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A sustainable alternative to antibiotics: Using acidifiers in fisheries & aquaculture

Antibiotic growth promoters are being banned due to their hazardous effect on human health and the environment. In this context, acidifiers offer a safe, ecologically friendly and sustainable alternative for achieving high levels of aquaculture productivity.

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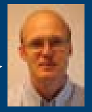

Since the early 1980s, yearly growth rates of around 10 percent have been reported for the aquaculture sector. Because of this situation, global production of farmed fish and shellfish has more than doubled in volume and value over the past 15 years (Naylor et al., 2000). The contribution of aquaculture to total fish production directly consumed by humans is currently more than 25 percent. Not only is it the only growing segment within the fishing industry, it is also reputed to be the fastest growing food production sector in the world.

Williams et al. (2000) pointed out that if aquaculture industry sustainability is to be achieved, it will necessitate the promotion of environmentally sound practices in all fields of fish and shrimp production. With this in mind, antibiotics have long had a valuable, yet controversial role in making of animal protein. In the field of aquaculture it is a well established fact that the inclusion of antibiotics into the diets of fish (Ahmad and Matty, 1989) promotes growth and feed conversion.

Despite their obvious usefulness, the long-term biological and environmental affects of antibiotic use has been a source of increasing concern. Growing awareness from consumers and aquaculture producers has resulted in calls for responsible and sustainable aquaculture, particularly in South East Asia's shrimp farming industry (Verbeeke, 2001; Feedinfo, 2005). With public opinion and regulatory authorities in exporting countries focussing on the misuse of antibiotics in aquaculture, public attention has shifted towards production methods (Lückstädt, 2005).

Since using low levels of antibiotics in animal feed risks transferring antibiotic immunity to pathogenic bacteria (Liem, 2004), the EU banned all antibiotic growth promoters (AGP) from >>

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

Location	Effective Form	Effects
Feed	H$^+$	pH reduction, reduction of acid binding capacity
	H$^+$ and Anion	reduction of microbial growth antibacterial effects
Intestinal tract	H$^+$	pH reduction in stomach and duodenum, improved pepsin activity
	Anion	complexing agents for cations (eg. Ca^{++}, Mg^{++}, Fe^{++}, Cu^{++}, Zn^{++})
	H$^+$ and Anion	antibacterial effects, change in microbial concentrations
Metabolism		energy supply

after Kirchgessner and Roth, 1988

livestock production with effect from January 2006. Consequently, alternatives to AGPs needed to be found.

Several feed additives, including acidifiers 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 acidifiers and their salts exert their performance promotion effects via three different ways, as currently reviewed by Freitag (2007). They augment performance via the feed itself, within the gastro-intestinal tract and due to metabolic effects within the animal itself (table 1).

Even with good hygiene and 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 percent) due to high humidity in a warm environment. Conserving agents such as acidifiers can reduce microbial growth and thereby lower the uptake of possibly pathogenic organisms by the fish or shrimp.

Acidifiers' modes of action

Besides hygienic effects, reducing the acid binding capacity of feed ingredients can promote animal performance. Feed with high crude protein content ensures rapid growth in juvenile fish but also generates a high dietary buffering capacity, thereby reducing free hydrochloric acid in the stomach. Consequently, pepsin activation and pancreatic enzyme secretion are reduced, thereby impairing nutrient digestion. In this context, lowering the dietary buffering capacity with an in-feed acidifier has beneficial effects on feed digestion (Eidelsburger, 1997).

Organic acids have two modes of action in the intestinal tract. On one hand, they reduce pH-levels in the stomach and particularly in the small intestine. On the other hand, acid dissociation in the bacterial cell and the accumulation of salt anions inhibits the growth of gram-negative bacteria.

As mentioned above, inadequate pH reduction in the stomach inhibits pepsin activity and thus impairs protein digestion. Effective proteolytic activity requires a pH below 4 and increases further at lower pH values. The positive effects of organic acids on protein hydrolysis have been demonstrated (Mroz et al., 2000). Similarly, 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 nitrogen retention and higher overall nutrient digestibility (Øverland et al., 2000; Kluge et al., 2004).

