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The use of acidifiers in fish nutrition

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Abstract

It is well established in the field of aquaculture that the use of antibiotic growth promoters (AGPs) as feed additives in the diets of fish and shrimp can improve live weight gain (LWG), feed conversion ratio (FCR) and survival rates. However, scientific knowledge and public concerns, especially in the EU, over the development of cross-resistance to antibiotics of importance to human health have led to a ban or a reduction in the use of such substances worldwide. Consequently, the aquafeed industry has turned its research attention to other additives in order to maintain performance and high survival rates in aquaculture. This review shows that acidifiers are an example of a group of additives which can play an important role in future in aquaculture diets. A number of studies, in cold-water and tropical species, indicate that a broad range of organic acids, their salts or admixtures can improve growth, feed utilization and disease resistance in fish.

Keywords: Acidifiers, Fish, Nutrition, Organic acids, Aquaculture

Review Methodology: The following databases were searched: CAB Abstracts, World Aquaculture Society database, Google Scholar and Scopus. Keyword search terms used were acidifiers, organic acids, fish, aquaculture, fish feed. In addition, the references from the articles obtained thereby were used to search for additional relevant material. Furthermore, the knowledge gained from editing the book 'Acidifiers in Animal Nutrition' was used. Colleagues were consulted and asked for any upcoming studies not yet published.

Introduction

Routine use of antibiotics as growth promoters is a subject for debate in animal farming and feed and food industries. The use of low levels of antibiotics in animal feeds creates the possibility of transferring immunity to antibiotics used against bacterial pathogens in animals and humans [1]. As a result of such concerns, the EU banned the prescription-free use of all the antibiotic growth promoters (AGPs) from livestock production with effect from January 2006. Public opinion and regulatory authorities in most exporting countries now focus on the misuse of antibiotics in aquaculture and public attention has shifted towards production methods. Therefore, alternatives to AGP are sought worldwide in a variety of forms. The earliest studies that showed that organic acids are able to positively influence animal performance when added to diets were published more than 30 years ago [2]. Acidifiers consisting of organic acids and their salts present a promising alternative, and they have received much attention as a potential replacement, for improving the performance and the health of the livestock. In animal nutrition, acidifiers exert their effects on performance via three different mechanisms [3]: (a) in the feed; (b) in the gastro-intestinal tract of the animal; and (c) in effects on the animal's metabolism (Table 1) (modified from [4]).

Role in Feed Hygiene

A certain level of contamination with fungi, bacteria or yeasts is unavoidable in nutrient-rich products like feeds. Under favourable conditions such microbes multiply rapidly during storage, especially at higher moisture levels (>14%) in warm environments. Acidifiers function as

Table 1 Effects of organic acids and their salts in animal nutrition (after [4])

Site of action	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^{2+}, Mg^{2+}, Fe^{2+}, Cu^{2+}, Zn^{2+})
	H^+ and Anion	Antibacterial effects Change in microbial concentrations
Metabolism		Energy supply

conserving agents by reducing the pH of the feed, thereby inhibiting microbial growth and thus lowering the uptake of possibly pathogenic organisms and their toxic metabolites by the farm animals [3]. Malicki *et al.* [5] found that a mixture of formic and propionic acid (1% dosage) can act synergistically against *Escherichia coli* in stored fishmeal, which is an often-used ingredient in aqua feeds.

Role in the Intestinal Tract

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

During periods of high feed intake, such as when the animals are young or when the feeds are high in protein, hydrochloric acid concentrations in the stomach are reduced. This reduction negatively impacts pepsin activation and pancreatic enzyme secretion and impairs digestion. Providing acidifiers in the feed addresses this problem and aids feed digestion [6]. Positive effects of organic acids on protein hydrolysis have been demonstrated [7]. Similarly, feed supplementation with organic acids has been shown to lead to lower duodenal pH, improved nitrogen retention and increased nutrient digestibility [8, 9].

The growth rates of many Gram-negative bacteria, such as *E. coli* or *Salmonellae*, are reduced below pH 5. Low pH also forms a natural barrier against microbes ascending from the ileum and large intestine. Moreover, low-molecular-weight acids are lipophilic and can diffuse across the cell membranes of Gram-negative bacteria. In the more alkaline cytoplasm, they dissociate and reduce the pH. This reduction alters cell metabolism and enzyme activity, thus inhibiting the growth of intralumenal microbes, especially that of pathogens. Several studies have demonstrated a reduction in bacterial counts in the stomach [9] and the duodenum [10–12], while acid-tolerant, beneficial *Lactobacilli* seem to be unaffected or may even be enhanced in number [12].

Table 2 Gross energy content of selected organic acids and their salts used in aquaculture feeds (modified from [3])

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

Role in Metabolism

Most organic acids have high gross energy values (Table 2) (modified from [3]). Short-chain organic acids are generally absorbed through the intestinal epithelia by passive diffusion and they can be used in various metabolic pathways for energy generation, for instance, for ATP generation in the citric acid cycle. As the energy content of organic acids is completely used in metabolism it should be included in the energy content of feed rations. For example, propionic acid contains one to five times more energy than wheat [13].

Organic Acids in Aquaculture

The acid preservation of fish and fish viscera in the production of fish silage has been a common practice with widespread use in fish feeds and reported beneficial effects [14, 15]. According to Batista [16], fish silage production was initiated in the 1930s, initially with

Table 3 Formulae, physical and chemical characteristics of organic acids used as dietary acidifiers in aquaculture (modified from [19])

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

sulphuric and hydrochloric acid preservation of fish waste. The production of acid-preserved fish silage can also be achieved either with organic or inorganic acids or blends. If inorganic acids are used, the pH of the silage has to be lowered to $\leqslant$pH 2 in order to obtain a fully preserved product. Therefore, before feeding this type of silage to animals, the pH must be neutralized. On the other hand, if organic acids such as formic or propionic acid are used, the silage is stable at pH levels of 3.5–4.0, enabling direct feeding without neutralization. Hence, most silage producers now use organic acids. Fish silage or liquefied fish protein is an effective way to convert fish by-catch and fish processing byproducts into nutritious feedstuffs for a wide variety of animals, such as poultry [17]. In aquaculture [18], 2.2% formic acid was used to produce sardine (*Sardine pilchardus*) fish hydrolysates for start-feeding of sea bass (*Dicentrarchus labrax*) larvae. The hydrolysate was incorporated in the diet at 10 and 19%. Performance results showed that the inclusion of fish hydrolysate gave similar growth results 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, leading to the investigation of the effects of these short-chain acids in fish feeds. Several studies have been conducted with different species including carnivores such as rainbow trout (*Oncorhynchus mykiss*), Atlantic salmon (*Salmo salar*) and Arctic charr (*Salvelinus alpinus*), herbivorous filter feeders (tilapia), omnivorous fish (carp, catfish) and shrimp.

Following the experiments in pig and poultry feeding, a wide variety of organic acids, their salts and admixtures has been tested in aquaculture diets (Table 3) (modified from [19]).

