Review

The use of acidifiers in fish nutrition

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Abstract

It is well established in the field of aquaculture that the use of antibiotic growth promoters (AGPs) as feed additives in the diets of fish and shrimp can improve live weight gain (LWG), feed conversion ratio (FCR) and survival rates. However, scientific knowledge and public concerns, especially in the EU, over the development of cross-resistance to antibiotics of importance to human health have led to a ban or a reduction in the use of such substances worldwide. Consequently, the aquafeed industry has turned its research attention to other additives in order to maintain performance and high survival rates in aquaculture. This review shows that acidifiers are an example of a group of additives which can play an important role in future in aquaculture diets. A number of studies, in cold-water and tropical species, indicate that a broad range of organic acids, their salts or admixtures can improve growth, feed utilization and disease resistance in fish.

Keywords: Acidifiers, Fish, Nutrition, Organic acids, Aquaculture

Review Methodology: The following databases were searched: CAB Abstracts, World Aquaculture Society database, Google Scholar and Scopus. Keyword search terms used were acidifiers, organic acids, fish, aquaculture, fish feed. In addition, the references from the articles obtained thereby were used to search for additional relevant material. Furthermore, the knowledge gained from editing the book 'Acidifiers in Animal Nutrition' was used. Colleagues were consulted and asked for any upcoming studies not yet published.

Introduction

Routine use of antibiotics as growth promoters is a subject for debate in animal farming and feed and food industries. The use of low levels of antibiotics in animal feeds creates the possibility of transferring immunity to antibiotics used against bacterial pathogens in animals and humans [1]. As a result of such concerns, the EU banned the prescription-free use of all the antibiotic growth promoters (AGPs) from livestock production with effect from January 2006. Public opinion and regulatory authorities in most exporting countries now focus on the misuse of antibiotics in aquaculture and public attention has shifted towards production methods. Therefore, alternatives to AGP are sought worldwide in a variety of forms. The earliest studies that showed that organic acids are able to positively influence animal performance when added to diets were published more than 30 years ago [2]. Acidifiers consisting of organic acids and their salts present a promising alternative, and they have received much attention as a potential replacement, for improving the performance and the health of the livestock. In animal nutrition, acidifiers exert their effects on performance via three different mechanisms [3]: (a) in the feed; (b) in the gastro-intestinal tract of the animal; and (c) in effects on the animal's metabolism (Table 1) (modified from [4]).

Role in Feed Hygiene

A certain level of contamination with fungi, bacteria or yeasts is unavoidable in nutrient-rich products like feeds. Under favourable conditions such microbes multiply rapidly during storage, especially at higher moisture levels (>14%) in warm environments. Acidifiers function as

Table 1 Effects of organic acids and their salts in animal nutrition (after [4])

Site of action	Effective form	Effects
Feed	H^+	pH reduction Reduction of acid binding capacity Reduction of microbial growth
	H^+ and Anion	Antibacterial effects
Intestinal tract	H^+	pH reduction in stomach and duodenum Improved pepsin activity
	Anion	Complexing agents for cations (Ca^{2+}, Mg^{2+}, Fe^{2+}, Cu^{2+}, Zn^{2+})
	H^+ and Anion	Antibacterial effects Change in microbial concentrations
Metabolism		Energy supply

conserving agents by reducing the pH of the feed, thereby inhibiting microbial growth and thus lowering the uptake of possibly pathogenic organisms and their toxic metabolites by the farm animals [3]. Malicki *et al.* [5] found that a mixture of formic and propionic acid (1% dosage) can act synergistically against *Escherichia coli* in stored fishmeal, which is an often-used ingredient in aqua feeds.

Role in the Intestinal Tract

The mode of action of organic acids in the intestinal tract involves two different mechanisms: on the one hand they reduce the pH level in the stomach, particularly in the small intestine, through delivery of H^+ ions, and on the other hand they inhibit growth of Gram-negative bacteria through the dissociation of the acids and the production of anions inside bacterial cells.

