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The use of dietary acidifiers in salmonid nutrition A REVIEW

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Routine use of antibiotics as growth promoters is a matter of debate in the animal farming industry.

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). The EU banned all antibiotic growth promoters (AGP) from livestock production from January 2006.

Public opinion and regulation authorities in most of the export countries focusing now on the misuse of antibiotics in aquaculture and public attention have shifted towards production methods.

Therefore, alternatives to AGP are worldwide sought in a variety of forms.

Acidifiers consisting of organic acids and their salts present a promising alternative and they have received much attention as a potential replacement in order to improve the performance and the health of treated animals. In animal nutrition, acidifiers exert their effects on performance via three different ways (Freitag 2007): a) in the feed; b) in the gastro-intestinal tract of the animal; and c) due to effects on the animal's metabolism.

Role of feed

A certain amount of fungi, bacteria or yeast is unavoidable in feeds. Under favourable conditions such microbes multiply rapidly during storage, especially at higher moisture levels (>14 percent) in a warm environment.

Acidifiers function as conserving agents by reducing the pH of the feed, and thereby inhibiting microbial growth and thus lower the uptake of possibly pathogenic organisms and their toxic metabolites by the farmed animals (Freitag 2007). Malicki et al. (2004) found that a mixture of formic and propionic acid (one percent dosage) can act synergistically against E. coli in stored fishmeal, which is an often used ingredient in aquafeeds.

Role in intestinal tract

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

During periods of high feed intake such as when the animals are young, or when the feeds are high in protein, free hydrochloric acid levels in the stomach are reduced.

This reduction negatively impacts pepsin activation and pancreatic enzyme secretion and impairs digestion. Providing acidifiers in the feed tackles this problem and aid in feed digestion (Eidelsburger 1997).

Positive effects of organic acids on protein hydrolysis have been demonstrated (Mroz et al. 2000). Likewise feed supplementation with organic acids has been shown to lead to lower duodenal pH, improved nitrogen retention and overall increased nutrient digestibility (Øverland et al. 2000; Kluge et al. 2004).

Table 1: Gross energy content of selected organic acids and their salts used in aquaculture feeds (modified from Freitag 2007)

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
Potassium diformate	good	2725

Growth rates of many gram-negative bacteria, like E. coli or Salmonella ssp. are reduced below pH5.

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.

Table 2: 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	CH3COOH	60.05	1.05	liquid	4.76
Propionic	CH3CH2COOH	74.08	0.99	liquid	4.88
Butyric	CH3CH2CH2COOH	88.12	0.96	liquid	4.82
Lactic	CH3CH(OH)COOH	90.08	1.21	liquid	3.83
Sorbic	CH3CH:CHCH:CHCOOH	112.14	1.2	solid	4.76
Malic	COOHCH2CH(OH)COOH	134.09	1.61	solid	3.4, 5.1
Citric	COOHCH2C(OH)(COOH)CH2COOH	192.14	1.67	solid	3.13, 4.76, 6.4

In the more alkaline cytoplasm they dissociate and cause pH reduction. This 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).

Role in metabolism

Most organic acids have a considerable amount of energy (Table 1). Organic acids are generally absorbed through the intestinal epithelia by passive diffusion. Short chain acids can be used in various metabolic pathways for energy generation, for instance for 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).

Organic acids in aquaculture

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).

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 2).

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 percent) 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 utilisation of protein and energy.

Data from the 1990s obtained more promising results in the use of dietary acidifiers in a number of salmonid species (Table 3).

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 one percent added sodium lactate increased in weight from about 310g to about 630g in 84 days, while fish fed diets without either salts reached a final weight of only 520g ($P<0.05$). Inclusion of one percent 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 one percent sodium acetate (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 one percent 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 contrast 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 percent in the negative control to 15.2 percent in the lactate fed salmons.

The effect of organic acids on digestibility of minerals

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 10mL 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).

Table 3: 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	0	0.61	n.d.	Ringø, 1991
	Na-lactate	1	0.83*		
	Na-propionate	1	0.49*		
Arctic charr	Control	0	0.51	1.2	Ringø, 1992
	Na-formate	1	0.58	1.08	
	Na-acetate	1	0.70*	0.96	
Arctic charr	Control	0	0.79	1.3	Ringø et al., 1994
	Na-lactate	1	1.12	0.91	
Atlantic salmon	Control	0	0.97	n.d.	Gislason et al., 1994
	Na-lactate	1.5	0.97		
Arctic charr	Control	0	0.28	n.d.	Gislason et al., 1996
	Na-lactate	1.5	0.51*		
Atlantic salmon	Control	0	0.76	n.d.	Gislason et al., 1996
	Na-lactate	1.5	0.79		

†SGR (percent): Specific Growth Rate = ln Body Mass1 – ln Body Mass0 / 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

Apparent availabilities of calcium and phosphorous were also greatly affected by the inclusion of citric acid into the diet of rainbow trout. A five percent inclusion of citric acid led approximately to a reduction of 50 percent 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 five percent level of dietary acidification led to a marked reduction of feed intake in goldfish.

The inclusion of sodium citrate (five percent) 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 percent 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).

Organic acids and rainbow trout fingerlings

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 percent of an organic acid blend (formic acid and its salts as well as sorbic acid) and a diet containing an AGP (40ppm Flavomycin).

At the end of the trial, improvement in growth was observed with increasing level of organic acid inclusion.

Inclusion levels of 1.0 percent and 1.5 percent 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 percent of the acid blend were used. But fish fed the 1.5 percent acid blend tended to have a lower FCR compare to the group with in-feed antibiotics.

Unpublished information (personal communication: Karl Sacherer, 2006) revealed furthermore that the use of an acid blend consisting of formic and propionic acid and their salts on a sequential release medium is successfully used in grow out of Turkish rainbow trout.

Latest results on salmonids

Latest results in salmonids reveal that Atlantic salmon fed 1.4 percent 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 percent and 1.4 percent 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. Further trials on the effect of potassium diformate in salmon are recently carried out (personal communication: Margareth Øverland, 2008).

In summary

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 from the author on request