Effect of Diet Acidification in Salmonids

Early studies on the use of organic acids in fish diets included succinic and citric acids in diets for salmonids [20]. These included the partial substitution of protein (12%) by a single amino acid or an organic acid (succinic or citric) tested in rainbow trout diets. Trout that were fed the organic acid diets had lower voluntary feed intakes compared to the basal diets, or to a diet supplemented with purified protein. However, there was no large variation between treatments in the efficiency of protein and energy utilization.

Data from the 1990s showed more promising results from the use of dietary acidifiers to salmonids (Table 4). The effect of supplementing commercial diets with sodium salts of lactic and propionic acids were tested in Arctic charr in brackish water at 8°C [21]. Fish fed a diet with 1% sodium lactate added to it increased in weight from about 310 to about 630 g in 84 days, while fish fed diets without either salt 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 contents of Arctic charr fed a diet supplemented with sodium lactate contained less water, energy, lipid, protein and free amino acids. It has been observed that charr feeding on high doses of commercial feeds, as often found under aquaculture conditions, tend to cause diarrhoea. When charr were fed on diets containing sodium lactate, diarrhoea did not occur, probably indicating much lower amounts of residual nutrients and water in the gut. It was also proposed that the growth-promoting effect of dietary lactate in Arctic charr is the result of the relatively slow gastric emptying rate [22]. An increased holding time in the stomach augments the antibacterial potential of the lactic acid salt, which can therefore enhance the inhibition of pathogenic bacteria [23]. The improved growth of the Arctic charr did not affect its chemical composition [24].

A similar study by Ringø [25] proved the growth-promoting effect ($P<0.05$) of 1% sodium acetate as an additive for Arctic charr reared in brackish water, while 1% sodium formate gave only a non-significant numerical improvement versus a negative control. The stimulated growth of the fish which were fed sodium acetate to some extent may be explained by the higher feed intake, but enhanced digestibilities of dietary components might also contribute to the increased growth. Addition of 1%

Table 4 Effects of the sodium salt of different organic acids on the performance of Arctic charr and Atlantic salmon

Fish species	Acid/acid salt	Dose (%)	SGR (%)[1]	FCR[2]	Reference
Arctic charr	Control	0	0.61	n.d.	[21]
	Na-lactate	1	0.83[3]		
	Na-propionate	1	0.49[3]		
Arctic charr	Control	0	0.51	1.20	[25]
	Na-formate	1	0.58	1.08	
	Na-acetate	1	0.70[3]	0.96	
Arctic charr	Control	0	0.79	1.30	[24]
	Na-lactate	1	1.12	0.91	
Atlantic salmon	Control	0	0.97	n.d.	[26]
	Na-lactate	1.5	0.97		
Arctic charr	Control	0	0.28	n.d.	[22]
	Na-lactate	1.5	0.51[3]		
Atlantic salmon	Control	0	0.76	n.d.	[22]
	Na-lactate	1.5	0.79		

[1]SGR (%): specific growth rate=ln body mass$_1$ – ln body mass$_0$/culture period (d)×100.
[2]FCR: feed conversion ratio=feed intake/LWG.
[3]Significantly different from the control diet ($P<0.05$); n.d., – not determined.

sodium acetate to the diet significantly affected the digestibility coefficients ($P<0.05$) for both protein and total lipid, and for dietary fatty acids 14:0, 16:0, 18:1, 20:1, 22:1 and essential fatty acids 18:0 and 18:2(n-6).

Contrary to the significant results with 1% sodium lactate in Arctic charr, no such results were obtained with Atlantic salmon using the same dosage [22, 26]. One of the most notable differences between the two species, which probably explains the results, is the doubled retention time of dietary lactate in the stomach in Arctic charr. According to these authors, it seems likely that lactate or sodium lactic acid exerts its influence in the upper part of the digestive system and therefore any difference found here may explain the difference in growth response in the two species. There was, however, a benefit of mortality reduction the lactate-fed salmon from 19.9% in the negative control to 15.2%.

Further studies on salmonids again include rainbow trout. The effect of organic acids on mineral digestibility was tested in several studies. It was reported from pigs that the inclusion of dietary organic acids enhances mineral absorption [27]. Since the availability of phosphorous in particular from a fishmeal-based diet plays a vital role in salmonid aquaculture [28], different acidifiers have been tested under these conditions. Vielma and Lall [29] reported the effect of dietary formic acid on the availability of phosphorus in rainbow trout diets. These authors found that the apparent digestibility of phosphorus significantly increased ($P<0.05$) in fish fed a diet containing 10 ml/kg formic acid. Sugiura *et al.* [30] found that the availabilities of magnesium and calcium in fishmeal increased ($P<0.05$) by the dietary inclusion of formic acid. Apparent availabilities of calcium and phosphorus were also greatly affected by the inclusion of citric acid in the rainbow trout diet. Dietary inclusion of citric acid (5%) reduced phosphorus in the faeces of fish by approximately 50%, with no reduction in feed intake or appetite. Other apparent mineral availabilities increased by citric acid application include iron, magnesium, manganese and strontium. In contrast, mineral availabilities were not affected by citric acid use in agastric goldfish (*Carrasius auratus*), but a 5% inclusion of the dietary acidifier led to a marked reduction of feed intake. Inclusion of sodium citrate (5%) in the diet of rainbow trout also showed significantly improved availabilities of calcium and phosphorus, but less than that of pure citric acid.

Another study with rainbow trout used much lower dietary levels of citric acid [31]. In this study, diets were supplemented with 0, 0.4, 0.8 or 1.6% citric acid in different particle size fish-bone meals. Citric acid increased the whole-body ash content, but the body phosphorus content showed only a tendency to increase (P=0.07). On the other hand, dietary acidification significantly increased whole-body iron dose-dependently. Sugiura *et al.* [32] found that in high-ash diets for rainbow trout, feed acidification with citric acid decreased the effect of supplemental phytase, whereas in low-ash diets, it markedly increased the effect of the enzyme. In general, it can be concluded that adding citric acid to the diet of rainbow trout regulates chelation of calcium and phosphorus, thereby increasing the solubility of calcium phosphates and improving phosphorus and mineral availabilities [33].

More recent studies include experiments with rainbow trout fingerlings [34, 35], which were fed five experimental diets, a negative control, three diets containing 0.5, 1.0 and 1.5% of an organic acid blend (formic acid and its salts plus sorbic acid) and a diet containing an AGP (40 ppm Flavomycin®). After 3 months, improvement in growth was observed with increasing acid blend inclusion. The 1.0 and 1.5% dosages resulted in a significant

Table 5 Effects of potassium diformate supplementation in diets on the performance of tilapia challenged with *V. anguillarum* (modified from [37])

	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 ($P<0.05$).

improvement in specific growth rate (SGR) versus control ($P<0.05$). The improvement by the 1.5% acid blend was similar to that achieved by the AGP, but with a lower feed conversion ratio (FCR) than the antibiotic group. Unpublished information (Karl Sacherer, personal communication, 2006) also reveals that the use of an acid blend of formic and propionic acids and their salts on a sequential release medium is successfully used in the grow-out of Turkish rainbow trout.