During periods of high feed intake, such as when the animals are young or when the feeds are high in protein, hydrochloric acid concentrations in the stomach are reduced. This reduction negatively impacts pepsin activation and pancreatic enzyme secretion and impairs digestion. Providing acidifiers in the feed addresses this problem and aids feed digestion [6]. Positive effects of organic acids on protein hydrolysis have been demonstrated [7]. Similarly, feed supplementation with organic acids has been shown to lead to lower duodenal pH, improved nitrogen retention and increased nutrient digestibility [8, 9].

The growth rates of many Gram-negative bacteria, such as *E. coli* or *Salmonellae*, are reduced below pH 5. Low pH also forms a natural barrier against microbes ascending from the ileum and large intestine. Moreover, low-molecular-weight acids are lipophilic and can diffuse across the cell membranes of Gram-negative bacteria. In the more alkaline cytoplasm, they dissociate and reduce the pH. This reduction alters cell metabolism and enzyme activity, thus inhibiting the growth of intralumenal microbes, especially that of pathogens. Several studies have demonstrated a reduction in bacterial counts in the

Table 2 Gross energy content of selected organic acids and their salts used in aquaculture feeds (modified from [3])

Organic acid/salt	Solubility in water	Gross energy (kcal/kg)
Formic acid	Very good	1385
Acetic acid	Very good	3535
Propionic acid	Very Good	4968
Lactic acid	Good	3607
Citric acid	Good	2460
Calcium formate	Low	931
Sodium formate	Very good	931
Calcium propionate	Good	3965
Calcium lactate	Low	2436

stomach [9] and the duodenum [10–12], while acid-tolerant, beneficial *Lactobacilli* seem to be unaffected or may even be enhanced in number [12].

Role in Metabolism

Most organic acids have high gross energy values (Table 2) (modified from [3]). Short-chain organic acids are generally absorbed through the intestinal epithelia by passive diffusion and they can be used in various metabolic pathways for energy generation, for instance, for ATP generation in the citric acid cycle. As the energy content of organic acids is completely used in metabolism it should be included in the energy content of feed rations. For example, propionic acid contains one to five times more energy than wheat [13].

Organic Acids in Aquaculture

The acid preservation of fish and fish viscera in the production of fish silage has been a common practice with widespread use in fish feeds and reported beneficial effects [14, 15]. According to Batista [16], fish silage production was initiated in the 1930s, initially with

Table 3 Formulae, physical and chemical characteristics of organic acids used as dietary acidifiers in aquaculture (modified from [19])

Acid	Formula	MM (g/mol)	Density (g/ml)	Form	pK-value
Formic	$HCOOH$	46.03	1.22	Liquid	3.75
Acetic	CH_3COOH	60.05	1.05	Liquid	4.76
Propionic	CH_3CH_2COOH	74.08	0.99	Liquid	4.88
Butyric	$CH_3CH_2CH_2COOH$	88.12	0.96	Liquid	4.82
Lactic	$CH_3CH(OH)COOH$	90.08	1.21	Liquid	3.83
Sorbic	$CH_3CH{:}CHCH{:}CHCOOH$	112.14	1.20	Solid	4.76
Malic	$COOHCH_2CH(OH)COOH$	134.09	1.61	Solid	3.4, 5.1
Citric	$COOHCH_2C(OH)(COOH)CH_2COOH$	192.14	1.67	Solid	3.13, 4.76, 6.4

sulphuric and hydrochloric acid preservation of fish waste. The production of acid-preserved fish silage can also be achieved either with organic or inorganic acids or blends. If inorganic acids are used, the pH of the silage has to be lowered to $\leqslant$pH 2 in order to obtain a fully preserved product. Therefore, before feeding this type of silage to animals, the pH must be neutralized. On the other hand, if organic acids such as formic or propionic acid are used, the silage is stable at pH levels of 3.5–4.0, enabling direct feeding without neutralization. Hence, most silage producers now use organic acids. Fish silage or liquefied fish protein is an effective way to convert fish by-catch and fish processing byproducts into nutritious feedstuffs for a wide variety of animals, such as poultry [17]. In aquaculture [18], 2.2% formic acid was used to produce sardine (*Sardine pilchardus*) fish hydrolysates for start-feeding of sea bass (*Dicentrarchus labrax*) larvae. The hydrolysate was incorporated in the diet at 10 and 19%. Performance results showed that the inclusion of fish hydrolysate gave similar growth results after 33 days of feeding, compared to an enzymatic fish hydrolysate (except the low inclusion of fish silage, which had lower wet weights), but the fish silage could significantly improve ($P<0.05$) the survival rate of sea bass larvae orally challenged with *Vibrio anguillarum*.

