The use of acidifiers in fisheries and aquaculture

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Since the early 1980s, yearly growth rates of around 10% have been reported for the aquaculture sector. Because of this situation, global production of farmed fish and shellfish has more than doubled in volume and value in the past 15 years (Naylor *et al*., 2000). The contribution of aquaculture to total fish production directly consumed by humans is currently more than 25%. Aquaculture is at present the only growing sector within the fishing industry and is also reputed to be the fastest growing food production sector in the world.

Williams *et al*. (2000) pointed out main goals for the aquaculture industry if sustainability is to be achieved and this includes especially the promotion of environmentally sound practices in all fields of fish and shrimp production.

In the field of aquaculture it is well established so far that the inclusion of antibiotics into the diets of fish (Ahmad and Matty, 1989) can promote growth and feed conversion.

However recently, growing awareness from consumers and producers of aquaculture species has resulted in calls for responsible and sustainable aquaculture, also in the much debated shrimp production in South East Asia (Verbeeke, 2001; Feedinfo, 2005). Public opinion and regulation authorities in most export countries focus now on the misuse of antibiotics in aquaculture and public attention has shifted towards production methods (Lückstädt, 2005).

Furthermore, the EU has banned all antibiotic growth promoters (AGP) from livestock production with effect of January 2006, since the use of low levels of these antibiotics in animal feeds possesses the possibility to transfer bacterial immunity to species pathogenic in animals and humans (Liem, 2004).

Due to the above mentioned facts however, alternatives needed to be found.
Several feed additives, including acidifier consisting of organic acids and their salts may be a promising alternative for the use of in-feed antibiotics in aquaculture.

In animal nutrition organic acidifier and their salts exert their performance promotion effects via three different ways, as currently reviewed by Freitag (2006): in the feed, in the gastro-intestinal tract and due to effects onto the metabolism of the animal (table 1).

Table 1: Effects of organic acids and salts in animal nutrition*

	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^{++}, Mg^{++}, Fe^{++}, Cu^{++}, Zn^{++})
	H^+ and Anion	- antibacterial effects - change in microbial concentrations
Metabolism		- energy supply

**after Kirchgessner and Roth, 1988*

Even under good hygienic environmental conditions aqua-feed may be infected with a certain amount of fungi, bacteria or yeast. In favourable conditions such microbes can multiply rapidly during storage, especially at higher moisture levels (>14%) due to a high humidity and in a warm environment. Conserving agents reduce microbial growth and thus lower the uptake of possibly pathogenic organisms by the fish or shrimp. Besides hygienic effects, the reduction of the acid binding capacity of feed ingredients can promote animal performance. A high crude protein content of feed ensures usually a rapid fish growth in juveniles, but generates as well a high dietary buffering capacity at the same time and is thus reducing free hydrochloric acid in the stomach. Pepsin activation and pancreatic enzyme secretion are therefore reduced and nutrient digestion is impaired. Lowering the dietary buffering capacity with in-feed acidifier has beneficial effects on feed digestion (Eidelsburger, 1997).

The mode of action of organic acids in the intestinal tract acts in two different ways: on the one hand they reduce the pH-level in the stomach and particularly in the small intestine and on the other hand acid dissociation in the bacterial cell and accumulation of salt anions inhibit growth of gram-negative bacteria.

As mentioned above, inadequate pH reduction in the stomach inhibits pepsin activity and thus protein digestion is impaired. Effective proteolytic activity requires a pH below 4 and is still increased at lower pH values. Positive effects of organic acids on protein hydrolysis have been demonstrated (Mroz *et al.*, 2000). Likewise duodenal secretion of pancreatic enzymes is reduced at high pH values thus impairing overall digestion in monogastric animals. Feed supplementation with organic acids can lead to lower duodenal pH, improved N retention and overall increased nutrient digestibility (Øverland *et al.*, 2000; Kluge *et al.,* 2004).

