2002; Bovee-Oudenhoven *et al.*, 2003; Silva *et al.*, 2004; Thitaram *et al.*, 2005). When the caecal *Bifidobacterium* population in broilers was increased by isomalto-oligosaccharide addition to the feed, and the animals were infected with a high dose of *Salmonella* Typhimurium, large reductions in caecal colonization were observed (Thitaram *et al.*, 2005).

Increases in lactic acid bacterial counts in the gut are correlated with increases in butyric acid concentrations (Kleessen *et al.*, 2001; Humblot *et al.*, 2005), and *Salmonella* colonization is decreased when butyric acid concentrations in the gut are increased (Van Immerseel *et al.*, 2004, 2005). Bifidobacteria increase butyric acid concentrations, but these bacteria do not produce butyric acid themselves. Lactic acid bacteria, such as lactobacilli and bifidobacteria, stimulate proliferation of butyric acid producing bacteria. This mechanism is called cross-feeding. It has been shown that lactic acid, produced *in vitro* by *Bifidobacterium adolescentis* with starch as sole carbon source, is used by *Anaerostipes caccae* and *Eubacterium hallii* (in co-culture) for the production of large concentrations butyric acid (Duncan *et al.*, 2004). Another approach would be a direct stimulation of butyric acid producing bacteria. In human gut samples, butyric acid producers are anaerobic bacteria belonging to the phylogenetic Clostridium clusters IV and XIVa (Pryde *et al.*, 2002), and species related to *Roseburia*, *Eubacterium*, *Faecalibacterium* and *Coprococcus* can also produce butyrate (Pryde *et al.*, 2002). Many of the butyrate producing microbiota that are identified are net consumers of acetate (Duncan *et al.*, 2004). It is not clear whether similar mechanisms exist in poultry gut microbiota. Random cloning and sequencing of 16S rDNA sequences isolated from chicken caeca revealed more than 85% of the clones belonging to eubacteria and clostridia (Bjerrum, 2005). Approximately 10% of the clones had high similarity with *Faecalibacterium prausnitzii*, a species that produces butyric acid in the human gut (Bjerrum, 2005).

## Conclusive Remarks

It is now evident that the addition of organic acids can have a beneficial effect on the quality of poultry by decreasing *Salmonella* and possibly other potentially pathogenic bacteria. The idea that probiotic and prebiotic applications, or simply rational design of feed composition could lead to similar effects producing favourable SCFA patterns is an interesting hypothesis, but research on this matter in poultry is still lacking. Recent studies have just started to generally describe the caecal microbiota of chickens (Lu *et al.*, 2003a,b; Bjerrum, 2005).

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## References

- Al-Chalaby, Z.A.M., Hinton, M. and Linton, A.H. (1985). Failure of drinking water sanitisation to reduce the incidence of natural *Salmonella* in broiler chickens. *The Veterinary Record*, 116, 364-365.
- Al-Tarazi, Y.H. and Alshawabkeh, K. (2003). Effect of dietary formic and propionic acids on *Salmonella* Pullorum shedding and mortality in layer chicks after experimental infection. *Journal of Veterinary Medicine B*, 50, 112-117.
- Asahara, T., Nomoto, K., Shimizu, K., Watanuki, M. and tanaka, R. (2001). Increased resistance of mice to *Salmonella* enterica serovar Typhimurium infection by symbiotic administration of



