The use of antibiotics in livestock or fish feed as a growth promoter or feed additive has become a hot topic for debate among farmers, researchers and planners. As a result, an alternative to antibiotics is highly sought after and has lead many researchers to divert their efforts in this direction.

Recently, probiotics added to fish feed has received considerable interest among aquaculturists, but the utility of their use in the field has been mixed. Some practitioners have a favourable opinion regarding probiotics, whereas others are yet to be convinced of their performance. Against this backdrop, the introduction of organic acid as an alternative to antibiotics in fish feed may present an interesting alternative.

Some promising results using organic acids have already been documented in livestock and poultry diets, although their use in aquafeed has been very limited to date. Hence, this study compared organic acids in livestock diets with that of fish feed. Indeed, in theoretical terms it would be more logical to use organic acids in fish, especially the agastric species, given that they lack the acidic conditions provided by the presence of a true stomach.

Antibiotics fed to farm animals as growth promoters have been associated with the spreading of bacterial resistance. This potential risk, combined with consumer demand for a medicinal-product free food chain, necessitates a reduction in the use of antibiotics in food production. Consequently, alternative strategies to control microbial activity in the gastro-intestinal tract of monogastric animals, including fish, require exploration. Organic acids can play a vital role in this respect. Experience with pigs has shown that several organic acids, such as citric acid, fumaric acid, formic acid, lactic acid and propionic acid have a positive influence on growth performance and on intestinal proteolytic processes.

Dietary acidification lowers gastric pH, maintains a more desirable microflora and enhances the digestion process. Promising results were found using organic acids in weanling piglets and chicks, where the stomach is not yet fully developed and functional, and also in fishes (Baruah et al 2006). Hence, the use of organic acids in fish feed may be beneficial, especially in agastric species (carp), as they lack a true stomach.

A recent trial with a commercial product (Raafres AQ, Guybro Chemicals, Mumbai, India) containing organic acids and their salts along with _-glucan and mannan oligosaccharides and other “anti-stress” factors has shown a reduction in the pH of the feed and in the gastrointestinal tract of _Macrobrachium rosenbergii_.

Further, inhibition of the growth of pathogenic bacteria like _E coli_, _Vibrio_ and _Salmonella_ was demonstrated, as was an improvement in the absorption of nutrients through better digestion of complex food molecules and by increasing the size of villi in the intestine. In contrast, evidence in rat and human subjects suggests that a more acidic diet can result in the dissociation of calcium bone, which could lead to a reduction in bone mineralisation.

An important objective of dietary acidification is the inhibition of intestinal bacteria competing with the host for available nutrients and reduction of toxic bacterial metabolites like ammonia and amines, thus improving the weight gain of the target animal. Furthermore, the growth inhibition of potential pathogenic bacteria and zoonotic bacteria like _E coli_ and _Salmonella_, both in the feed and in the gastrointestinal tract, has a clear benefit to animal health.

A decrease in total aerobes and _E coli_ was also observed by feeding citric acid to weanling pigs. Beside these, gastric acidity enhances solubility of minerals in the gastrointestinal tract and utilisation by animals. This is beneficial for agastic fishes like cyprinids, as better absorption of Ca and P takes place in acidic condition. Baruah et al 2007 reported increased mineral availability in _Labeo rohita_ fed plant-based diets. An increase in the apparent availability of Ca, P, Mg, Mn and Fe in rainbow trout fed fishmeal-based diets supplemented with citric acid has also been reported.

**MECHANISM OF ACTION**

Antibacterial activity increases with decreasing pH value. Organic acids are lipid-soluble in their undisassociated form – they are also able
to enter the microbial cell. However, a carrier-mediated transport mechanism seems to be involved in the membrane transport of these organic acids. Once in the cell, the acid releases the proton in the more alkaline environment, thereby decreasing intracellular pH (Figure 1). This influences the metabolism of microbes inhibiting the action of important enzymes and forces the bacterial cell to use energy to release protons, leading to an intracellular accumulation of acid anions; this is dependent on the pH gradient across the membrane.

The acid anion seems to be very important with respect to the antibacterial effect of organic acids and their salts. Several investigations have shown a strong bactericidal effect of organic acid without significantly decreasing the pH value in the gastrointestinal tract. In general, lactic acid bacteria are able to grow at a relatively low pH, which means that they are more resistant to organic acid than other bacteria species, e.g. E. coli. An explanation for this may be that gram-positive bacteria have a high intracellular potassium ion concentration, which counteracts the acid anion (Figure 2).

