Effects of dietary acidifiers in aquaculture – a review

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It is a well established fact in the field of aquaculture, that the use of antibiotic growth promoters as an in-feed additive for the diets of fish and shrimp may promote growth and feed conversion as well as improve survival rates. However public concerns, especially in the EU, on the development of cross-resistances to humans have led to a ban or decrease in the use of such substances worldwide. Consequently, research focused on other additives in order to maintain performance parameter and high survival rates in aquaculture. The scope of this review is to show that in-feed acidifiers maybe an example for a group of additives which can play an important role in future aquaculture diets. A number of studies, covering cold-water as well as tropical species, indicate that a broad range of organic acids, their salts or mixtures of those can improve growth, feed utilization and disease resistance in fish.

Acid preservation of fish and fish viscera to produce fish silage has been a common practice and its final product has been widely used in fish feeds with reported beneficial effects (Gildbert and Raa 1977; Åsgård and Austreng 1981). According to Batista (1987) the fish silage production was initiated in the 30ies of the last century first with sulphuric and hydrochloric acid preservation of fish waste. The production of acid-preserved fish silage can be achieved either with organic or inorganic acids or blends of those. If inorganic acids are used the pH of the silage has to be lowered to a pH of 2 or below in order to obtain a fully preserved product. Therefore, before feeding this type of silage to animals neutralization needs to take place. On the other hand, if organic acids like formic or propionic acid are used, the silage is stable at pH levels of 3.5-4.0 enabling the silage to be directly fed without neutralization. Due to this advantage, most silage producer's use recently organic acids. Fish silage or liquefied fish protein is an effective way to convert fish by-catch and fish processing by-products into nutritious feedstuff for a wide variety of animals, such as poultry (Balios 2003). Kotzamanis (2007) used 2.2% formic acid inclusion to produce sardine (*Sardine pilchardus*) fish hydrolysates for start feeding sea bass (*Dicentrarchus labrax*) larvae. The hydrolysate was incorporated into the diet at two different levels – 10% and 19% of total ingredients. Results on performance showed that the inclusion of fish hydrolysate gave similar results on growth 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 to investigate the effects of these short-chain acids onto the fish feed directly. Several studies have been conducted with different species including carnivores like rainbow trout *Oncorhynchus mykiss*, Atlantic salmon *Salmo salar* and artic charr *Salvelinus alpinus*, herbivorous filter feeders (tilapia), omnivorous fish (carp, catfish) and shrimp.

Following experiences in swine and poultry feeding, a wide variety of organic acids, their salts – as well as blends of those is and was tested in aquaculture diets (Table 1).

Table 1: Formulas, physical and chemical characteristics of organic acids used as dietary acidifiers in aquaculture (modified from Foegeding and Busta 1991)

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

Effect of acidification in salmonids

Early studies on organic acids in fish diets included succinic and citric acid in salmonids (Fauconneau 1988). In this study the partial substitution of protein (12%) by a single amino acid or an organic acid (either succinic or citric acid) was tested in rainbow trout (*Oncorhynchus mykiss*) diets. Trout which fed the organic acid diets had a lower voluntary feed intake, compared to the basal diet, or to a diet additionally supplemented with purified protein. However there was no large variation between the tested groups in the efficiency of utilization of protein and energy.

Data from the 90ies of the last century obtained more promising results in the use of dietary acidifiers in a number of salmonid species (Table 2). The effect of supplementation of commercial diets with sodium salts of lactic and propionic acid was tested in Arctic charr (*Salvelinus alpinus*) in brackishwater at 8°C (Ringø 1991). Fish fed the diet with 1% added sodium lactate increased in weight from about 310 g to about 630 g in 84 days, while fish fed diets without either salts 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 content from Arctic charr fed the sodium lactate supplemented diet contained lower amounts of water, energy, lipid, protein and free amino acids. It has been observed that charr feeding on high doses of commercial feeds, as it often appears under aquaculture conditions, have a tendency for diarrhoea. When charr was feeding on diets containing sodium lactate, diarrhoea did not occur, probably indicating much lower amounts of remaining nutrients and water in the gut. Furthermore, it was proposed that the growth promoting effect of dietary lactate in Arctic charr is caused by the relatively slow gastric emptying rate (Gislason *et al*. 1996). An increased holding time in the stomach augments the antibacterial potential of the lactic acid salt and can have therefore a larger inhibition effect against possible pathogenic bacteria (Sissons 1989). The improved growth of the Arctic charr did not affect the chemical composition of the fish (Ringø *et al*. 1994).