- Bifidobacteria and transgalactosylated oligosaccharides. *Journal of Applied Microbiology*, 91, 985-996.
- Berchieri, A. Jr., and Barrow, P.A. (1996). Reduction in incidence of experimental fowl typhoid by incorporation of a commercial formic acid preparation (Bio-Add™) into poultry feed. *Poultry Science*, 75, 339-341.
- Bjerrum, L. (2005). The intestinal microbiota of broiler chickens. PhD Thesis, Aarhus, Denmark.
- Bovee-Oudenhoven, I.M., Ten Bruggencate, S.J., Lettink-Wissink, M.L., Van Der Meer, R. (2003). Dietary fructo-oligosaccharides and lactulose inhibit intestinal colonisation but stimulate translocation of *Salmonella* in rats. *Gut*, 52, 1572-1578.
- Buddington, K.K., Donahoo, J.B. and Buddington, R.K. (2002). Dietary oligofructose and inulin protect mice from enteric and systemic pathogens and tumor inducers. *Journal of Nutrition*, 132, 472-477.
- Byrd, J. A., Hargis, B.M., Caldwell, D.J., Bailey, R.H., Herron, K.L., McReynolds, J.L., Brewer, R.L., Anderson, R.C., Bischoff, K.M., Callaway, T.R. and Kubena, L.F. (2001). Effect of lactic acid administration in the drinking water during preslaughter feed withdrawal on *Salmonella* and *Campylobacter* contamination of broilers. *Poultry Science*, 80, 278-283.
- Chaveerach, P., Keuzenkamp, D.A., Lipman, L.J. and Van Knapen, F. (2004). Effect of organic acids in drinking water for young broilers on *Campylobacter* infection, volatile fatty acid production, gut microflora and histological cell changes. *Poultry Science*, 83, 330-334.
- Chaveerach, P., Keuzenkamp, D.A., Urlings, H.A., Lipman, L.J. and Van Knapen, F. (2002). In vitro study on the effect of organic acids on *Campylobacter jejuni/coli* populations in mixtures of water and feed. *Poultry Science*, 81, 621-628.
- Clark, D.P. and Cronan, J.E. (2005). Two-carbon compounds and fatty acids as carbon sources. In : Neidhardt, F.C., *Escherichia coli* and *Salmonella* : Cellular and Molecular Biology, Web edition : <http://www.ecosal.org/ecosal/index.jsp>.
- Diez-Gonzalez F, Russell JB (1997) The ability of *Escherichia coli* O157:H7 to decrease its intercellular pH and resist the toxicity of acetic acid. *Microbiology* 143, 1175-1180
- Desmidt, M., Ducatelle, R. and Haesebrouck, F. (1997). Pathogenesis of *Salmonella* Enteritidis phage type four after experimental infection of young chickens. *Veterinary Microbiology*, 56, 99-109.

- Dirusso, C.C. and Black, P.N. (2004). Bacterial long chain fatty acid transport: gateway to a fatty acid-responsive signalling system. *Journal of Biological Chemistry*, 279, 49563-49566.
- Duncan, S.H., Holtrop, G., Lobley, G.E., Calder, A.G., Stewart, C.S. and Flint, H.J. (2004). Contribution of acetate to butyrate formation by human faecal bacteria. *British Journal of Nutrition*, 91: 915-923.
- Durant, J.A., Lowry, V.K., Nisbet, D.J., Stanker, L.H., Corrier, D.E. and Ricke, S.C. (1999). Short-chain fatty acids affect cell-association and invasion of Hep-2 cells by *Salmonella* Typhimurium. *Journal of Environmental Science and Health B*, 34, 1083-1099.
- Durant, J.A., Corrier, D.E. and Ricke, S.C. (2000). Short-chain volatile fatty modulate the expression of the *hilA* and *invF* genes of *Salmonella* Typhimurium. *Journal of Food Protection*, 63, 573-578.
- Engberg, R.M., Hedemann, M.S. and Jensen, B.B. (2002). The influence of grinding and pelleting of feed on the microbial composition and activity in the digestive tract of broiler chickens. *British Poultry Science*, 43, 569-579.
- Flythe MD, Russell JB (in press) The ability of fermentation acids to inhibit the deamination of *Clostridium sporogenes* MD1 via a mechanism that does not involve protonmotive force dissipation. *Microbiology*
- Gantois, I., Ducatelle, R., Pasmans, F., Haesebrouck, F., Hautefort, I., Thompson, A., Hinton, J.C. and Van Immerseel, F. (2006). Butyrate specifically down-regulates *Salmonella* pathogenicity island I gene expression. *Applied and Environmental Microbiology*, 72, 946-949.
- Gimenez, R., nunez, M.F., Badia, J., Aguilar, J. and Baldoma, L. (2003). The gene *yjcG*, cotranscribed with the gene *acs*, encodes an acetate permease in *Escherichia coli*. *Journal of Bacteriology*, 185, 6448-6455.
- Hensel, M. (2004). Evolution of pathogenicity islands of *Salmonella enterica*. *International Journal of Medical Microbiology*, 294, 95-102.
- Heres, L., Engel, B., Van Knapen, F., Wagenaar, J.A. and Urlings, B.A.P. (2003). Effect of fermented feed on the susceptibility for *Campylobacter jejuni* colonisation in broiler chickens with and without concurrent inoculation of *Salmonella* Enteritidis. *International Journal of Food Microbiology*, 87, 75-86.