The antimicrobial effect of organic acids increases with increasing concentration and the increasing length of the carbon chain. However, gram-negative bacteria are able to take up and metabolise long and medium-chain organic acids. Furthermore, vegetative cells are more sensitive to organic acids than the corresponding spore forms.

**SITE OF ACTION**

Organic acids exert their antimicrobial action both in the feed and in the gastrointestinal tract of the animal. Following dietary intake, organic acids are only recovered from the proximal part of the pig's gastrointestinal tract, the stomach and small intestine. This explains that the strongest effect of organic acids with respect to digesta pH and antimicrobial activity is found in the stomach and small intestine.

In poultry, pathogenic bacteria like Salmonella enter the gastrointestinal tract via the crop. The environment of the crop with respect to microbial composition and pH seems to be very important in relation to the resistance to pathogens. High lactobacilli concentrations and low pH in the crop have shown to decrease the occurrence of salmonella in poultry. Also, the antibacterial effect of dietary organic acids in chickens is believed to take place mainly in the upper part of the digestive tract, the crop and gizzard.

Following the addition of a combination of formic and propionic acid, a high concentration of these acids could be recovered from the crop and gizzard. A study on the metabolism of dietary added propionic acid reveals that only a little, if any, dietary propionic acid reaches the lower digestive tract and the caeca. It seems that a similar mechanism may work in fish, although very little work has been done in this area.

**FUNCTION OF ORGANIC ACIDS IN ANIMAL AND FISH NUTRITION**

**Citric acid (CA)**

Dietary supplementation of CA has been shown to influence the availability of minerals in rainbow trout, Oncorhynchus mykiss and rohu, Labeo rohita. However, studies on other fish species are limited and the findings on gastrointestinal tract pH is inconsistent. CA addition (1.5 percent) to control diet did not significantly affect the pH, the concentration of volatile or non-volatile fatty acids or microflora (total aerobes, lactobacilli, clostridia, E. coli) in contents from the stomach, jejunum, caecum or lower colon of weanling pigs (Risley et al 1993). There are reports regarding the use of CA in fish feed (Baruah et al 2005) but its effect on gut microflora has not been studied.

**Propionic acid (PA)**

Luprosil-NC (product contains 53.5 percent PA) at levels of 0.3 and 1 percent did not affect the pH, lactic acid concentration or short-chain fatty acid concentration in the stomach and small intestine of piglets, but decreased E. coli counts in the stomach at a concentration of 1 percent but not at 0.3 percent. In broilers, the inclusion of 0.4 percent and 0.8 percent Luprosil-NC decreased the number of coliforms and E. coli in the small intestine without any effect on intestinal pH (Izat et al 1990).

Following periodic dosage of Salmonella typhimurium, the same authors observed a reduced number of salmonella on post-chill broiler carcasses when 0.4 percent Luprosil-NC was added to the diet. No such study has been done in fish. Propionic acid is used in moist fish feed as a preservative and also in the preparation of fish silage, which is an ideal ingredient in fish feed.

**Fumaric acid (FA)**

In experiments with weanling pigs, it was observed that the dietary addition of FA has no influence on pH, the concentration of volatile fatty acid and microflora (counts of total anaerobic bacteria, lactobacilli, clostridia and E. coli) in the entire gastrointestinal tract (Sutton et al 1991). The concentration of FA in the stomach and jejunum was increased when a control diet was supplemented with 1.5 percent FA.

However, studies (Gedek et al 1992) demonstrated a significant decreasing effect of 1.8 percent FA on lactobacilli in the duodenum, jejunum, ileum, caecum and colon; eubacteria in the duodenum, jejunum and ileum; enterococci in the duodenum and jejunum; and E. coli in the jejunum weanling piglets. There are also no reports on the use of FA in fish feed.

**Lactic acid (LA)**

Lactic acid addition at the concentration of 0.8 percent to a control weaner diet effectively reduces the level of E. coli in the duodenum and jejunum of eight-week-old piglets. Moreover, in contrast to the control-fed animals, piglets fed the acid-added diets had only non-haemolytic E. coli. Piglets fed diets supplemented with 0.7, 1.4 or 2.8 percent of lactic acid also showed changes in gastrointestinal characteristics (Maribo et al 2000).