A similar study by the same author (Ringø 1992) proved the growth promoting effect of 1% sodium formate ($P<0.05$) given as an additive to Arctic charr reared in brackishwater, while the same dosage of sodium formate had only a numerical improvement compared to a negative control. The stimulated growth of the fish which fed the acetate additive may be explained to some extend by a higher feed intake, but the enhanced digestibilities of dietary components might also contribute to the increased growth. Addition of 1% sodium acetate to the diet affected significantly ($P<0.05$) the digestibility coefficients for both protein and lipid, and for the dietary fatty acids 14:0, 16:0, 18:1, 20:1, 22:1 including the essential fatty acids 18:0 and 18:2(n-6).

In contrary to the significant results with sodium lactate in Arctic charr, no such results could be determined with Atlantic salmon (*Salmo salar*), using the same dosage (Gislason et al. 1994, 1996). One of the most notable differences between the two species, probably explaining the results, is the difference in the retention of dietary lactate in the stomach, which was twice as long in Arctic charr. According to the authors, it seems likely that lactate or lactic acid exerts its influence in the upper part of the digestive system and therefore any difference found here may explain the variation in growth rate between the two fish species. However, a beneficial result of the acidified diet may be the numerical reduction of mortality from 19.9% in the negative control to 15.2% in the lactate fed salmons.

Table 2: Effect of the sodium salt of different organic acids on the performance of Arctic charr and Atlantic salmon

Fish species	**Acid / acid salt**	**Dose (%)**	**SGR (%)**†	**FCR**††	**Reference**
Arctic charr	Control Na-lactate Na-propionate	0 1 1	0.61 0.83* 0.49*	n.d.	Ringø, 1991
Arctic charr	Control Na-formate Na-acetate	0 1 1	0.51 0.58 0.70*	1.20 1.08 0.96	Ringø, 1992
Arctic charr	Control Na-lactate	0 1	0.79 1.12	1.30 0.91	Ringø *et al*., 1994
Atlantic salmon	Control Na-lactate	0 1.5	0.97 0.97	n.d.	Gislason *et al*., 1994
Arctic charr	Control Na-lactate	0 1.5	0.28 0.51*	n.d.	Gislason *et al*., 1996
Atlantic salmon	Control Na-lactate	0 1.5	0.76 0.79	n.d.	Gislason *et al*., 1996

†SGR (%): Specific Growth Rate = ln Body Mass$_1$ – ln Body Mass$_0$ / Culture period (d) x 100

††FCR: Feed Conversion Ratio = Feed intake / Live weight gain

*significantly different from the control diet (P<0.05); n.d. – not determined

Further studies on salmonids included again the rainbow trout *Oncorhynchus mykiss*. The effect of organic acids on digestibility of minerals was tested in several studies. It was reported from pigs, that the inclusion of dietary organic acids enhances the mineral absorption (Ravindran and Kornegay 1993). Since especially the availability of phosphorous from a fishmeal-based diet plays a vital role in salmonid aquaculture (Åsgård and Shearer 1997), different acidifiers were tested on such possibilities. Vielma and Lall (1997) reported the effect of dietary formic acid on the availability of phosphorous in such diets for rainbow trout. It was found that the apparent digestibility of phosphorous was significantly increased (P<0.05) in fish fed a diet containing 10 mL kg^{-1} formic acid. Furthermore, also the availability of magnesium and calcium was increased (P<0.05) due to the inclusion of formic acid into the diet of fish (Sugiura et al. 1998a). Apparent availabilities of calcium and phosphorous were also greatly affected by the inclusion of citric acid into the diet of rainbow trout. A 5% inclusion of citric acid led approximately to a reduction of 50% phosphorous in the feces of fish. This very high dietary supplement did not reduce feed intake or appetite of rainbow trout. Further minerals which increased with citric acid application in apparent availability in trout include iron, magnesium, manganese and strontium Contrary to these results, the mineral availabilities were not affected by citric acid in agastric goldfish (*Carrasius auratus*), but the 5% level of dietary acidification led to a marked reduction of feed intake in goldfish. The inclusion of sodium citrate (5%) to the diet of rainbow trout showed as well significantly improved availabilities for calcium and phosphorous, but not to the same extend as the pure citric acid. Another study with rainbow trout used much lower levels of citric acid application (Vielma et al. 1999). In this study differently grounded fish bone meals were supplemented with 0, 0.4, 0.8 or 1.6% of citric acid. Citric acid increased the whole-body ash content but the influence of citric acid on the body phosphorous content was only a tendency (P=0.07). On the other hand, dietary acidification significantly increased whole-body iron in a dose dependent fashion. Sugiura et al. (2001) found that in high-ash diets for rainbow trout in-feed acidification with citric acid decreased the effect of supplemented phytase, whereas in low-ash diets, acidification markedly increased the effect of the enzyme. In general, it can be concluded, that adding citric acid to the diet of rainbow trout regulates the chelation and formation of calcium and phosphorous, which increases the solubility of calcium phosphates and thereby improves phosphorous and mineral utilization (Suguira et al. 1998b).

