The Use of Organic Acids and Phytogenics to Promote Intestinal Tract Health

PEER REVIEWED

Introduction

The modern poultry industry demands increasing productivity and efficiency. Historically, antibiotics, particularly for growth promotion (AGPs), have made a significant contribution to achieving the modern levels of production. However, globally, the use of antibiotics is under great scrutiny and changes to legislation will require the poultry industry to review production practices with much less reliance on antibiotics. The 2006 European Union ban on such use is one obvious example, while some countries in Europe now require producers to review and decrease therapeutic use of antibiotics. AGP removal invariably leads to reduced animal performance and increased disease problems. Moreover, there is a need to reduce the incidence of human enteropathogen infections derived from food animals.

These factors have increased the interest in natural alternatives with the potential to fill the void created by the reduction in antibiotic use. Two groups of compounds where there is particular interest are organic acids and phytogenics.

Organic acids (often referred to as volatile fatty acids, fatty acids, carboxylic acids or weak acids) are naturally-occurring, carbon-containing (hence organic) compounds with acidic properties. Phytogenics (often referred to as essential oils, plant extracts, botanicals or phytobiotics) are also naturally-occurring compounds derived from plants. This grouping represents a diverse range of compounds, of which the oils are probably of greatest focus.

Organic Acids

Interest in microbes and their fermentation end products (e.g. organic acids), for food preservation purposes, has been apparent for many centuries, if not millennia. There is evidence of cheese-making dating back to 6000 BC (Ross *et al*., 2002). The historic use of fermentation and the selective growth of some bacteria at the expense of others is, in itself, clear proof of the differing susceptibility of microorganisms to organic acids. Microorganisms can regulate cytoplasmic pH and thus can ameliorate the effects of a degree of acid stress but the extent to which this is achievable varies between different microbes. For example, and as an indication of this, growth of *Escherichia coli (E. coli)* was inhibited at a formic acid concentration of less than 1/10th of that required to inhibit lactobacilli (minimum inhibitory concentrations (MICs) of 2.20 and 26.0 mg/L, respectively) (Nakai and Siebert, 2003).

The antimicrobial effects of organic acids are well documented. An organic acids pKa value defines the pH at which half the acid is dissociated. It is the undissociated, uncharged, lipophilic portion of the acid that is believed to diffuse freely across the microbial cell membrane. At a pH higher than the organic acid's pKa value, more of the acid will be in the dissociated state. Thus, once in the more neutral pH of the cell cytoplasm (than say the acidic parts of the intestine), more of the acid dissociates. The resulting H+ ions acidify the cytoplasm, stressing the pH regulatory mechanisms of the cell, whilst the anion portion accumulates in the cell. Both disrupt microbial cell physiology and metabolism; for example, through denaturing and causing oxidative damage to proteins/enzymes, energy expenditure, osmotic stress and compromising membrane integrity/function, all of which inhibit growth and/or lead to cell death (Theron and Rykers Lues, 2011). Additionally, in bacteria, SCFAs have the potential to regulate virulence gene expression. For example, pre-treatment of *Salmonella enterica* serovar Enteritidis with butyrate reduces invasion of avian/chicken intestinal and caecal epithelial cells (Van Immerseel *et al*., 2004), while high levels of butyrate inhibit virulence factor production in *Listeria monocytogenes* (Sun and O'Riordan, 2013).

In-vivo Efficacy of Organic Acids

In the 1960s, there were concerns about Salmonella contamination of animal by-product meals (e.g. meat and bone) and cross-contamination to mixed feeds. It was reported that low molecular weight volatile fatty acids were particularly bacteriostatic or bactericidal against Salmonella (Khan and Katamay, 1969). Hinton (1988) demonstrated that feeding chickens feed that had been artificially contaminated with Salmonella resulted in intestinal colonisation and excretion of the Salmonella; whilst Iba and Berchieri (1995) reported that a mixture of formic and propionic acid, added to feed inoculated with strains of either S. Enteritidis, Typhimurium or Agona, reduced the numbers of Salmonella in the caeca by approximately 7 Log10 units, and had a strong bactericidal

effect against a strain of *S. Typhimurium* up to 28 days post feed manufacture. Whilst initial work focussed primarily on Salmonella, other work with organic acids has shown wider effects on the intestinal microbiota. Using PCR-based approaches, Nava *et al.* (2009) reported that a formic and propionic acid-based blend, added to the drinking water at 0.0525 %, resulted in much greater numbers of lactobacilli in the lumen of the ileum.

Indeed, recent work we have conducted has confirmed that differing concentrations and combinations of formic and propionic acids, and their salts, on different silica-based carriers, reduced caecal *E. coli* by up to 1.8 Log10 CFU/g contents and increased lactobacilli by up to 2.0 Log10 CFU/g contents in broilers orally challenged with a subclinical dose of *E. coli* K88 (Khodambashi Emami *et al.*, 2015).

Organic acids are known to have positive effects on intestinal morphology and butyric acid is a preferred energy substrate for colonocytes. Small intestine villus height is important as it determines the functional maturity of enterocytes arriving at the villus tip. With shorter villi, enterocytes reach the villus apex earlier, when their enzyme secretory capacity is less developed, leading to reduced digestive and absorptive efficiency. Work by Garcia et al. (2007) and Senkoylu *et al.* (2007) showed that formic acid supplementation of the diet at up to 1.0 %, or a formic and propionic acid-based product at 0.3 %, increased villus height by up to 15 %, villus surface area by up to 28 % and performance by up to 12 % in broilers. A larger surface area provides an increased surface for digestive and absorptive processes.

