The use of ergotropics in aquaculture; A sustainable solution for antibiotic-free feeding in fish and shrimp

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The current situation in world food supply calls for supreme efforts to ensure the increasing requirements of the growing world population for staple diets and high-quality food, as well as to bridge the widening gap between food demand and supply, especially in developing areas. Setbacks in any food production sector will place greater pressure on other sectors for supplying the increasing urban and rural populations, particularly in less developed countries.

About one billion people are dependent on fish as their main protein resource, and this number likely will increase further (Becker and Focken 1998), because the world population is increasing at an estimated annual rate of 2 percent. Aquaculture now provides more than 22 percent of consumable aquatic products (Guillaume *et al.* 2001). Most aquaculture production occurs in developing countries and, mainly, in Asia. Between 1987 and 1996, aquaculture production of food fish increased by 148 percent (Tomasso and New 1999), while livestock meat and fisheries have grown yearly only by 3 percent and 1.6 percent, respectively. 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.

Since the early 1980s, yearly growth rates of around 10 percent have been reported for the aquaculture sector, but it has increased much more rapidly in the developing world than in developed countries, especially as a result of the development in Asia. 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). If products from aquaculture that are not directly used for human consumption are included, for instance seaweed, then the world's aquaculture production more than tripled by weight and value between 1984 and 1996 (Dagoon 2000). The contribution of aquaculture to total fish production directly consumed by humans is currently more than 25 percent.

Aquaculture has to be recognized as a part of the natural environment and the different farming systems operate inside larger ecosystems, using available natural resources, including water supply, natural food and oxygen supply, and releasing harvested animals and also degraded resources (Bagarinao 1999). Folke and Kautsky (1992) described aquaculture as an economic subsystem of the overall ecosystem in which the existing ecosystem is used as a source for energy and farm inputs and additionally as a sink for the waste outputs.

Aquaculture production differs greatly between countries and human preferences, climatic zones and local conditions as well as types of farmed animals. Therefore, the production practices and the resulting impact on the ecosystem vary widely. 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.

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 southeast 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 from livestock production effective January 2006, because the use of low levels of these antibiotics in animal feeds poses the possibility of transferring bacterial immunity to species pathogenic in animals and humans (Liem 2004). In the field of aquaculture, it is well established that the inclusion of antibiotics in the diets of fish (Ahmad and Matty 1989) can promote growth and feed conversion. Because of the afore mentioned facts however, alternatives needed to be found.

Because the use of fish silage from preserved fish and fish viscera included the acid preservation (Gildbert and Raa 1977, Åsgård and Austreng 1981) this group of additives came into scientific observation, too. Also, it was interesting to investigate the effects of these short-chain acids onto the fish directly. First with carnivore species, like rainbow trout *Oncorhynchus mykiss*, Atlantic salmon *Salmo salar* and Artic charr *Salvelinus alpinus*,

Table 1. Influence of dietary treatment with an organic acid blend on Rainbow trout *Oncorhynchus mykiss* performance compared to an antibiotic growth promoter (AGP), (de Wet 2005)

Parameter	Control	AGP	5 kg / t acidifier	10 kg / t acidifier	15kg/ t acidifier
Initial weight (g)	40.3	42.3	40.0	37.3	37.2
Final weight (g)	184.8[a]	235.4[b]	205.6[ab]	231.2[b]	231.4[b]
FCR	1.22	1.10	1.09	1.08	1.04
SGR (%)	1.23[a]	1.37[b]	1.23[a]	1.29[ab]	1.37[b]
Survival (%)	82.7	88.8	85.0	85.8	89.6

[ab]*within rows, means without common superscripts are significantly different ($p<0.05$)*

but also with herbivorous filter feeders, such as tilapia and shrimp.