Organic acids and their salts exert their growth inhibiting effects on stomach and gut microbes through pH reduction and anion and proton effects in the microbial cell. Growth rates of many gram-negative bacteria, like *E. coli* or *Salmonella ssp*. are reduced below pH 5. Low pH also forms a natural barrier against ascending microbes from the ileum and large intestine. Moreover, small acids are lipophilic and can diffuse across the cell membrane of gram-negative bacteria. In the more alkaline cytoplasm they dissociate and the released protons will subsequently lower the internal pH. pH reduction alters cell metabolism and enzyme activity thus inhibiting growth of intraluminal microbes, especially pathogens. Several investigations demonstrate a reduction in bacterial count in the stomach (Kluge *et al*., 2004) and the duodenum (Kirchgessner and Roth, 1991; Hebeler *et al*., 2000; Hellweg *et al*., 2006), while acid tolerant beneficial *Lactobacillus spp.* seem to be unaffected or may even be enhanced in number (Hellweg *et al.*, 2006).

Finally, most organic acids have a considerable amount of energy (table 2). Organic acids are generally absorbed through the intestinal epithelia by passive diffusion. Short chain acids can be used for instance for the ATP generation in the citric cycle. As the energy content of organic acids is completely used in metabolism it should be considered in the energy calculation of feed rations. For example, propionic acid contains one to five times more energy than wheat (Diebold and Eidelsburger, 2006).

Table 2: Gross energy content of selected organic acids and their salts*

Organic acid / salt	Solubility in water	Gross energy (kJ / g)
Formic acid	very good	5.8
Acetic acid	very good	14.8
Propionic acid	very good	20.8
Lactic acid	good	15.1
Fumaric acid	low	11.5
Citric acid	good	10.3
Calcium formate	low	3.9
Sodium formate	very good	3.9
Calcium propionate	good	16.6
Calcium lactate	low	10.2

**after Freitag, 2006*

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). It is a common practice in Norway to preserve fish-by-products as well as freshly caught "industrial fish" for further fish meal or fish oil production with formic acid or potassium diformate in order to prolong fishing time or to extend the storage duration of those fish. These 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 carnivore species, like rainbow trout *Oncorhynchus mykiss*, Atlantic salmon *Salmo salar* and artic charr *Salvelinus alpinus*, but also with herbivorous filter feeders, like tilapia, or omnivorous catfish and also shrimp.

The effect of supplementation of commercial diets with sodium salts of lactic and propionic acid (10 kg / t of feed) was tested in Arctic charr under brackish water conditions at 8°C (Ringø, 1991). Fish fed the diet with added Na-lactate increased their weight from around 310 g to about 630 g within 84 days of the experiment, while the difference to the negative control group (final weight of fish: 520 g) was significantly ($P<0.05$). The inclusion of Na-propionate however had a growth depressing effect compared to the control. The gut content from Arctic charr fed the sodium-lactate supplemented diet contained significantly ($P<0.05$) 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 Na-lactate no nutritive diarrhoea appeared, probably because of 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 Artic charr did not affect the chemical composition of the fish (Ringø *et al.*, 1994).

Recently, a trial with organic acid salts was also carried out with Rainbow trout *Oncorhynchus mykiss* (de Wet, 2005). This study aimed to evaluate an organic acid blend (5 – 15 kg / t), mainly consisting of formate and sorbate, for its use in trout nutrition to improve performance parameters and compared it with some commonly used AGP (40 ppm Flavomycin). Rainbow trout fingerlings feeding on 10 and 15 kg acidifier per ton of feed had significantly higher final weights compared to the negative control group after three months of feeding (three times a day to apparent satiety), while there was no difference to the group treated with Flavomycin. Feed conversion ratio tended to be lower with increasing dosages of the acid blend, even if compared to the antibiotic treated group.

The results of this study showed that the application of the acidifier at 15 kg / t improved weight gain and feed conversion ratio in trout compared to a negative control by 20.1% and 14.8% respectively and was furthermore an alternative in the use of the AGP.