Organic acids are reported to have effects on other bodily systems. Of particular interest are effects on the immune system. Khodambashi Emami *et al.* (2013) showed that organic acid addition (0.2 %), to an apparently phosphorus-sufficient diet, changed components of the primary and secondary humoral immune response to sheep red blood cells in broilers, and Ghasemi *et al.* (2014) reported that an organic acid blend at 0.4 % of the diet increased primary antibody titres to both infectious bursal disease virus and infectious bronchitis virus following vaccination. Another area of interest is reproductive function and progeny performance. Araujo *et al.* (2015) reported that providing broiler breeder hens, from 25 weeks of age, with a commercial organic acid product, based on the combination of formic and propionic acids at 0.2 % improved fertility at 35 and 45 weeks of age. Moreover, the performance of progeny from supplemented hens was also improved. Given ascending infections of the oviduct from the cloaca are well accepted (De Buck *et al.*, 2004) and that microorganisms can infect hatching eggs at any point during development (De Reu *et al.*, 2006), it is not unreasonable to suggest that a more favourable intestinal microbiota can directly impact reproductive tract health, function and progeny development.

Phytogenics

Animals and humans have been using plant material for 'medicinal' purposes for probably as long as they have existed. As modern medicine became more advanced, a tendency to be sceptical of the potential benefits that may be derived from the plant kingdom undoubtedly evolved. Phrases such as 'hocus-pocus' and 'witchcraft' have commonly been associated with the practice of promoting the use of plant-derived compounds for health benefits. However, we do not need to look far to find examples of plant components used in modern medicine. Morphine, an opiate originally isolated from the poppy plant around 200 years ago, is used routinely for both acute and chronic pain. Another example is Paclitaxel, a well-known compound derived from the bark of the Pacific yew tree (*Taxus brevifolia*), which is used in the treatment of various cancers (National Cancer Institute, USA). Moreover, it is believed that humans first started using herbs and spices in food, particularly in warmer climates, to help control microbes that cause intestinal infections, and various plant compounds have been demonstrated to have strong antimicrobial properties. It is clear that plants contain powerful compounds.

Phytogenics can be very effective antimicrobials and work has shown that compounds such as oregano, specifically the components carvacrol and thymol, may be particularly effective (Mith *et al*., 2014). Generally, phytogenics are considered to be slightly more efficacious against gram-positive compared to gram-negative bacteria (Zeng *et al*., 2015) and the individual components of phytogenic compounds may have additive or synergistic effects. Carvacrol and thymol (two main components of oregano) were shown to have additive effects against *Staphylococcus aureus* and *Pseudomonas aeruginosa* (Lambert *et al*., 2001). Whilst the precise antimicrobial mode of action(s) of phytogenic compounds is not yet fully understood, a number of mechanisms have been proposed. One suggestion is the ability to penetrate the microbial cell membrane and inhibit internal cell functioning; whilst phenols are understood to disrupt the cell membrane, alter permeability and cause leakage of cell contents (Calo *et al*., 2015).

In-vivo Efficacy of Phytogenics

Whilst interpretation of average performance responses to phytogenic supplementation can be difficult due to the range of phytogenic compounds used, their exact composition and dose rate, on average, phytogenic use in poultry has been reported to improve both weight gain and feed conversion by 3 % (Zeng *et al*., 2015). Some of these studies have reported improvements of up to 15 % and 8 % for weight gain and FCR, respectively.

The key, *in-vivo* benefits of phytogenic compounds are believed to primarily result from their antimicrobial activity. Various studies have been conducted, mainly in broilers, which report reductions in *Clostridium perfringens, E. coli, Enterobacteriaceae*, etc., mainly in the caeca, with various phytogenic compounds/blends (e.g. Jamroz, 2005; Placha, 2014). The antimicrobial activity of phytogenics would appear to be quite broad and a consistent effect seems to be to reduce the impact of coccidiosis in broilers. In a challenge study with *Eimeria acervulina* and *Eimeria* maxima (at 14 days of age), broilers receiving diets supplemented with oregano (Orego-Stim) at 0.03 or 0.06 % had similar performance to both the non-challenged control and a group receiving a standard coccidiostat (salinomycin) (Tsinas, 2011). The performance of all these groups was better than the challenged control. The reductions in intestinal lesion score associated with coccidiosis, and in oocyst excretion, would further confirm the benefit of oregano supplementation for the prevention and control of coccidiosis. Similar effects have been seen in other studies (e.g. Mohiti-Asli and Ghanaatparast-Rashti, 2015).

Various other *in-vivo* benefits have been reported with phytogenics. Sarica *et al.* (2014) showed that oregano at 0.025 or 0.050 % of the diet improved intestinal morphology in broilers, and there have been a number of reports of improvements in nutrient digestibility with phytogenics (e.g. Basmacioglu Malayoglu, 2010). Moreover, Eleiwa *et al.* (2011) showed that supplementing the drinking water of E. coli-infected broilers with oregano oil (0.3 ml/L) modulated immune parameters and reduced the impact of the infection on clinical signs and bird performance.

Conclusion

The drive for increased productivity, but with less reliance on antibiotics, requires credible alternatives. Organic acids (e.g. formic and propionic acids) have a long history of antimicrobial activity for food preservation purposes, as well as in farmed animals for microbial control, particularly Salmonella. The use of phytogenic compounds in farmed animals is, perhaps, a bit more recent but their historic value to humans (and animals) for medicinal purposes and maintenance of 'health' is equally important. However, the more work that is done with both organic acids and phytogenic compounds reveals a diverse range of benefits. Most of these undoubtedly emanate from the antimicrobial activity of the compounds and, therefore, positive effects on gut health and intestinal function. There are numerous organic acids and even more phytogenic compounds. A better understanding of the compounds, their effects and how they can be optimised in poultry will enhance the efficacy and consistency of products available to the industry.

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