Ringø (1991) fed Arctic charr commercial diets with or without the supplementation of the sodium salts of lactic and propionic acid in brackish water at 8° C. Inclusion rate of the sodium salts was 10 kg / t of feed. 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 significant. 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 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 is often the case under aquaculture conditions, have a tendency for diarrhea. When charr was feeding on Na-lactate no nutritive diarrhea 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 was 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).

Feeding Na-lactate to Atlantic salmon juveniles (15 kg / t) however, did not show such a prolonged effect (Ringø *et al.* 1994, Gislason *et al.* 1996) compared to charr. Ringø *et al.* (1994) found slightly increased survival rates in salmon feeding on lactate (84.8 percent compared to 80.1 percent), while Gislason *et al.* (1996) determined a higher specific growth rate SGR ((ln final weight – ln initial weight) x 100 / days of culture period). However, none of those differences were statistically significant. These findings may suggest that the influence of lactate is a result of some differences in digestive physiology between the two fish species, for instance a longer retention time of lactate in the stomach in charr. But lower bacterial challenge, as a result of the use of the organic acid salt, may have lead to the tendency of higher survival rates.

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 to some commonly used antibiotic growth promoters (40 ppm Flavomycin). Rainbow trout fingerlings (ca. 40 g) were kept in flow-through ponds and fed three times daily to apparent satiety. The experiment lasted for three months (Table 1).

Fish feeding on 10 and 15 kg acidifier per ton of feed had significantly higher final weights compared to the negative control group, while there was no difference to the group treated with AGP. Feed conversion ratio tended to be lower with increasing dosages of the acid blend, even if compared to the AGP group.

The results of this study show 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 percent and 14.8 percent, respectively. These data proved that an organic acid inclusion was suitable for use in rainbow trout grower feeds at and above levels of 10 kg acidifier per ton of finished feed and that this level could be an effective alternative compared to the use of AGP's in trout aquaculture.

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 2). In this study, fish were fed over a period of 85 days 6 times a day different concentrations of potassium-diformate (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.

Over the whole feeding period from day 1 to day 85, potassium-diformate significantly increased the weight gain and feed efficiency

Table 2. Effects of potassium-diformate on growth performance in tilapia challenged with *V. anguillarum*; (Ramli *et al.* (2005).

Parameter	Control acidifier	2 kg/t acidifier	3 kg/t acidifier	5 kg/t
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* ($p<0.05$)

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 percent and 8.2 percent, respectively and, furthermore, indicate that the chosen acidifier is able to counteract bacterial infections in tilapia. Finally, a recent report[2] suggests that a dosage of 2.5 kg / t Ca-formate can 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.

Based on the above mentioned studies and trials it 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 that might lead to higher survival rates. The use of acidifier in aquaculture can be, therefore, an efficient tool to achieve sustainable and economical fish and shrimp production.

The production of carnivorous aquatic species however, such as salmon and shrimp, is supported by a large input of fish meal or fish oil to the farming system, mainly from ocean fisheries. In this case, aquaculture systems use 2 to 5 times more fish protein, supplied through fish meal, to feed the cultured fish than is supplied by the farmed species (Tacon 1996). New and Csavas (1995) asked in this connection: "Will there be enough fish meal for fish meals?" Almost one third of the total world fish harvest of 142 million tons in the year 2000 was converted into fishmeal or fish oil for use as animal feeds. Therefore, aquaculture competes, particularly in the case of carnivorous species such as trout and salmon, with other livestock production sectors for the limited fishmeal resources. It is, therefore, of great imprtance to replace fish meal by plant based protein sources (Mabahinzireki *et al.* 2001). Because around 70 percent of the total phosphorous sources in plant ingredients is bound as phytate and, therefore, practically not available for fish (Lall 1991), the use of phytase came into consideration as well. Liebert and Portz (2005) showed the growth improving effect of microbial phytase in levels between 750 and 1250 FTU / kg in Nile tilapia *Oreochromis niloticus*, which was in line with a significantly increased energy-, protein- and phosphorous utilization as well. Similar effects have been reported for rainbow trout also (Sugiura *et al.* 2001). Not only acidifiers were tested by the scientific community and on a commercial scale to overcome the use of antibiotics in aquaculture.