The pH in the gastrointestinal tract was reduced by the acid addition and the lactobacilli numbers were lower in the small intestine (1.4 percent LA) and higher in the caecum and colon (0.7 percent LA) of pigs. Furthermore, LA decreased the counts of coliforms and increased the count of yeasts along the gastrointestinal tract. Unpublished data from Mikkelsen and Jensen showed that the addition of 0.9, 1.8 or 2.7 percent LA to liquid feeds offered to weaners decreased gastric pH, increased the number of lactobacilli and yeast and decreased the counts of coliform bacteria along the gastrointestinal tract.

Increased amounts of dietary LA (0.25, 0.5, 1 and 2 percent) in
ORGANIC ACIDS AS NON-ANTIBIOTIC NUTRACEUTICALS IN FISH AND PRAWN FEED

broiler chicken diets did not offer protection from caecal Salmonella colonisation or carcass contamination following oral challenge with Salmonella typhimurium.

Although there are no reports on the direct use of LA in fish feed, numerous studies have been carried out on the effect of LA-producing bacteria fed as probiotics. Probiotic feeding in fish and shrimp has shown promising results in terms of enhancing growth rate and immunity and functions by reducing the number of pathogenic bacteria and/or enhancing the absorption rate of nutrients. However, the effects of probiotics in fish diets are still under investigation and more research is required before any definitive conclusions can be made.

Formic acid

Vielma and Lall (1997) reported that an increase in dietary formic acid had no significant difference in the pH of the food chyme collected from proximal intestine of rainbow trout, whereas in the distal intestine, the pH was found to be higher but the change in microflora was not studied. In contrast, other authors observed a lower pH in the stomach of piglets following the addition of 0.35 percent formic acid. There was no effect along the gastrointestinal tract, due to the addition of formic acid.

However, when compared to animals fed the controlled diet, lower LA concentration in the stomach and lower counts of E coli were found following the addition of 1.2 percent formic acid. There was no effect on the number of yeasts recovered from the gastrointestinal tract.

In poultry, formic acid alone or in combination with propionic acid (68 percent formic acid and 20 percent propionic acid) at a concentration of 0.6 percent was effective in preventing Salmonella kedougou and Salmonella gallinarum infection.

Benzoic acid (BA)

Benzoic acid is not yet approved as an additive or preservative for pig, chicken or fish feed, but is extensively used as a preservative in human food. Experiments with weaning pigs fed two percent BA in starter feed showed that BA was detected in considerable amounts in the stomach and in smaller amounts in the small intestine, indicating that BA may not be metabolised as fast as other organic acids. The supplementation of feed with BA resulted in significantly lower counts of lactic acid bacteria, lactobacilli and yeast throughout the entire gastrointestinal tract (Maribo et al 2000). The number of coliform bacteria was numerically lower compared to the control diet.

CONCLUSIONS

The results from the literature indicate that the effects of different organic acids on intestinal bacteria vary substantially and depend on the dose of the respective organic acid in the feed. It is obvious that the majority of the organic acid inhibits the growth of coliform bacteria in most cases.

In conclusion, feeding a diet fortified with organic acids may offer the possibility of reducing the level of undesired microbes in the gut and can facilitate an enhanced growth rate. It seems that this is area shows potential but requires further research.

In summary, the addition of organic acid in fish feed may have the following advantages. It may:

- reduce the unwanted pathogenic microbial load in feed and the gut of fish
- reduce toxic microbial metabolites by reducing the pathogenic microbes
- enhance nutrient absorption due to proliferation of mucosal epithelium of the intestine
- reduce the discharge of phosphorus in water thereby preventing aquatic pollution, and
- reduce the risk of antibiotic residue in fish and prawns in those regions where antibiotics are used.

Mineral absorption may also be enhanced due to more solubilisation of Ca, P and Mg etc from animal protein sources, especially in stomach-less fishes. The bio-availability of P from plant ingredients increases in acidic medium due to phytase supplementation.

SUGGESTIONS FOR FURTHER RESEARCH

Further research in this area should include:

- the species specificity of organic acids should be tested in a range of commercially relevant fish and shrimp species
- the optimum dose of organic acid should be standardised with response to gastric and agastric species
- the efficacy of a mixture of organic acids should be tested in fish feed
- supplementation of dietary phytase along with different concentrations of organic acid should be explored in fish and shrimp feed
- a range of organic acids should be identified for use in fish feed
- any negative effects of dietary organic acid should be thoroughly studied
- the effect of dietary organic acids on fish metabolism should be studied in terms of haematological and enzymatic parameters, and
- the effect of dietary organic acid on feed intake should be studied.

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