The beneficial effects of acid-preserved products caught the attention of the scientific community, leading to the investigation of the effects of these short-chain acids in fish feeds. Several studies have been conducted with different species including carnivores such as rainbow trout (*Oncorhynchus mykiss*), Atlantic salmon (*Salmo salar*) and Arctic charr (*Salvelinus alpinus*), herbivorous filter feeders (tilapia), omnivorous fish (carp, catfish) and shrimp.

Following the experiments in pig and poultry feeding, a wide variety of organic acids, their salts and admixtures has been tested in aquaculture diets (Table 3) (modified from [19]).

Effect of Diet Acidification in Salmonids

Early studies on the use of organic acids in fish diets included succinic and citric acids in diets for salmonids [20]. These included the partial substitution of protein (12%) by a single amino acid or an organic acid (succinic or citric) tested in rainbow trout diets. Trout that were fed the organic acid diets had lower voluntary feed intakes compared to the basal diets, or to a diet supplemented with purified protein. However, there was no large variation between treatments in the efficiency of protein and energy utilization.

Data from the 1990s showed more promising results from the use of dietary acidifiers to salmonids (Table 4). The effect of supplementing commercial diets with sodium salts of lactic and propionic acids were tested in Arctic charr in brackish water at 8°C [21]. Fish fed a diet with 1% sodium lactate added to it increased in weight from about 310 to about 630 g in 84 days, while fish fed diets without either salt reached a final weight of only 520 g ($P<0.05$). Inclusion of 1% sodium propionate in the diet however had a growth-depressing effect compared to the control ($P<0.05$). The gut contents of Arctic charr fed a diet supplemented with sodium lactate contained less water, energy, lipid, protein and free amino acids. It has been observed that charr feeding on high doses of commercial feeds, as often found under aquaculture conditions, tend to cause diarrhoea. When charr were fed on diets containing sodium lactate, diarrhoea did not occur, probably indicating much lower amounts of residual nutrients and water in the gut. It was also proposed that the growth-promoting effect of dietary lactate in Arctic charr is the result of the relatively slow gastric emptying rate [22]. An increased holding time in the stomach augments the antibacterial potential of the lactic acid salt, which can therefore enhance the inhibition of pathogenic bacteria [23]. The improved growth of the Arctic charr did not affect its chemical composition [24].

A similar study by Ringø [25] proved the growth-promoting effect ($P<0.05$) of 1% sodium acetate as an additive for Arctic charr reared in brackish water, while 1% sodium formate gave only a non-significant numerical improvement versus a negative control. The stimulated growth of the fish which were fed sodium acetate to some extent may be explained by the higher feed intake, but enhanced digestibilities of dietary components might also contribute to the increased growth. Addition of 1%

Table 4 Effects of the sodium salt of different organic acids on the performance of Arctic charr and Atlantic salmon

Fish species	Acid/acid salt	Dose (%)	SGR (%)[1]	FCR[2]	Reference
Arctic charr	Control	0	0.61	n.d.	[21]
	Na-lactate	1	0.83[3]		
	Na-propionate	1	0.49[3]		
Arctic charr	Control	0	0.51	1.20	[25]
	Na-formate	1	0.58	1.08	
	Na-acetate	1	0.70[3]	0.96	
Arctic charr	Control	0	0.79	1.30	[24]
	Na-lactate	1	1.12	0.91	
Atlantic salmon	Control	0	0.97	n.d.	[26]
	Na-lactate	1.5	0.97		
Arctic charr	Control	0	0.28	n.d.	[22]
	Na-lactate	1.5	0.51[3]		
Atlantic salmon	Control	0	0.76	n.d.	[22]
	Na-lactate	1.5	0.79		

[1]SGR (%): specific growth rate=ln body mass$_1$ – ln body mass$_0$/culture period (d)$\times$100.
[2]FCR: feed conversion ratio=feed intake/LWG.
[3]Significantly different from the control diet ($P<0.05$); n.d., – not determined.

sodium acetate to the diet significantly affected the digestibility coefficients ($P<0.05$) for both protein and total lipid, and for dietary fatty acids 14 : 0, 16 : 0, 18 : 1, 20 : 1, 22 : 1 and essential fatty acids 18 : 0 and 18 : 2(n-6).