The latest results in salmonids reveal that Atlantic salmon fed a fishmeal enriched with 1.4% potassium diformate (a potassium salt of formic acid) tended (P=0.055) to a higher SGR versus negative control [36]. Furthermore, groups fed 0.8 and 1.4% potassium diformate via fishmeal had a significantly better feed conversion and improved uniformity within fish groups. This was confirmed in older data (Rune Christiansen, personal communication, 1996 and 1998), where salmon fed diets containing potassium diformate-treated fishmeal had significantly higher growth rates, and improved protein and fat digestibilities.

In-Feed Acidifier in Tropical Aquaculture Species

Ramli *et al*. [37] tested potassium diformate as a growth promoter in tilapia grow-out in Indonesia (Table 5). In this study, fish were fed six times a day diets containing different concentrations of potassium diformate (0, 0.2, 0.3 and 0.5%) over a total period of 85 days. The diets contained 32% crude protein, 25% carbohydrate, 6% lipid and 10% fibre. The fish were challenged orally from day 10 with *Vibrio anguillarum* at 10^5 CFU/day for 20 days.

From day 1 to day 85, potassium diformate significantly improved feed intake ($P<0.01$), live weight gain (LWG) ($P<0.01$), FCR ($P<0.01$) and protein efficiency ratio (PER) ($P<0.05$). Furthermore, PER also significantly improved due to the addition of the formic acid salt ($P<0.05$). The improvement was greater for 0.2 and 0.5% formate addition. Survival rates of fish after the challenge with *V. anguillarum* on days 10–30 were also significantly higher than the negative control, and this effect was dose-dependent ($P<0.01$). The authors concluded that the use of potassium diformate at 0.2% is an efficient tool to control *V. anguillarum* in tropical tilapia culture.

Another study in tilapia (*Oreochromis niloticus*) investigated feeding behaviour in the fish using different organic acids [38], as sometimes reported for some organic acids or their salts in piglets [39]. Citric acid at a concentration of 10^{-2}–10^{-6} M and lactic acid at 10^{-2}–10^{-5} M stimulated feeding, as recorded automatically using the frequency of feeding 'bites' of the fish, whereas *O. niloticus* tended to avoid acetic acid at 10^{-3} M, while acetic acid at 10^{-5} M had no significant effects.

A more recent trial [40] determined the effects of an acid/salts blend, (containing of calcium formate, calcium propionate, calcium lactate, calcium phosphate and citric acid) at different levels (0.5, 1.0 and 1.5%) on the growth performance of tilapia. Fish were fed to appetite twice a day for 8 weeks, using a pelleted diet containing 31% crude protein. Despite a lack of statistically significant data for LWG and FCR, the blend at 1.5% resulted in a numerical increase in LWG of 11% versus negative control, with results similar to the AGP-supplemented diet (0.5% oxytetracycline). Such organic acid salts and blends may therefore be especially useful during grow-out period in tilapia culture [41].

More research on the potential growth-promoting effects on tilapia is currently being carried out with various single and blended organic acids at various dietary levels (Ng, Wing-Keong, personal communication, 2008). The effects of dietary organic acids on gut and faecal microflora population as well as survival of tilapia challenged with *Streptococcus agalactiae* or *Aeromonas hydrophila* are also being investigated.

Further research has been devoted to sea bream (*Pagrus major*), in order to determine the phosphorus utilization after feeding dietary organic acids, as observed in previous studies with other fish species [42]. The use of 1% each of citric acid, malic acid and lactic acid in three different dietary groups showed significantly better LWGs and FCRs in the citric acid group versus negative control, but malic or lactic acid did not improve performance. Phosphorus excretion in the citric, malic and lactic acids fed bream groups also significantly reduced, indicating a better phosphorus utilization. The higher absorption of phosphorus in diets supplemented with organic acids agrees with other reports that citric acid can increase the apparent digestibility of many minerals, including phosphorus, in fishmeal [33, 43].

Despite their lack of success in agastric goldfish in Europe, acidifiers have also been tested in agastric Indian carp (*Labeo rohita*). Baruah *et al.* [44] determined the interactions of dietary protein level, microbial phytase and citric acid inclusion on bone mineralization in *Labeo* juveniles. Their data showed that the addition of 3% citric acid to either a low- (25%) or high-protein diet (35%) resulted in a significantly decreased pH of the feed and intestinal digesta. Furthermore, bone ash content significantly increased, suggesting a better bioavailability of minerals. The mineral content of bones is in close agreement with these findings, since, for example, the phosphorus retention in the skeleton after citric acid supplementation significantly increased. Debnath *et al.* [45] suggest synergistic effects between microbial phytase and organic acids in this respect. A follow-up study [46] investigated the synergistic effects of citric acid and phytase on nutrient digestibility and growth performance in Indian carp, again in low (25%)- and high (35%)-protein diets. Citric acid in both diets significantly increased LWG and SGR in carp juveniles, while FCR reduced. No effects were observed on PER and apparent net protein utilization (ANPU). However, a significant interaction between citric acid and microbial phytase (500 units/Kg) was found for LWG, SGR, PER and ANPU, further supporting the findings of Debnath *et al.* [45]. Finally, it was found [47] that citric acid and microbial phytase have a synergistic effect on mineral bioavailability, as measured in the whole body and in the plasma. This effect was more prominent in low-protein diets.

Other omnivorous fish species have also been fed diets supplemented with acidifiers. In a recent trial, Owen *et al.* [48] tested sodium butyrate as a feed additive in the tropical catfish (*Clarias gariepinus*) added at 0.2% to two diets differing in their major protein source (fishmeal or defatted soya). Slightly higher growth and a concomitant reduction in FCR were observed in catfish fed the fishmeal diet supplemented with sodium butyrate, compared with the control diet, while fish receiving defatted soya together with 0.2% Na-butyrate showed no improvement. The SGR surplus in the fishmeal plus butyrate group was 4.7%, while the improvement in FCR was 4.1%. However, both indices differed insignificantly from the control. Sodium butyrate supplementation also appeared to increase the proportion of gram-positive bacteria in the hindgut of *C. gariepinus*, though this increase was not statistically significant.

Microbials and organic acids in fish

A different approach was taken by Vazquez *et al.* [49], who studied the effect of lactic acid bacteria cultures on pathogenic microbiota from turbot (*Scophthalmus maximus*). According to their results, inhibition of pathogenic species in fish by the use of lactic acid bacteria was achieved due to the presence of lactic and acetic acids, rather than bacteriocins, in all the cases studied. In other words, these bacteria cultures are only effective if they supply the turbot host with organic acids.