- Heres, L., Engel, B., Urlings, H.A.P., Wagenaar, J.A. and Van Knapen, F. (2004). Effect of acidified feed on susceptibility of broiler chickens to intestinal infection by *Campylobacter* and *Salmonella*. *Veterinary Microbiology*, 99, 259-267.
- Hinton, M., Linton, A.H., and Perry, F.G. (1985). Control of *Salmonella* by acid disinfection of chicks' food. *The Veterinary Record*, 116, 502.
- Hinton, M. (1988). *Salmonella* infection in chicks following the consumption of artificially contaminated feed. *Epidemiology and Infection*, 100, 247-256.
- Hinton, M. and Linton, A.H. (1988). Control of *Salmonella* infections in broiler chickens by the acid treatment of their feed. *The Veterinary Record*, 123, 416-421.
- Hinton, M. (1990). Antibacterila activity of short-chain organic acids. *The Veterinary Record*, 126, 370.
- Holt, P.S. (2003). Molting and *Salmonella* enterica serovar Enteritidis infection : the problem and some solutions. *Poultry Science*, 82, 1008-1010.
- Horswill, A.R. and Escalante-Semerana, J.C. (1999). *Salmonella* Typhimurium LT2 catabolizes propionate via the 2-methylcitric acid cycle. *Journal of Bacteriology*, 181, 5615-5623.
- Hsiao, C. and Siebert, K.J. (1999). Modeling the inhibitory effects of organic acids on bacteria. *International Journal of Food Microbiology*, 47, 189-201.
- Humblot, C., Bruneau, A., Sutren, M., Lhoste, E.F., Dore, J., Andrieux, C. and Rabot, S. (2005). Brussels sprouts, inulin and fermented milk alter the faecal microbiota of human microbiota-associated rats as shown by PCR-temporal temperature gradient gel electrophoresis using universal, *Lactobacillus* and *Bifidobacterium* 16S rRNA gene primers. *British Journal of Nutrition*, 93, 677-684.
- Hume, M.E., Corrier, D.E., Ambrus, S., Hinton, A. and DeLoach, J.R. (1993). Effectiveness of dietary propionic acid in controlling *Salmonella* Typhimurium colonization in broiler chicks, *Avian Diseases*, 37, 1051-1056.
- Humphrey, T.J. and Lanning, D.G. (1988). The vertical transmission of *Salmonella* and formic acid treatment of chicken feed. *Epidemiology and Infection*, 100, 43-49.
- Iba, A.M. and Berchieri, A. (1995). Studies on the use of a formic acid-propionic acid mixture (Bio-Add™) to control experimental *Salmonella* infection in broiler chickens. *Avian Pathology*, 24, 303-311.