Contrary to the significant results with 1% sodium lactate in Arctic charr, no such results were obtained with Atlantic salmon using the same dosage [22, 26]. One of the most notable differences between the two species, which probably explains the results, is the doubled retention time of dietary lactate in the stomach in Arctic charr. According to these authors, it seems likely that lactate or sodium lactic acid exerts its influence in the upper part of the digestive system and therefore any difference found here may explain the difference in growth response in the two species. There was, however, a benefit of mortality reduction the lactate-fed salmon from 19.9% in the negative control to 15.2%.

Further studies on salmonids again include rainbow trout. The effect of organic acids on mineral digestibility was tested in several studies. It was reported from pigs that the inclusion of dietary organic acids enhances mineral absorption [27]. Since the availability of phosphorous in particular from a fishmeal-based diet plays a vital role in salmonid aquaculture [28], different acidifiers have been tested under these conditions. Vielma and Lall [29] reported the effect of dietary formic acid on the availability of phosphorus in rainbow trout diets. These authors found that the apparent digestibility of phosphorus significantly increased ($P<0.05$) in fish fed a diet containing 10 ml/kg formic acid. Sugiura *et al.* [30] found that the availabilities of magnesium and calcium in fishmeal increased ($P<0.05$) by the dietary inclusion of formic acid. Apparent availabilities of calcium and phosphorus were also greatly affected by the inclusion of citric acid in the rainbow trout diet. Dietary inclusion of citric acid (5%) reduced phosphorus in the faeces of fish by approximately 50%, with no reduction in feed intake or appetite. Other apparent mineral availabilities increased by citric acid application include iron, magnesium, manganese and strontium. In contrast, mineral availabilities were not affected by citric acid use in agastric goldfish (*Carrasius auratus*), but a 5% inclusion of the dietary acidifier led to a marked reduction of feed intake. Inclusion of sodium citrate (5%) in the diet of rainbow trout also showed significantly improved availabilities of calcium and phosphorus, but less than that of pure citric acid.

Another study with rainbow trout used much lower dietary levels of citric acid [31]. In this study, diets were supplemented with 0, 0.4, 0.8 or 1.6% citric acid in different particle size fish-bone meals. Citric acid increased the whole-body ash content, but the body phosphorus content showed only a tendency to increase (P=0.07). On the other hand, dietary acidification significantly increased whole-body iron dose-dependently. Sugiura *et al.* [32] found that in high-ash diets for rainbow trout, feed acidification with citric acid decreased the effect of supplemental phytase, whereas in low-ash diets, it markedly increased the effect of the enzyme. In general, it can be concluded that adding citric acid to the diet of rainbow trout regulates chelation of calcium and phosphorus, thereby increasing the solubility of calcium phosphates and improving phosphorus and mineral availabilities [33].

More recent studies include experiments with rainbow trout fingerlings [34, 35], which were fed five experimental diets, a negative control, three diets containing 0.5, 1.0 and 1.5% of an organic acid blend (formic acid and its salts plus sorbic acid) and a diet containing an AGP (40 ppm Flavomycin®). After 3 months, improvement in growth was observed with increasing acid blend inclusion. The 1.0 and 1.5% dosages resulted in a significant

Table 5 Effects of potassium diformate supplementation in diets on the performance of tilapia challenged with *V. anguillarum* (modified from [37])

	Potassium diformate inclusion in diet (%)			
	0	0.2	0.3	0.5
Initial weight (g)	16.7	16.7	16.7	16.7
Final weight (g)	218^{a}	258^{c}	246^{b}	252^{bc}
FCR	1.34^{a}	1.23^{b}	1.25^{b}	1.22^{b}
Mortality (%), day 10–85	33.0^{a}	20.8^{b}	18.4^{b}	11.0^{c}

^{abc}Within rows, means without common superscripts are significantly different ($P < 0.05$).

improvement in specific growth rate (SGR) versus control ($P < 0.05$). The improvement by the 1.5% acid blend was similar to that achieved by the AGP, but with a lower feed conversion ratio (FCR) than the antibiotic group. Unpublished information (Karl Sacherer, personal communication, 2006) also reveals that the use of an acid blend of formic and propionic acids and their salts on a sequential release medium is successfully used in the grow-out of Turkish rainbow trout.