More recent studies include experiments with rainbow trout fingerlings (de Wet 2005, 2006), which were fed five experimental diets. Those diets consisted of a control diet, three diets containing 0.5, 1.0 and 1.5% of an organic acid blend (formic acid and its salts as well as sorbic acid) and a diet containing an AGP (40 ppm Flavomycin). At the end of the trial, improvement in growth was observed with increasing level of organic acid inclusion. Inclusion levels of 1.0% and 1.5% resulted in significant improvement in specific growth rate of the fish when compared to the control (P<0.05). The improvement was similar to what was achieved with AGP inclusion, if 1.5% of the acid blend were used. But fish fed the 1.5% acid blend tended to have a lower FCR compare to the group with in-feed antibiotics.

Latest results in salmonids reveal that Atlantic salmon fed 1.4% potassium diformate enriched fishmeal tended (P=0.055) to have a higher specific growth rate compared to a negative control (Christiansen and Lückstädt 2008). Furthermore, groups fed 0.8% and 1.4% potassium diformate fishmeal had a significantly better feed conversion and improved the uniformity of fish groups. This was confirmed by older data (personal communication: Rune Christiansen, 1996 and 1998), where salmon fed on diets containing potassium diformate treated fishmeal had significantly higher growth rates, an improved protein digestibility and a significantly higher fat digestibility respectively.

In-feed acidifier in tropical aquaculture species

Ramli *et al.* (2005) tested potassium diformate (potassium salt of formic acid) as a growth promoter in tilapia grow-out in Indonesia (Table 3). In this study, fish were fed over a period of 85 days 6 times a day diets containing different concentrations of potassium diformate (0%, 0.2%, 0.3% and 0.5%). The diets contained 32% crude protein, 25% carbohydrates, 6% lipids and 10% fibre. The fish were challenged orally starting day 10 of the culture period with *Vibrio anguillarum* at 10^5 CFU per day over a period of 20 days.

Over the entire feeding period from day 1 to 85, potassium diformate significantly increased feed intake (P<0.01) and weight gain (P<0.01) as well as improved the feed conversion ratio significantly (P<0.01).

Furthermore, protein efficiency ratio was also significantly improved due to the addition of the formic acid salt (P<0.05). The improvement was best with 0.2% and 0.5% addition of the formate.

Survival rates of fish after the challenge with *V. anguillarum* on day 10 were also significantly higher compared to the negative control and the effect was dose dependent (P<0.01).

Table 3: Effects of potassium diformate supplementation in diets on performance of tilapia challenged with V. anguillarum *(modified from Ramli et al. 2005)*

	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 ($p<0.05$)

The authors concluded that the application of potassium diformate at 0.2% is an efficient tool to control bacterial infections in tropical tilapia culture.

Similar results were achieved by Zhou *et al.* (2008), who tested hybrid tilapia (*Oreochromis niloticus* x *Oreochromis aureus*) fingerlings (2.7 g initial weight) in a dose response study with potassium diformate (0%, 0.3%, 0.6%, 0.9% and 1.2%), while also comparing the results with an antibiotic growth promoter (8 mg / kg Flavomycin). During the 56 day trial period, tilapia fed all the potassium diformate enriched diets grew faster than the negative control (an increase of up to 11.6%), while fish fed 0.3% and 0.6% potassium diformate achieved even better weight gain than the fish in the positive control group. The authors speculated that dietary potassium diformate could stimulate a beneficial bacterial colonization of the intestine.

Another study on tilapia (*Tilapia nilotica*) searched on stimulating the feeding behaviour of the fish with different organic acids (Xie et al. 2003), as this is sometimes reported with different organic acids or their salts in pigs (Paulicks et al. 1996). The result showed that citric acid at a concentration of 10^{-2} to 10^{-6} M and lactic acid at 10^{-2} to 10^{-5} M stimulated feeding, as automatically recorded via the frequency of feeding “bites” of tilapia. On the other hand, fish tended to avoid acetic acid at 10^{-3} M. The inclusion of acetic acid at 10^{-5} M had no significant effects on fish feeding.