Acidifiers in Shrimps and Snails

Research in non-fish aquaculture species is somewhat limited. Tung *et al.* [50] reported that 0.5% sodium citrate with inactivated *Lactobacilli* boosted the growth of the Kuruma shrimp (*Masurpenaeus japonicus*). Further work suggests that a dose of 0.25% calcium formate can enhance giant tiger prawn (*Penaeus monodon*) survival in brackish water farms in Taiwan (Tan Seong Lim, personal communication, 2005). These results are to be evaluated again over more than just one grow-out season. Most recent data include the successful usage of acidifiers in the development of artificial diets for abalone culture in South Africa (Lourens de Wet, personal communication, 2007).

Conclusion/Summary

Despite the limited number of published studies on the use of acidifiers for the improvement of growth, feed efficiency, digestibility and mineral absorption in aquaculture, results from the available studies indicate promising potential and compel aquafeed manufacturers to consider the use of acidifiers in the diets they formulate. Furthermore, acidifiers can mitigate the impact of bacterial infections, thereby preventing diseases and thus affording higher survival rates. The use of acidifiers can be an efficient tool to achieve sustainable, economical and safe fish and shrimp production [51].

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Progress and perspectives of short-chain fatty acids in aquaculture

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Abstract

Gut microbiota is important and plays a crucial role in the host health and nutritional metabolism through multiple mechanisms. Short-chain fatty acids (SCFAs), which are carboxylic acids with aliphatic tails < 6 carbons, are mainly produced by anaerobic microbiota through fermentation of carbohydrates in the intestine. Acetate, propionate and butyrate are the most abundant SCFAs metabolites, important in energy homoeostasis, metabolism and the maintenance of gut health. In this review, we describe and document what is known about the production, absorption, transport and receptors as well as the factors that affect SCFA production in aquatic animals. Some evidence on the roles that SCFAs as feed additives play in improving growth performance, digestibility, survival rate, immune responses, disease resistance and structure and function of the intestinal tract and abundance of commensal microbiota in aquatic animals is summarized. In addition, the immune regulatory mechanism of SCFAs is highlighted. Although the effects of SCFAs in aquatic animals have been explored, further research is needed to profoundly investigate the mechanisms that by which SCFAs induce their effects on host metabolism.

Key words: dietary additives, disease resistance, gut microbiota, immunostimulant, metabolism, short-chain fatty acids.

Introduction

Aquatic animal products are an integral part of the human diet, with their global demand greater than that for beef, pork and poultry products (Tacon & Metian 2013). Fish are an ideal source of human nutrients as they contain easily digestible and high-quality proteins, essential fats (such as long-chain omega-3 fatty acids), vitamins (D, A and B) and minerals (FAO 2016). According to the Food and Agricultural Organization (FAO), the share of world fish production used for direct human consumption has remarkably increased from 67% in the 1960s to 87% (more than 146 million tonnes) in 2014 (FAO 2016). Growth in aquaculture fish production now replaces capture fishery and has helped to meet the increase in human fish consumption (Guerreiro *et al.* 2018). A number of issues come up under culture conditions, including increasing growth performance, digestibility, survival rate, improving immune function as well as maintaining good health of the cultured animals (Hoseinifar *et al.* 2015, 2016a; Nawaz *et al.* 2018), all of which require studies to fully understand. Intriguingly, the intestine of aquatic animals is an ideal environment for colonization and proliferation of symbiotic microbes (Han *et al.* 2010; Tran *et al.* 2017; Wang *et al.* 2018). Gut microbiota plays important roles in host health and nutritional metabolism through multiple mechanisms (Ríos-Covián *et al.* 2016; Li *et al.* 2018a; Ringø *et al.* 2018). For instance, microbial metabolism involved in carbohydrate fermentation converts indigestible dietary carbohydrates into short-chain fatty acids (SCFAs) (Piazzon *et al.* 2017), thereby affecting the host's physiology. SCFAs are carboxylic acids with aliphatic tails (< 6 carbons) and comprise of both straight- and branched-chain conformation (Layden *et al.* 2013; Ríos-Covián *et al.* 2016). In aquatic animals, the most predominant straight-chain SCFAs include acetic acid (C2), propionic acid (C3)

and butyric acid (C4), with the levels of valeratic acid, isobutyric acid and isovaleric acid being low (i.e. accounting for only 2–9% of the total SCFA production) (Clements *et al.* 1994; Smith *et al.* 1996; German 2009; Hao *et al.* 2017a,b). The measurement of intestinal SCFAs in omnivorous and herbivorous marine fish species has been early started, with SCFAs being confirmed for the first time as bacterial metabolic by-products in the intestine of sea chubs, *Kyphosus cornelii* and *K. sydneyanus*, in 1987 (reviewed in Smith *et al.* (1996) and Burr *et al.* (2005)). Attempts at understanding the beneficial roles of SCFAs in regulating metabolism in human and other animals have been going on for decades. SCFAs play an important role in maintaining gut homoeostasis and serve to monitor the relationship with the host, its immune system and intestinal commensal microbes (Trachsel *et al.* 2016). In aquaculture, SCFAs and their salts have been used as growth promoters (Ringø *et al.* 1994; Gao *et al.* 2011; Robles *et al.* 2013; Liu *et al.* 2014; Silva *et al.* 2016b) and immune stimulators (Liu *et al.* 2014; Estensoro *et al.* 2016; Hoseinifar *et al.* 2016b, 2017a; Safari *et al.* 2016; Silva *et al.* 2016b; Tian *et al.* 2017), indicating a promising application of SCFAs as dietary supplements in farmed aquatic animals. To date, only a few studies regarding the application of SCFAs as feed additives in aquaculture have been reviewed in details (Hoseinifar *et al.* 2017b). The current review, therefore, aims at summarizing the basic information about SCFAs, including their production, absorption, transport, receptors and the factors that affect SCFA production in aquatic animals. The application of SCFAs in aquaculture and their regulation mechanisms in immune responses will also be discussed. Suggestions on further applications of SCFAs in aquaculture will be given. It is envisioned that the information provided here would guide future directions in the use of feed supplements (including SCFAs) in improving growth performance, health status and disease resistance of farmed aquatic animals.