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, 2007

Organic acids and their salts exert their growth inhibiting effects on stomach and gut microbes through pH reduction, anion and proton effects in the microbial cell. Growth rates of many gram-negative bacteria including E. coli or Salmonella ssp. are reduced when pH is below 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. The resulting pH reduction alters cell metabolism and enzyme activity, thereby inhibiting growth of intraluminal microbes, especially pathogens. Several studies demonstrate a reduced bacterial count in both the stomach (Kluge et al., 2004) and the duodenum (Kirchgessner and Roth, 1991; Hebeler et al., 2000; Hellweg et al., 2006). At the same time, beneficient, acid tolerant Lactobacillus spp. seem to be unaffected or may even be enhanced in number (Hellweg et al., 2006).

Finally, most short chain organic acids contain a considerable amount of energy (see table 2) that can be used, for example, to generate ATP during the citric cycle. They are usually absorbed through the intestinal epithelia by passive diffusion. Since the organic acids' energy is completely used up in metabolism, it should be included in feed rations' energy content calculations. For example, propionic acid contains one to five times more energy than wheat (Diebold and Eidelsburger, 2006).

Acid preservation of fish and fish viscera to produce fish silage is 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 scientific community's attention and motivated it to investigate the effects of applying short-chain acids directly into aqua feed. Several studies have been conducted with a variety of different species. These include cold water carnivores such as rainbow trout (Oncorhynchus mykiss), Atlantic salmon (Salmo salar) and Artic charr (Salvelinus alpinus). Studies were also done in tropical, warm water fish including herbivorous filter feeders such as tilapia, omnivorous catfish (Clarias gariepinus) and Kuruma shrimp (Masurpenaeus japonicus).

Sodium lactate & Arctic charr

The effect of supplementing commercial diets with sodium salts of lactic and propionic acid (10kg/tonne of feed) was tested in Arctic charr under brackish water conditions at 8°C (Ringø, 1991). Fish fed a diet with added sodium lactate increased their weight from approximately 310g to an average of 630g within 84 days, with a significantly less weight gain of 520g in the negative control group ($P<0.05$). On the other hand, the inclusion of sodium propionate depressed fish growth compared to the control group population.

The gut content from Arctic charr fed a sodium lactate supplemented diet contained significantly ($P<0.05$) lower amounts of water, energy, lipids, protein and free amino acids. It has also been observed that Arctic charr fed high doses of the commercial feeds used in aquaculture have a tendency for diarrhoea. In the case of Arctic charr raised on sodium lactate supplemented feed, no nutritive diarrhoea appeared. This is probably due to the much lower proportion of residual nutrients and water left 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) it induces. An increased holding time in the stomach augments the antibacterial potential of lactic acid salt, thereby augmenting its inhibitory effect against pathogenic bacteria (Sissons, 1989). In addition, it was ascertained that the improved growth of Arctic charr did not change the fish's chemical composition (Ringø et al., 1994).

Formate-sorbate & trout, salmon and tilapia

Recently, a trial with organic acid salts was also carried out on rainbow trout (de Wet, 2005). This study sought to evaluate an organic acid blend (consisting mostly of formate and sorbate at 5–15kg/tonne) for its capacity to improve fish performance parameters compared to a commonly used AGP (40 ppm Flavomycin). For three months, they were fed 10kg/tonne and 15kg/tonne acidifier per ton of feed three times a day to apparent satiety. At the end of the study, these fingerlings had significantly higher final weights compared to the negative control group. There was virtually no difference in weight gain between the group given organic acid salts and those treated with Flavomycin. Moreover, the feed conversion ratio tended to be lower with increasing dosages of the acid blend, even when compared to the AGP treated group.

This study demonstrated that compared to the control group, applying the acidifier at 15kg/tonne improved the weight gain and feed conversion ratio in rainbow trout by 20.1 percent and 14.8 percent respectively. It also showed that this organic acid combination is a viable, effective alternative to using AGPs.

Similar, additional studies were carried out with other species including Atlantic salmon (Christiansen and Lückstädt, 2008). Salmon with a mean weight of 270g 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 percent) for a total experimental period of 126 days. Fish fed pelletted diets containing potassium diformate (KDF) enriched fishmeal

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gained significantly more weight (17 percent and 19 percent for 0.8 percent and 1.4 percent KDF inclusion respectively). The Specific Growth Rate (SGR) of fish fed 1.4 percent 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 also observed that the uniformity of fish fed KDF treated fishmeal was improved.