A more recent trial (Petkam et al. 2008) determined the effects of an acid blend, containing of calcium formate, calcium propionate, calcium lactate as well as calcium phosphate and citric acid at different levels (0.5%, 1.0% and 1.5%) on the growth performance of tilapia (*Oreochromis niloticus*). Fish were fed to satiation two times a day during an 8 week period, using a pelletized diet containing 31% crude protein. Despite a lack of statistical significant data on growth and FCR, the inclusion of the acidifier at 1.5% of the diet resulted in a numerical 11% increase in body weight when compared to the negative control and achieved similar results as the AGP-supplemented diet (0.5% Oxytetracycline). Organic acid salt may be therefore especially during the grow-out period of high importance for tilapia culture (Lückstädt, 2008).

Further research was devoted too sea bream *Pagrus major* in order to determine the phosphorous utilization after feeding dietary organic acids, as this was seen in previous studies with different fish species before (Hossain et al. 2007). The use of 1% citric acid, 1% malic acid and 1% lactic acid in 3 different dietary groups led to significantly better weight gain and feed conversion ratio in the citric acid supplemented group, compared to the negative control. The use of malic acid and lactic acid did not improve the performance of sea bream. The phosphorous excretion in the citric acid fed bream, as well as in the malic acid and lactic acid fed groups were also significantly reduced, suggesting a better utilization of this mineral in the fish. The higher absorption of phosphorous in the diet with supplemented organic acid is in agreement with other reports that citric acid might increase the apparent digestibility of many minerals including phosphorous in fishmeal (Suguira et al. 1998b, Sarker et al. 2005).

Despite the lack of success in agastric goldfish in Europe, acidifiers were tested in agastric Indian carp (*Labeo rohita*). A study carried out by Baruah et al. (2005) determined the interactions of dietary protein level, microbial phytase and citric acid on bone mineralization of Labeo juveniles. Data proved that the addition of 3% citric acid to either a low (25%) or high protein diet (35%) resulted in a significantly decreased feed pH and intestinal digesta pH. Furthermore bone ash content was significantly increased, suggesting a better bioavailability of minerals. Likewise, the minerals in bones are in close agreement with these finding, since for instance the phosphorous retention in the skeleton after citric acid supplementation was significantly increased as well. Debnath et al. (2005) are suggesting synergistic effects between microbial phytase and organic acids in this respect. A follow up study (Baruah et al. 2007a) investigated the synergistically effect of citric acid and phytase onto the the nutrient digestibility and growth performance in Indian carp, again in low- and high protein diets. Addition of citric acid in both diets significantly increased the weight gain (WG) and the SGR of carp juveniles, while it reduced the FCR. No effects where observed on protein efficiency ratio (PER) and apparent net protein utilization (ANPU). However, a significant interaction between citric acid and microbial phytase (at 500 U kg^{-1}) was found on WG, SGR, PER and ANPU, supporting findings from Debnath et al. (2005) even further. Finally it was found (Baruah et al. 2007b) that citric acid and microbial phytase have a synergistic effect on mineral bioavailability, as measured in the whole body and the plasma, and this effect was more prominent in low protein diets.

Other omnivorous fish species were supplementary fed with acidifiers as well. In a recent trial, Owen et al. (2006) tested the sodium salt of butyric acid as a feed additive in the tropical catfish *Clarias gariepinus* at 0.2% in two diets: the main protein source in one diet was fishmeal and in the other diet was defatted soya. Slightly higher growth and concomitant reduction in FCR were observed in catfish fed fishmeal diet supplemented with sodium butyrate when compared with the control diet, while fish receiving defatted soya together with 0.2% Na-butyrate did not showed any improvement at all. The SGR surplus in the fishmeal fed butyrate group was more than 4%, while the improvement in feed efficiency was about 4% too, however both indices differed not significantly from the control. Sodium butyrate supplementation did appear to increase the proportion of gram positive bacteria in the hindgut of *C. gariepinus*, though this increase was not statistically significant.

Research in non-fish species in the aquaculture sector is somewhat limited. Tung et al. (2006) reported that 0.5% sodium citrate next to inactivated Lactobacilla boosted the growth of the Kuruma shrimp *Masurpenaeus japonicus*. Further work with shrimps suggests that a dosage of 0.25% calcium formate can enhance giant tiger prawn (*Penaeus monodon*) survival in brackishwater farms in Taiwan (Lückstädt, unpublished). Additional work on acidifiers was done in Abalone. Goosen et al. (2008) found, that organic acids may work as potential growth promoters in South African Abalone culture.

Though there are only a limited number of published studies on the use of acidifiers for growth promotion, feed efficiency as well as mineral absorption and disease prevention in aquaculture, results from those studies indicate promising potential and compel aqua feed manufacturers to consider using acidifiers in their diets. The use of acidifiers can be an efficient tool to achieve sustainable, economical, and safe fish and shrimp production (Lückstädt, 2007).

References

Available on request from the author