To withstand the high stocking densities currently used, for instance, in shrimp production with hatcheries and pond grow-out, and related stress situations, such as water parameters, including low dissolved oxygen contents, directly fed probiotics are a promising sustainable additive to stimulate shrimp growth and secure a low disease response. In shrimp grow-out, Massam (2005) found directly fed probiotics to be an effective tool to boost survival, while Decamp *et al.* (2005) studied the effect of probiotics on one Asian shrimp hatchery. Data from Lückstädt (2006) support those findings in a wider range of shrimp hatcheries. The concept of probiotics in aquaculture is outlined below:

Mode of action of probiotics in aquaculture

- **Production of inhibitory compounds**
  Probiotic bacteria release chemicals, which have a bactericidal or bacteriostatic effect. Some of these chemicals are: Bacteriosins, lysozyme, proteases, organic acids (pH-change)
- **Competition for available energy (nutrients)**
  Microbial competition for organic substrates (carbon and energy sources) in the intestinal tract of shrimp means that by increasing the relative numbers of probiotic bacteria, nutrients are consumed that would otherwise be available for the growth of pathogenic bacteria.
- **Competition for adhesion sites**
  Bacteria also compete for gut adhesion sites. Adhesion is a prerequisite to colonization in the intestinal tract. By applying a high number (10^{12}) of beneficial bacteria (probiotics), harmful bacteria (pathogens) are not able to adhere and, thus, cannot pro-

Table 3:. urvival in Vietnam hatcheries using a probiotic blend* until PL 12 in *Penaeus monodon* (Lückstädt 2006).

Hatcheries	Control containing antibiotics	Probiotic additive
1. province, 15 hatcheries	50.0%	53.3%
2. province, 12 hatcheries	45.0%	45.8%
3. province, 9 hatcheries	55.0%	58.3%
4. province, 20 hatcheries	50.0%	51.3%

**DynaGain LARVAEbiotic from Mangrove Coast Ltd. was used*

liferate.

- **Enhancement of immune response** There are many publications available on immune stimulating substances. Most of these derive from the cell walls of various microorganisms, such as ß-glucans, lipopolysaccharides (LPS) and peptidoglucan (PG). These substances are the first challenge that approaches the shrimp's immune system in response to invading microorganisms.
- **Improvement of water quality** This is usually associated with *Bacillus* species. In comparison to gram negative, bacteria gram positive strains (*Bacillus subtilis*) are better converters of organic matter thus producing CO_2. This results in lower levels of residues in the pond, so the BOD (biological oxygen demand) and the COD (chemical oxygen demand) are reduced.
- **Enzymatic contribution of digestion** Certain bacterial species (*Bacillus subtilis*) are known to produce and release enzymes, such as amylase and protease, that are able to improve the digestive process in shrimp.

During the year 2005 several trials were performed in South Vietnam in a wide range of hatcheries using a single-strain fermented probiotic feed additive, with three different strains of *Bacillus* sp., *Enterococcus* sp. and *Lactobacillus* sp., designed to improve bioavailability in shrimp larvae used from early Zoea to later Post Larvae stages. The trials were done in 4 provinces in 56 hatcheries from Nauplii state until PL 12. The probiotic was poured once a day into the larval tank at 10 g per 5000 L of water.

Average results from all farms are shown in Table 3 and based on triplicates in each farm. Based on these results it could be stated that the chosen additive can be an alternative for an antibiotic-free hatchery operation under the circumstances in Vietnam.

As a conclusion, the use of different ergotropics in aquaculture can play a vital role in replacing directly fed antibiotics to a wide range of aquaculture relevant species and following therefore the requests of officials and more important the consumers.

Notes

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[2]information about the use of Ca-formate in Taiwanese *P. monodon* culture available from the author

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