- Izat, A.L., Tidwell, N.M., Thomas, R.A., Reiber, M.A., Adams, M.H., Colberg, M. and Waldroup, P.W. (1990a). Effects of a buffered propionic acid in diets on the performance of broiler chickens and on microflora in the intestine and carcass. *Poultry Science*, *69*, 818-826.
- Izat, A.L., Adams, M.H., Cabel, M.C., Colberg, M., Reiber, M.A., Skinner, J.T. and Waldroup, P.W. (1990b). Effects of formic acid or calcium formate in feed on performance and microbiological characteristics of broilers. *Poultry Science*, *69*, 1876-1882.
- Jenkins, L.S. and Nunn, W.D. (1987a). Regulation of the ato operon by the atoC genes in *Escherichia coli*. *Journal of Bacteriology*, *169*, 2096-2102.
- Jenkins, L.S. and Nunn, W.D. (1987b). Genetic and molecular characterization of the genes involved in short-chain fatty acid degradation in *Escherichia coli*: the ato system. *Journal of Bacteriology*, *169*, 42-52.
- Jones, B.D. (2005). *Salmonella* invasion gene regulation: a story of environmental awareness. *Journal of Microbiology*, *43*: 110-117.
- Kashket, E.R. (1987). Bioenergetics of lactic acid bacteria: cytoplasmic pH and osmotolerance. *FEMS Microbiology Reviews*, *46*, 233-244.
- Khan, M. and Katamay, M. (1969). Antagonistic effects of fatty acids against *Salmonella* in meat and bone meal. *Applied Microbiology*, *17*, 402-404.
- Kleessen, B., Hartmann, L. and Blaut, M. (2001). Oligofructose and long-chain inulin: influence on the gut microbial ecology of rats associated with a human faecal flora. *British Journal of Nutrition*, *86*, 291-300.
- Kubena, L.F., Byrd, J.A., Moore, R.W., Ricke, S.C. and Nisbet, D.J. (2005). Effects of drinking water treatment on susceptibility of laying hens to *Salmonella* Enteritidis during forced molt. *Poultry Science*, *84*, 204-211.
- Kwon, Y.M. and Ricke, S.C. (1998). Induction of acid resistance of *Salmonella* Typhimurium by exposure to short-chain fatty acids. *Applied and Environmental Microbiology*, *64*, 3458-3463.
- Lawhon, S.D., Maurer, R., Suyemoto, M and Altier, C. (2002). Intestinal short-chain fatty acids alter *Salmonella* Typhimurium invasion gene expression and virulence through BarA/SirA. *Molecular Microbiology*, *46*, 1451-1464.
- Lostroh, C.P. and Lee, C.A. (2001). The *Salmonella* pathogenicity island-1 type III secretion system. *Microbes and Infection*, *3*, 1281-1291.

- Louis, P., Duncan, S.H., McCrae, S.I., Millar, J., Jackson, M.S. and Flint, H.J. (2004). Restricted distribution of the butyrate kinase pathway among butyrate-producing bacteria from the human colon. *Journal of Bacteriology*, *186*, 2099-2106.
- Lu, J., U. Idris, B. Harmon, C. Hofacre, J. J. Maurer, R. and M. D. Lee. (2003). Diversity and succession of the intestinal bacterial community of the maturing broiler chicken. *Applied and Environmental Microbiology*, *69*, 6816-6824.
- McHan, F. and Shotts, E.B. (1992). Effect of feeding selected short-chain fatty acids on the in vivo attachment of *Salmonella* Typhimurium in chick ceca. *Avian Diseases*, *36*, 139-142.
- Morgan-Jones, S.C. (1980). The occurrence of *Salmonellae* during the rearing of broiler chicks. *British Poultry Science*, *21*, 463-470.
- Ohmomo S, Tanaka O, Kitamoto HK, Cai Y (2002) Silage and microbial performance, old story but new problems. *Japan Agricultural Research Quarterly* *36*, 59-71
- Padan I, Zilberstein D, Schuldiner S (1981) pH homeostasis in bacteria. *Biochimica Biophysica Acta*, *650*, 151-166
- Pryde, S.E., Duncan, S.H., Hold, G.L., Stewart, C.S. and Flint, H.J. (2002). The microbiology of butyrate formation in the human colon. *FEMS Microbiology Letters*, *217*, 133-139.
- Russell, J.B. and Diez-Gonzalez, F. (1998). The effects of fermentation acids on bacterial growth. *Advances in Microbial Physiology*, *39*, 205-234.
- Russell, J.B. (1991). Resistance of *Streptococcus bovis* to acetic acids at low pH: anion accumulation versus uncoupling. *Journal of Applied Bacteriology*, *73*, 363-370.
- Russell, J.B. and Strobel, H.J. (1990). ATPase-dependent energy spilling by the ruminal bacterium, *Streptococcus bovis*. *Archives in Microbiology*, *153*, 378-383.
- Silva, A.M., Barbosa, F.H., Duarte, R., Vieira, L.Q., Arantes, R.M. and Nicoli, J.R. (2004). Effect of *Bifidobacterium longum* ingestion on experimental salmonellosis in mice. *Journal of Applied Microbiology*, *97*, 29-37.
- Smyser, C.F. and Snoeyenbos, G.H. (1979). Evaluation of organic acids and other compounds as *Salmonella* antagonists in meat and bone meal. *Poultry Science*, *58*, 50-54.
- Sprong, R.C., Hulstein, M.F.E. and Van der Meer, R. (2001). Bactericidal activities of milk lipids. *Antimicrobial Agents and Chemotherapy*, *45*, 1298-1301.