Furthermore, organic acids were not only tested in cold water fish such as trout and salmon but also on tropical, warm-water species such as tilapia and shrimp. Ramli et al. (2005) tested KDF as alternative to AGPs on tilapia raised in Indonesia (see table 3). In this study, tilapia were fed different concentrations of KDF (0, 2, 3 and 5 kg/tonne of feed) over a period of 85 days, 6 times a day. In addition, starting on the tenth day of the culture period, fish were challenged orally with the fish pathogen bacteria Vibrio anguillarum at 10^5 CFU per day for a period of 20 consecutive days.

Over the whole feeding period from day 1 to day 85, KDF significantly improved tilapia's weight gain and the feed's efficiency by 18.6 percent and 8.2 percent respectively (see table 3). Survival rates of fish after the challenge with Vibrio anguillarum on day 10 were also significantly higher compared to the negative control group. Moreover, the survival rate enjoyed a proportionate relationship with the KDF dose. The results strongly imply that this particular acidifier is able to counteract bacterial infections in tilapia.

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

Parameter	Control Group	2kg / tonne KDF	3kg / tonne KDF	5kg / tonne KDF
Initial weight (g)	16.7	16.7	16.7	16.7
Final weight (g)	218a	258c	246b	252bc
FCR	1.34a	1.23b	1.25b	1.22b
Percent mortality rate for days 10 to 85	33.0a	20.8b	18.4b	11.0c

abc Note: rows without common superscripts are significantly different (P<0.05)

In a more recent trial with Nile tilapia (Oreochromis niloticus) (Petkam et al. 2008), an acid blend containing calcium formate, calcium propionate, calcium lactate, calcium phosphate and citric acid was tested at 3 different inclusion levels (0.5 percent, 1 percent and 1.5 percent). It was compared against a negative control group and also positive control given an AGP (0.5 percent oxytetracycline) over a growth period of 8 weeks.

The inclusion of this acidifier at 1.5 percent led to an 11 percent increase in body weight gain when compared to the fish fed a control diet (33.6g vs. 30.2g) and achieved better results than fish given oxytetracycline (33.6 g vs. 33.0g). Apparently, the inclusion of dietary organic acid blends may be a good alternative to using AGPs when farming tilapia.

Sodium butyrate, sodium citrate, catfish & shrimp

Owen et al. (2006) tested sodium butyrate as a feed additive for omnivorous tropical catfish (Clarias gariepinus) at 2kg/tonne in both a fish meal based diet and in a defatted soya concentrates diet. No significant differences were found while supplying sodium butyrate when compared with a negative control group. However, particularly in the catfish fed fish meal, the SGR was slightly higher. The body weight gain observed was 131.3 percent and 141.4 percent for the control group and sodium butyrate group respectively, coupled with a concomitant reduction in the FCR of supplemented fish. Apparently, sodium butyrate supplementation also raised the proportion of gram positive bacteria in hindgut of catfish, though this increase was not statistically significant.

In a similar vein, the beneficial application of organic acid salts was also proven by Tung et al. (2006), who used 5kg/tonne sodium citrate with inactivated Lactobacilla to boost the growth of the Kuruma shrimp Masurpenaeus japonicus. Finally, a recent report (Lückstädt, unpublished data) suggests that a dosage of 2.5kg/tonne calcium formate enhances the survival rate of brackish water shrimp grown in Taiwan. However, the above mentioned result must be evaluated in more than just one growing season.

Conclusion

All the above mentioned studies and trials strongly demonstrate that using organic acids or acid blends is a viable, sustainable option for optimising the performance of numerous aquaculture species worldwide. It is furthermore implied, that these acidifiers mitigate the impact of bacterial infections, thereby leading to higher survival rates. Having demonstrated that their effectiveness equals or exceeds that of AGPs, it must be concluded that acidifiers are a sustainable, ecologically friendly means of achieving economical fish and shrimp aquaculture.

***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.**