Short-chain fatty acids and production

SCFAs are the end products of microbial anaerobic metabolism in aquatic animals, with the highest concentrations found in the posterior region of the gut (85% of the total SCFAs) (Clements *et al.* 1994), where they are absorbed and used as a potential energy source (Titus & Ahearn 1988; Smith *et al.* 1996; Mountfort *et al.* 2002; German 2009). The most abundant SCFAs in the gut of fish are acetate, propionate and butyrate (Clements *et al.* 1994; German 2009; Hao *et al.* 2017a,b). Nondigested proteins or peptides are also fermented to give a range of metabolites, including branched-chain fatty acids, amines, phenols, indoles and thiols (Canfora *et al.* 2015). The branched-chain SCFAs, isobutyrate, isovalerate and 2-methylbutyrate, are typically produced from deamination of the amino acids valine, leucine and isoleucine respectively (this constitutes only 2–9% of the total SCFAs in fish) (Clements *et al.* 1994; Smith *et al.* 1996; Hao *et al.* 2017b). It has been reported that the concentrations of SCFAs increased towards the distal intestine of fish (Mountfort *et al.* 2002; Leenhouwers *et al.* 2007). For example, in *K. sydneyanus*, it was shown that the levels of acetate, propionate and butyrate were 1.1, 0.0 and 0.0 mM (in the stomach section), 37.4, 13.9 and 2.1 mM (in the proximal section) and 37.5, 12.8 and 1.3 mM (in the distal gut) respectively (Mountfort *et al.* 2002). The concentrations of the SCFAs, acetate, propionate and butyrate in terms of molar ratio, are different among fish and gut regions within a species and are presented by the ratio acetate:propionate:butyrate:valerate, for which the posterior intestine is 83:8:9:1 (*Odax cyanomelas*), 64:21:14:1 (*Odax pullus*) and 74:17:9:0 (*Crinodus lophodont*) (Clements *et al.* 1994). However, it is somewhat difficult to assess the ratios of SCFAs since their production involves the other types of fermentation substrates (Ohira *et al.* 2017). Obviously, the synthesis of SCFAs depends on the microbial composition and environmental conditions (i.e. pH, hydrogen partial pressure and available substrates) (Louis *et al.* 2014) as well as species of fish (herbivorous freshwater fish have lower SCFA levels in the gut than that of their marine counterparts, while carnivorous fish have relatively higher concentrations than that of herbivorous and omnivorous species) (Smith *et al.* 1996; Clements *et al.* 2014; Hao *et al.* 2017a). Consistent with this observation, the concentration of total SCFAs in the hindguts of herbivorous freshwater grass carp (*Ctenopharyngodon idellus*) (5.04 mM) (Hao *et al.* 2017a) was found to be lower than that of two herbivorous marine fish, *K. sydneyanus* and *O. pullus* (both >37 mM) (Mountfort *et al.* 2002).

Fermentation relates to many reactions and metabolic processes in the anaerobic microbial breakdown of organic matter, with the contribution of specific species of bacteria. Although much is known about the biosynthesis of SCFAs in humans and other mammals (Louis *et al.* 2014; Flint *et al.* 2015; Koh *et al.* 2016; Reichardt *et al.* 2018) (Fig. 1), limited data are available for aquatic animals. In mammals, acetate is produced from pyruvate *via* acetyl-CoA or *via* the Wood–Ljungdahl pathway or from arabinoxylan by enteric bacteria (*Bifidobacterium* spp.) (Wolfe 2005; Louis *et al.* 2014; Reichardt *et al.* 2018). Propionate is synthesized from lactate (through the acrylate pathway), succinate (succinate pathway) or deoxyhexose sugars (fucose and rhamnose) (propanediol pathway) (Louis *et al.* 2014; Reichardt *et al.* 2014, 2018; Flint *et al.* 2015; Ríos-Covián *et al.* 2016). Butyrate is formed from either butyryl-CoA (Louis *et al.* 2014; Reichardt *et al.* 2018) or a combination of lactate with acetate (Flint *et al.* 2015) or mammalian cells *via* fatty acid (FA) oxidation and glucose metabolism (Bourassa

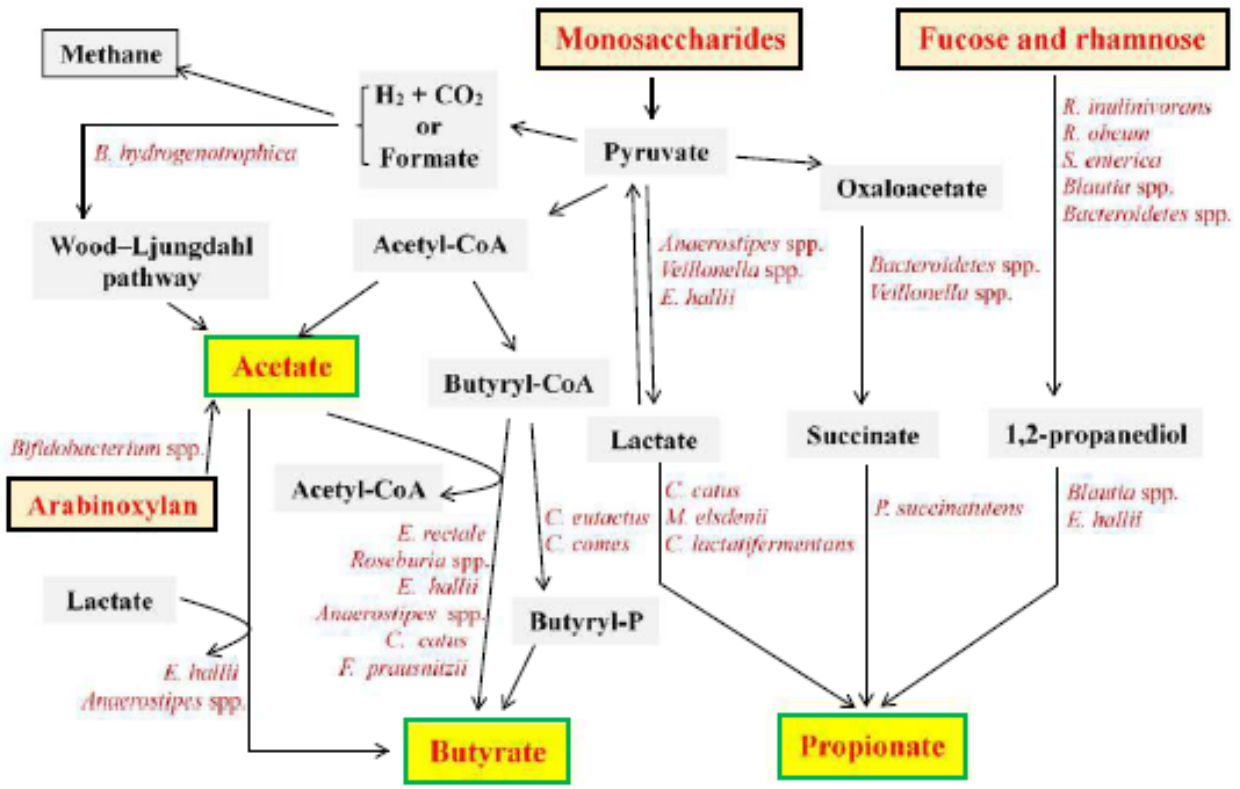

Figure 1 An overview of the production of acetate, propionate and butyrate by microbial fermentation in the intestine (Louis *et al.* 2014; Flint *et al.* 2015; Reichardt *et al.* 2018). Bacterial species involved in the production of SCFAs are *Blautia hydrogenotrophica*, *Bifidobacterium* spp., *Eubacterium hallii*, *Anaerostipes* spp., *Eubacterium rectale*, *Roseburia* spp., *Coprococcus catus*, *Faecalibacterium prausnitzii*, *Coprococcus eutactus*, *Coprococcus comes*, *Veillonella* spp., *Megasphaera elsdenii*, *Clostridium lactatifermentans*, *Bacteroidetes* spp., *Phascolarctobacterium succinatutens*, *Roseburia inulinivorans*, *Ruminococcus obeum*, *Salmonella enterica* and *Blautia* spp.

et al. 2016). With a number of reaction pathways involved in the metabolism of SCFAs means that it merits further investigations in aquatic animals.