The latest results in salmonids reveal that Atlantic salmon fed a fishmeal enriched with 1.4% potassium diformate (a potassium salt of formic acid) tended (P=0.055) to a higher SGR versus negative control [36]. Furthermore, groups fed 0.8 and 1.4% potassium diformate via fishmeal had a significantly better feed conversion and improved uniformity within fish groups. This was confirmed in older data (Rune Christiansen, personal communication, 1996 and 1998), where salmon fed diets containing potassium diformate-treated fishmeal had significantly higher growth rates, and improved protein and fat digestibilities.

In-Feed Acidifier in Tropical Aquaculture Species

Ramli *et al.* [37] tested potassium diformate as a growth promoter in tilapia grow-out in Indonesia (Table 5). In this study, fish were fed six times a day diets containing different concentrations of potassium diformate (0, 0.2, 0.3 and 0.5%) over a total period of 85 days. The diets contained 32% crude protein, 25% carbohydrate, 6% lipid and 10% fibre. The fish were challenged orally from day 10 with *Vibrio anguillarum* at 10^{5} CFU/day for 20 days.

From day 1 to day 85, potassium diformate significantly improved feed intake ($P < 0.01$), live weight gain (LWG) ($P < 0.01$), FCR ($P < 0.01$) and protein efficiency ratio (PER) ($P < 0.05$). Furthermore, PER also significantly improved due to the addition of the formic acid salt ($P < 0.05$). The improvement was greater for 0.2 and 0.5% formate addition. Survival rates of fish after the challenge with *V. anguillarum* on days 10–30 were also significantly higher than the negative control, and this effect was dose-dependent ($P < 0.01$). The authors concluded that the use of potassium diformate at 0.2% is an efficient tool to control *V. anguillarum* in tropical tilapia culture.

Another study in tilapia (*Oreochromis niloticus*) investigated feeding behaviour in the fish using different organic acids [38], as sometimes reported for some organic acids or their salts in piglets [39]. Citric acid at a concentration of 10^{-2}–10^{-6} M and lactic acid at 10^{-2}–10^{-5} M stimulated feeding, as recorded automatically using the frequency of feeding 'bites' of the fish, whereas *O. niloticus* tended to avoid acetic acid at 10^{-3} M, while acetic acid at 10^{-5} M had no significant effects.

A more recent trial [40] determined the effects of an acid/salts blend, (containing of calcium formate, calcium propionate, calcium lactate, calcium phosphate and citric acid) at different levels (0.5, 1.0 and 1.5%) on the growth performance of tilapia. Fish were fed to appetite twice a day for 8 weeks, using a pelleted diet containing 31% crude protein. Despite a lack of statistically significant data for LWG and FCR, the blend at 1.5% resulted in a numerical increase in LWG of 11% versus negative control, with results similar to the AGP-supplemented diet (0.5% oxytetracycline). Such organic acid salts and blends may therefore be especially useful during grow-out period in tilapia culture [41].

More research on the potential growth-promoting effects on tilapia is currently being carried out with various single and blended organic acids at various dietary levels (Ng, Wing-Keong, personal communication, 2008). The effects of dietary organic acids on gut and faecal microflora population as well as survival of tilapia challenged with *Streptococcus agalactiae* or *Aeromonas hydrophila* are also being investigated.

Further research has been devoted to sea bream (*Pagrus major*), in order to determine the phosphorus utilization after feeding dietary organic acids, as observed in previous studies with other fish species [42]. The use of 1% each of citric acid, malic acid and lactic acid in three different dietary groups showed significantly better LWGs and FCRs in the citric acid group versus negative control, but malic or lactic acid did not improve performance. Phosphorus excretion in the citric, malic and lactic acids fed bream groups also significantly reduced, indicating a better phosphorus utilization. The higher absorption of phosphorus in diets supplemented with organic acids agrees with other reports that citric acid can increase the apparent digestibility of many minerals, including phosphorus, in fishmeal [33, 43].