Further studies were carried out for instance with Atlantic salmon *Salmo salar* (Christiansen and Lückstädt, 2008). Salmon with a mean weight of 270 g were randomly distributed between 9 fibre glass tanks ($1m^3$), with 50 fish in each tank. The tanks were supplied with 20 litres per minute of sea water (30-32 ‰) for a total experimental period of 126 days. Fish fed pelleted diets containing potassium diformate (KDF) enriched fishmeal had a numerically increased body weight gain (17% and 19% for 0.8% and 1.4% KDF inclusion rate respectively). The SGR of fish fed 1.4% KDF tended to be higher (P=0.055) compared to the negative control. Furthermore, both groups treated with KDF had a significantly better feed conversion ratio (P<0.05). It was seen as well, that the uniformity of fish fed KDF treated fishmeal was improved.

The use of organic acids however was not only tested in Salmoniformes, but also in tropical warm-water species, like tilapia. Ramli *et al.* (2005) tested the use of potassium diformate as a non-antibiotic 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 different concentrations of KDF (0, 2, 3 and 5 kg / t feed). Furthermore, 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.

Table 3: Effects of potassium diformate (KDF) on growth performance in tilapia challenged with *V. anguillarum*; data from Ramli *et al.* (2005)

Parameter	Control	2 kg / t KDF	3 kg / t KDF	5 kg / t KDF
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)

Over the whole feeding period from day 1 to day 85 KDF significantly increased the weight gain and feed efficiency in fed tilapia. 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 furthermore dose dependent.

The 2 kg / t inclusion of the potassium salt of the formic acid lead to an improvement in weight gain and feed conversion ratio in tilapia by 18.6% and 8.2% respectively and indicate furthermore that the chosen acidifier is able to counteract bacterial infections in tilapia.

In a more recent trial with *Oreochromis niloticus* (Petkam *et al*. 2008) the effect of an acid blend, containing Ca-formate, Ca-propionate, Ca-lactate, Ca-phosphate and citric acid was tested at 3 different inclusion levels (0.5%, 1% and 1.5%) against a negative control and a positive control containing an AGP (0.5% Oxytetracycline) during a growth period of 8 weeks. Overall, there were no statistical significant differences in weight gain or FCR measurements. Nevertheless, despite the lack of statistical significances between treatments, the inclusion of the acidifier at 1.5% of the diet resulted in a numerical 11% increase in body weight gain when compared to the fish fed the control diet (33.6 g vs. 30.2 g) and achieved better results than the inclusion of the AGP (33.6 g vs. 33.0 g). The inclusion of dietary organic acid blends may be a good alternative in order to further reduce the application of AGP's and attain economic tilapia culture.

Owen *et al.* (2006) tested the sodium salt of butyric acid as a feed additive in the omnivorous tropical catfish *Clarias gariepinus* at 2 kg / t in a fish meal based diet and in a defatted soya concentrates diet. No significant differences were found while supplying sodium butyrate if compared with the negative control. However, especially in the catfish fed on fish meal diet the SGR was seen to be slightly higher in the supplemented fish (% body weight gain 131.3% and 141.4% for control group and Na-butyrate group respectively) with a concomitant reduction in the FCR of the supplemented fish. Subjectively sodium butyrate supplementation did appear to increase the proportion of gram positive bacteria in the hindgut of *C. gariepinus*, even though this increase was not statistically.

The beneficial application of organic acid salts was also proven by Tung *et al.* (2006) who used 5 kg / t Na-citrate next to inactivated Lactobacilla to boost the growth of the Kuruma shrimp *Masurpenaeus japonicus*.

Finally, a recent report (Lückstädt, unpublished data) suggests that a dosage of 2.5 kg / t Ca-formate can also enhance the survival rates in brackishwater shrimp grow-out in Taiwan. However, those achieved results must be evaluated in more than just one grow-out season.

Out of the above mentioned studies and trials can be concluded that the use of organic acid salts or acid blends is an interesting option to promote the performance of a wide variety of aquaculture species worldwide. It is furthermore suggested, that the impact of bacterial infections can be reduced which might lead to higher survival rates. The use of acidifier in aquaculture can be therefore an efficient tool to achieve a sustainable and economical fish and shrimp production.

*partly based on the book chapter "Effect of organic acid containing additives in worldwide aquaculture – sustainable production the non-antibiotic way"; in: "Acidifiers in Animal Nutrition", Ed. C. Lückstädt, Nottingham University Press, 2007