Factors influencing the formation of SCFAs

As mentioned above, SCFAs are the primary end products of non-digestible carbohydrate fermentation by gut microbiota in the intestinal tract. The effects of dietary intake (including non-digestible dietary carbohydrates, proteins and fats) on the formation of SCFAs have previously been reported (Flint *et al.* 2015). It has been shown that the composition of SCFAs is dictated primarily by the chemical structures of the substrates and the microbial composition and activities (Macfarlane & Gibson 1997; Flint *et al.* 2015; Ríos-Covián *et al.* 2016). For instance, the amount and type of fibre consumed have an extreme influence on the type and amount of SCFAs produced in human and mammals (den Besten *et al.* 2013; Canfora *et al.* 2015), which is also found in aquatic animals (Hao *et al.* 2017a,b). The total SCFA concentrations in the gut of grass carp decreased by almost 50% with dietary change (dietary shift from animal-based to plant-based diets) (Hao *et al.* 2017b). An increase in the abundance of some potential SCFA-producing microbiota (i.e. *Roseburia*, *Coprococcus*, *Blautia*, *Dialister*, *Lactobacillus*, *Propionibacterium*, *Bifidobacterium* and *Clostridium*) was recently observed in the intestine of *Nibea coibor* fed on a formulated diet compared with those fed on fish-trash diets (Li *et al.* 2018b). In a recent study, the levels of total SCFAs and acetate were also observed in a recent study to decrease in Sudan grass-fed grass carp compared with those on compound feed and fish meal (Hao *et al.* 2017a). The question is, to what extent does the high fermentation by gut microbiota of fish fed on fishmeal have on the production of high levels of SCFAs. It has been observed that, in situations of insufficient fibre for fermentation, microbes switch to less energy favourable sources (amino acids from dietary or endogenous proteins or dietary fats) for proliferation (Cummings *et al.* 1987; Koh *et al.* 2016). A study on the carnivorous red sea bream (*Pagrus major*) revealed that total SCFAs in the hindgut (< 12 mM) were lower than that of herbivorous teleosts (Kihara 2008).

The composition of gut microbiota, which is mainly influenced by diet and disease, can change the metabolic

profile (Llewellyn *et al.* 2014; Carding *et al.* 2015; Flint *et al.* 2015). Monosaccharides converted from dietary fibre in the gut are associated with a complex process mediated by the enzyme repertoire of specific microbial members (Koh *et al.* 2016). In grass carp, a positive correlation between the levels of acetate and the total bacterial count was observed (Hao *et al.* 2017a). The high levels of acetate, butyrate and propionate in Siberian sturgeon fed AXOS involved an increase in the relative abundance of *Eubacterium*, *Clostridium*, *Lactobacillus*, *Bacillus* and *Lactococcus* present in the hindgut of the sturgeon (Geraylou *et al.* 2012, 2013). There is, therefore, a positive relationship between the diversity and composition of gut microbiota, especially SCFA producers, and production of SCFAs in aquatic animals. However, it remains to be seen precisely which microbial species are mainly responsible for SCFA production in fish (Llewellyn *et al.* 2014). Furthermore, stress-stimulated microbiota dysbiosis may be a factor influencing the production of SCFA in the gut. A recent study revealed that gut dysbiosis in Tsumura Suzuki obese diabetes (TSOD) mice leads to a reduction in total plasma SCFA levels, where acetate was decreased, with an increase in propionate and butyrate, while valerate and hexanoate were absent in the TSOD mice compared with controls (Nishitsuji *et al.* 2017). In aquatic animals such as Atlantic salmon (*Salmo salar*) and Atlantic cod (*Gadus morhua*), supplementation with soybean-derived protein (Ringø *et al.* 2006; Green *et al.* 2013) as well as in largemouth bronze gudgeon (*Coreius guichenoti*) and grass carp, which suffer from infections (Li *et al.* 2016; Tran *et al.* 2018), resulted in significant changes in gut microbiota. It is unclear whether the changes of microbiota structure in these studies directly affected the levels of SCFA in the host gut. However, in grass carp on a changed diet (from animal-based to plant-based diet), total prokaryote and bacteria counts decreased after the dietary shift, which resulted in a decline in SCFA levels (Hao *et al.* 2017a,b). This study clearly shows a positive correlation between the concentration of SCFA and the total bacterial count in grass carp gut.

Prebiotics are non-digestible food ingredients that modulate the composition and/or activity of gut microbiota, which is beneficial to the host (Bindels *et al.* 2015; Valcheva & Dieleman 2016). Prebiotics selectively promote the growth of beneficial microbiota (including SCFA-producing bacteria), which most likely stimulates gut fermentation and SCFA production (Burr *et al.* 2005; Geraylou *et al.* 2013; Nawaz *et al.* 2018). Some prebiotics, including α-starch, gelatinized starch, inulin, maize, barley, wheat, rye, lactosucrose, arabinoxylooligosaccharides (AXOS), fructooligosaccharide (FOS) and pre-gelatinized tapioca starch, have all been proved to be capable of influencing the production of SCFAs in the gut of aquatic animals *in vivo* (summarized in Table 1). Although many studies have reported good results on the effects of prebiotic supplementation on the production of SCFAs in the gut of aquatic animals, *in vitro* fermentation has been used as a simpler approach. *In vitro* cultures of arabinoxylan, whole wheat, soybean-oligosaccharides, isomalto-oligosaccharides, raffinose, gentiobiose, lactosucrose, AXOS, oligofructose, xylose or fructose using inocula of fish gut microbes have yielded high production of major SCFAs in fish species such as common carp (*Cyprinus carpio*) (Kihara & Sakata 2002), Nile tilapia (*Oreochromis niloticus*) and European seabass (*Dicentrarchus labrax*) (Leenhouwers *et al.* 2008) and Siberian sturgeon and African catfish (Geraylou *et al.* 2014). Additionally, organic acids and their salts, including sodium butyrate, acetate, propionate, formate or citrate, have been shown to decrease the gut SCFAs in red hybrid tilapia (*Oreochromis* sp.) (Romano *et al.* 2016; Ebrahimi *et al.* 2017). Results from these studies have revealed that the type of prebiotic substrates, fermentation activity and the microbial populations associated with the process strongly affect the composition of fermentation end products.