Despite their lack of success in agastric goldfish in Europe, acidifiers have also been tested in agastric Indian carp (*Labeo rohita*). Baruah *et al.* [44] determined the interactions of dietary protein level, microbial phytase and citric acid inclusion on bone mineralization in *Labeo* juveniles. Their data showed that the addition of 3% citric acid to either a low- (25%) or high-protein diet (35%) resulted in a significantly decreased pH of the feed and intestinal digesta. Furthermore, bone ash content significantly increased, suggesting a better bioavailability of minerals. The mineral content of bones is in close agreement with these findings, since, for example, the phosphorus retention in the skeleton after citric acid supplementation significantly increased. Debnath *et al.* [45] suggest synergistic effects between microbial phytase and organic acids in this respect. A follow-up study [46] investigated the synergistic effects of citric acid and phytase on nutrient digestibility and growth performance in Indian carp, again in low (25%)- and high (35%)-protein diets. Citric acid in both diets significantly increased LWG and SGR in carp juveniles, while FCR reduced. No effects were observed on PER and apparent net protein utilization (ANPU). However, a significant interaction between citric acid and microbial phytase (500 units/Kg) was found for LWG, SGR, PER and ANPU, further supporting the findings of Debnath *et al.* [45]. Finally, it was found [47] that citric acid and microbial phytase have a synergistic effect on mineral bioavailability, as measured in the whole body and in the plasma. This effect was more prominent in low-protein diets.

Other omnivorous fish species have also been fed diets supplemented with acidifiers. In a recent trial, Owen *et al.* [48] tested sodium butyrate as a feed additive in the tropical catfish (*Clarias gariepinus*) added at 0.2% to two diets differing in their major protein source (fishmeal or defatted soya). Slightly higher growth and a concomitant reduction in FCR were observed in catfish fed the fishmeal diet supplemented with sodium butyrate, compared with the control diet, while fish receiving defatted soya together with 0.2% Na-butyrate showed no improvement. The SGR surplus in the fishmeal plus butyrate group was 4.7%, while the improvement in FCR was 4.1%. However, both indices differed insignificantly from the control. Sodium butyrate supplementation also appeared to increase the proportion of gram-positive bacteria in the hindgut of *C. gariepinus*, though this increase was not statistically significant.

Microbials and organic acids in fish

A different approach was taken by Vazquez *et al.* [49], who studied the effect of lactic acid bacteria cultures on pathogenic microbiota from turbot (*Scophthalmus maximus*). According to their results, inhibition of pathogenic species in fish by the use of lactic acid bacteria was achieved due to the presence of lactic and acetic acids, rather than bacteriocins, in all the cases studied. In other words, these bacteria cultures are only effective if they supply the turbot host with organic acids.

Acidifiers in Shrimps and Snails

Research in non-fish aquaculture species is somewhat limited. Tung *et al.* [50] reported that 0.5% sodium citrate with inactivated *Lactobacilli* boosted the growth of the Kuruma shrimp (*Masurpenaeus japonicus*). Further work suggests that a dose of 0.25% calcium formate can enhance giant tiger prawn (*Penaeus monodon*) survival in brackish water farms in Taiwan (Tan Seong Lim, personal communication, 2005). These results are to be evaluated again over more than just one grow-out season. Most recent data include the successful usage of acidifiers in the development of artificial diets for abalone culture in South Africa (Lourens de Wet, personal communication, 2007).

Conclusion/Summary

Despite the limited number of published studies on the use of acidifiers for the improvement of growth, feed efficiency, digestibility and mineral absorption in aquaculture, results from the available studies indicate promising potential and compel aquafeed manufacturers to consider the use of acidifiers in the diets they formulate. Furthermore, acidifiers can mitigate the impact of bacterial infections, thereby preventing diseases and thus affording higher survival rates. The use of acidifiers can be an efficient tool to achieve sustainable, economical and safe fish and shrimp production [51].

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