- Thitaram, S.N., Chung, C.H., Day, D.F., Hinton, A.Jr., Bailey, J.S., Siragusa, G.R. (2005). Isomaltooligosaccharides increases cecal Bifidobacterium population in young broiler chickens. *Poultry Science*, 84, 998-1003.
- Thompson, J.L. and Hinton, M. (1997). Antibacterial activity of formic and propionic acids in the diet of hens on *Salmonellas* in the crop. *British Poultry Science*, 38, 59-65.
- Thormar, H., Hilmarsson, H. and Bersson, G. (2006). Stable concentrated emulsions of the 1-monoglyceride of capric acid (monocaprin) with microbicidal activities against the food-borne bacteria *Campylobacter jejuni*, *Salmonella* spp., and *Escherichia coli*. *Applied and Environmental Microbiology*, 72, 522-526.
- Van Den berg, B. (2005). The FadL family : unusual transporters for unusual substrates. *Current Opinions in Structural Biology*, 15, 401-407.
- Van Der Wielen, P.W., Biesterveld, S., Notermans, S., Hofstra, H., Urlings, B.A. and Van Knapen, F. (2000). Role of volatile fatty acids in development of the cecal microflora in broiler chickens during growth. *Applied and Environmental Microbiology*, 71, 2206-2207.
- Van Immerseel, F., De Buck, J., Meulemans, G., Pasmans, F., Velge, P., Bottreau, E., Haesebrouck, F. and Ducatelle, R. (2003) Invasion of *Salmonella* Enteritidis in avian intestinal epithelial cells *in vitro* is influenced by short-chain fatty acids, *International Journal of Food Microbiology*, 85, 237 – 248.
- Van Immerseel, F., De Buck, J., De Smet, I., Pasmans, F., Haesebrouck, F. and Ducatelle, R. (2004). Interactions of butyric acid and acetic acid-treated *Salmonella* with chicken primary cecal epithelial cells *in vitro*. *Avian Diseases*, 48, 384-391.
- Van Immerseel, F., De Buck, J., Boyen, F., Bohez, L., Pasmans, F., Volf, J., Sevcik, M., Rychlik, I., Haesebrouck, F. and Ducatelle, R. (2004b). Medium-chain fatty acids decrease colonization and invasion shortly after infection with *Salmonella* Enteritidis in chickens through *hila* suppression. *Applied and Environmental Microbiology*, 70, 3582-3587.
- Van Immerseel, F., Fievez, V., De Buck, J., Pasmans, F., Martel, A., Haesebrouck, F. and Ducatelle, R. (2004c). Microencapsulated short-chain fatty acids in feed modify colonization and invasion early after infection with *Salmonella* Enteritidis in young chickens. *Poultry Science*, 83, 69-74.

- Van Immerseel, F., Boyen, F., Gantois, I., Timbermont, L., Bohez, L., Pasmans, F., Haesebrouck, F. and Ducatelle, R. (2005). Supplementation of coated butyric acid in the feed reduces colonization and shedding of *Salmonella* in poultry. *Poultry Science*, 84, 1851-1856.
- Van Staden, J.J., Van Der Made, H.N. and Jordaan, E. (1980). The control of bacterial contamination in carcass meal with propionic acid. *Onderstepoort Journal of Veterinary Research*, 47, 77-82.
- Williams, J.E. (1981). *Salmonella* in poultry feed : a worldwide review. *World's Poultry Science Journal*, 37, 6-19.