Probiotics can also serve as modulators of SCFA formation in the human gut (Hemalatha *et al.* 2017). Similar inferences have been observed in fish species (Allameh *et al.* 2017; Duan *et al.* 2017; Asaduzzaman *et al.* 2018). Recent studies have revealed that *Enterococcus faecalis* increased the production of propionic and butyric acid in the posterior intestine of Javanese carp (*Puntius gonionotus*) (Allameh *et al.* 2017). *Alcaligenes* sp. AFG22 brought effects to Malaysian mahseer (*Tor tambroides*) in enhancing the levels of acetate and total SCFA (Asaduzzaman *et al.* 2018). A diet supplemented with *Clostridium butyricum* increased acetic, propionic and butyric acid production in whiteleg shrimp (*Litopenaeus vannamei*) (Duan *et al.* 2017). Based on their results, the authors suggested that increases in levels of SCFAs are involved in the probiotic supplementation.

pH has a profound influence on the transport of SCFAs from the lumen to the colonocytes (Cook & Sellin 1998) and also influences the growth of SCFA-producing bacteria (Walker *et al.* 2005). On the basis of this, a low inoculum pH (approximately 5–6) in an *in vitro* fermentation of glucose and native wheat starch with the intestinal contents of Nile tilapia and European sea bass (Leenhouwers *et al.* 2008) has been reported to favour the growth of lactic acid producers (such as *Lactobacillus*, *Bifidobacteria* and *Eubacteria* species) and the conversion of lactic acid produced into acetic, propionic, butyric and longer chain fatty acids. This should be confirmed in future research conducted in aquatic animal models, with the high pH in the gut (usually > 7) (Ray & Ringø 2014; Ban *et al.* 2017).

Other factors such as fish species, living environments, seasons, intestinal morphology, intestinal regions, gut transit time, rate of SCFA transport across the gut epithelium

Table 1 Summary of the effects of prebiotics on the production of short-chain fatty acids (SCFAs) in the gut of aquatic animals

Prebiotic	Host species	Dose and period administered	Effects on SCFA production	References
α-starch	Nile tilapia (*Oreochromis niloticus*) (~50 g)	150 g kg^{-1} (14 days)	↑ Acetate, propionate, n-butyrate and total SCFA	Kihara and Sakata (1997)
Gelatinized starch	Nile tilapia (45 ± 1.5 g)	181.8 and 309 g kg^{-1} (8 weeks)	↑ Total SCFA production in the stomach ↓ Total SCFA production in the intestine	Amirkolaie *et al.* (2006)
Inulin	Siberian sturgeon (*Acipenser baeri*) (213 ± 0.7 g)	20 g kg^{-1} (1 month)	→ Total SCFA and lactate production ↓ Butyrate production	Mahious *et al.* (2006) (reviewed in Ringø *et al.* (2010)
Maize, barley, wheat or rye	Nile tilapia (70 g)	49% (60 days)	→ Total concentration and type of SCFA (acetic, propionic and butyric acid)	Leenhouwers *et al.* (2007)
Lactosucrose	Red sea bream (*Pagrus major*) (~70 g)	0.24% (2 months)	↑ Total SCFA (molar sum of acetic, propionic and n-butyric acids)	Kihara (2008)
AXOS	African catfish (*Clarias gariepinus*) (~20 g)	10 and 20 g kg^{-1} (10 weeks)	↑ Acetate, propionate and total SCFA production → Butyrate production	Rurangwa *et al.* (2008) (reviewed in Ringø *et al.* (2010))
	Siberian sturgeon (~20 g)	10 and 20 g kg^{-1} (10 weeks)	↑ Acetate, propionate and total SCFA production → Butyrate production	Rurangwa *et al.* (2008) (reviewed in Ringø *et al.* (2010)
	Siberian sturgeon (25.9 ± 0.9 g)	2% (12 weeks)	↑ Acetate, butyrate and total SCFA production → Propionate production	Geraylou *et al.* (2012)
	Siberian sturgeon (30 g)	2 or 4% (10 weeks)	↑ Acetate, butyrate, propionate and total SCFA production	Geraylou *et al.* (2013)
FOS	Freshwater prawn (*Macrobrachium rosenbergii*) (PL-12)	0.4–2% (56 days)	↑ Acetic and propionic acid production → Butyric acid production	Chen *et al.* (2017)
Pre-gelatinized tapioca starch	African catfish (6.2 ± 0.3 g)	25% (8 weeks)	↑ Acetic and butyric acid production → Propionic acid production	Romano *et al.* (2018)

Symbols indicate an increase (↑), decrease (↓) or no effect (→) on the concentrations of SCFA production; AXOS: arabinoxylooligosaccharides, FOS: fructooligosaccharide.

as well as species and number of microbiota also influence the production of SCFAs (Smith *et al.* 1996; Mountfort *et al.* 2002; German 2009; Clements *et al.* 2014; Canfora *et al.* 2015; Wu *et al.* 2015; Hao *et al.* 2017a,b). Clements *et al.* (2014) have suggested that herbivorous and omnivorous freshwater fish show shorter gut retention time and thus lower SCFA levels in the gut than their marine counterparts. Consistent with this observation, a lower level of SCFAs in the hindgut of grass carp has been found in comparison with that in marine fish and mammals (Hao *et al.* 2017a).

Absorption, transport and receptors of SCFAs

About 95–99% of SCFA produced is rapidly absorbed in the hindgut, which exceeds those of other solutes present in the gut (Titus & Ahearn 1988) and is used in different biosynthetic processes by the host (den Besten *et al.* 2013). SCFAs are mainly used within the vicinity of the gut, while a small proportion of propionate and acetate reaches the liver through the portal vein from the colon capillaries, where is used in the liver as substrates for the energy-producing tricarboxylic acid cycle and gluconeogenesis (Tan *et al.* 2014; Ohira *et al.* 2017). In fish, acetate is transported into the portal blood and used as energy source for skeletal muscle or for lipid synthesis (Asaduzzaman *et al.* 2018). Absorption of SCFAs in tilapia is largely driven by the anion exchange with bicarbonate (in a ratio of 4 : 1) between the intestinal lumen and the blood (Titus & Ahearn 1988, 1991, 1992). In human and other mammals, SCFAs are also thought to be absorbed through either diffusion of protonated SCFAs or monocarboxylate transporters [(monocarboxylate transporter-1 (MCT1) and sodium-dependent monocarboxylate transporter-1 (SMCT1)]

(Cook & Sellin 1998; Canfora *et al.* 2015; Corrêa-Oliveira *et al.* 2016). Once absorbed, butyrate is used as fuel for colonocytes, and the rest is transported to the liver. In the liver, propionate is used for hepatic gluconeogenesis, while acetate and butyrate are used for lipogenesis (den Besten *et al.* 2013; Morrison & Preston 2016; Ríos-Covián *et al.* 2016). Unabsorbed SCFAs are excreted (Tan *et al.* 2014).

SCFAs appear to be signalling molecules in cellular processes through the binding of receptors. In mammals, the four receptors G-protein-coupled receptors 41 (GPR41), GPR43, GPR109A and Olfactory receptor 78 (OLFR78) have been described (Natarajan & Pluznick 2014). GPR41 is primarily activated by propionate, followed by butyrate and acetate (Byrne *et al.* 2015), which has been detected in adipose tissues, peripheral blood mononuclear cells, pancreas, spleen, bone marrow and lymph nodes (Byrne *et al.* 2015). GPR43 is efficiently triggered by acetate, propionate, butyrate and other SCFAs (Kimura *et al.* 2014) and is expressed in immune cells (eosinophils, basophils, neutrophils, monocytes, dendritic cells and mucosal mast cells), skeletal muscle, heart, spleen and adipose tissues (Tan *et al.* 2014; Byrne *et al.* 2015). GPR109A is expressed in colonic epithelium, adipose tissues and immune cells (Blad *et al.* 2012; Ganapathy *et al.* 2013; Kasubuchi *et al.* 2015; Koh *et al.* 2016), and ligands niacin (Vitamin B3), β-hydroxybutryate and butyrate, but not acetate or propionate (Natarajan & Pluznick 2014). Additionally, OLFR78, a receptor for acetate and propionate but not butyrate (Pluznick 2014; Kasubuchi *et al.* 2015), is expressed in the kidney and is responsible for responding to SCFAs (Pluznick *et al.* 2009). In summary, these receptors are expressed in a variety of tissues and cell types, indicating that SCFAs are involved in the regulation of substrate and energy metabolism as well as immune response in the host. However, these receptors are still unknown in aquatic animals, which require detail further investigation.

Regulation of host immunity by SCFAs

SCFAs have been reported in human and other animals to be important in regulating metabolic disorders and immune system *via* the inhibition of HDAC and activation of GPCRs (Meijer *et al.* 2010; Corrêa-Oliveira *et al.* 2016; Morrison & Preston 2016; Sivaprakasam *et al.* 2016; Sun *et al.* 2017). Acetate, propionate and butyrate are rapidly absorbed from the gut lumen and perform their functions, including systemic autoimmune responses and participate in different steps of the inflammation process (Sun *et al.* 2017). The potential roles of SCFAs in various cellular processes, including gene expression, differentiation, chemotaxis, proliferation and apoptosis, have recently been demonstrated (Corrêa-Oliveira *et al.* 2016; Sun *et al.* 2017). SCFAs are used by the immune cells of the gut-associated lymphoid tissue (Hoseinifar *et al.* 2017b) by recognition through the receptors (GPR41, GPR43 and GPR109A) on the surface of colonocytes and immune cells or directly transported into host cells (Louis *et al.* 2014). Although studies have confirmed the existence of these SCFA receptors, this has not been conducted in aquatic animals; however, it seems the mucosal immune response in fish is attributed to the aforementioned mechanisms (Hoseinifar *et al.* 2017b).

Butyrate is reported to improve the epithelial barrier function and gut permeability through the modulation of tight junction proteins and mucin expression (Canfora *et al.* 2015). In grass carp, a diet supplemented with sodium butyrate (SB) was shown to enhance the activities of lysozyme and acid phosphatase activities as well as the contents of complement 3 (C3), complement 4 (C4) and immunoglobulin M (IgM), and the expression of β-defensin-1, hepcidin, liver-expressed antimicrobial peptide 2B (LEAP-2B) and mucin2, thereby improving the intestinal immune functions of fish (Tian *et al.* 2017). Similarly, in whiteleg shrimp, propionic acid has been reported to stimulate the expression of penaeidin-3a (Pen-3a) and crustin (Cru) genes in the hepatopancreas (Pourmozaffar *et al.* 2017). Butyrate inhibits the activity of histone deacetylases (HDACs) in the colonocytes and immune cells, promoting the hyperacetylation of histones that are associated with signal transduction, with this having multiple consequences on gene expression and cellular differentiation (Louis *et al.* 2014). In human, regulatory T cells (T_{reg} cells) are central regulators of antimicrobial immunity and tissue inflammation (Chaudhry & Rudensky 2013). Interestingly, SCFAs are associated with the anti-inflammatory effects by promoting the differentiation of T_{Reg} cells and IL-10-producing T cells, as well as by blocking activation of nuclear factor-κB (NF-κB), in addition to the induction of apoptosis by HDAC inhibition (Louis *et al.* 2014). Administration of SB in grass carp for 60 days was shown to induce the downregulation of the pro-inflammatory cytokines TNF-α, IFN-γ2, IL-1β, IL-6, IL-8, IL-15, IL-17D, IL-12p35 and IL-12p40, while there was upregulation of the anti-inflammatory cytokines IL-10, IL-11, TGF-β1, TGF-β2, IL-4/13A and IL-4/13B (Tian *et al.* 2017). Besides, microencapsulated sodium butyrate (MSB) significantly correlated with the expression of gut HSP70, pro-inflammatory cytokines (including IL-1β and TNF-α) and anti-inflammatory cytokines (TGF-β) within each gut segment, except for HSP70 in the distal gut and IL-1β in the foregut of common carp (*C. carpio*) (Liu *et al.* 2014). Prolonged administrations of SB induced the downregulation of NF-κB p65, c-Rel, IKKβ, IKKγ, p38MAPK and MAPKK6 genes, and the upregulation of IκBα gene in the intestinal tissue of young grass carp (Tian *et al.* 2017). Thus, SB supplementation depressed intestinal inflammation (Fig. 2). Since SCFAs

have benefits to the host immune responses in mammals, molecular tools could be used to study the interactions among the components of the immune system of aquatic animals' further studies.

Application of SCFAs in aquaculture

The important effects of SCFAs formed by microbial fermentation on the host metabolism and colonic immunity have been reviewed elsewhere (Louis *et al.* 2014; Natarajan & Pluznick 2014; Tan *et al.* 2014; Byrne *et al.* 2015; Corrêa-Oliveira *et al.* 2016; Koh *et al.* 2016; Morrison & Preston 2016). In fish, the positive effects of using SCFAs and their salts have been reported (Hoseinifar *et al.* 2017b). Previous studies have explored the main roles of SCFAs, including improving growth performance, feed efficiency, immune responses, disease resistance survival rate and intestinal microbiota in aquatic animals. A summary of the SCFAs used in aquaculture and the effects on their hosts is shown in Table 2.

Increase growth performance, digestibility and survival rate

In aquaculture, the benefits of dietary SCFAs supplementation have attracted attention since the 1990s. Early work on the influence of dietary SCFAs (including lactate and propionate) supplementation on the growth performance of Arctic charr (*Salvelinus alpinus*) was first carried out by Ringø (1991). Since then, many studies on this subject have been carried out (Ringø *et al.* 1994; Gao *et al.* 2011; Robles *et al.* 2013; Liu *et al.* 2014; Silva *et al.* 2016b). In rainbow trout (*Oncorhynchus mykiss*), supplementation of dietary organic acid salt blend (10 g acid moiety kg^{-1} of a mixture of

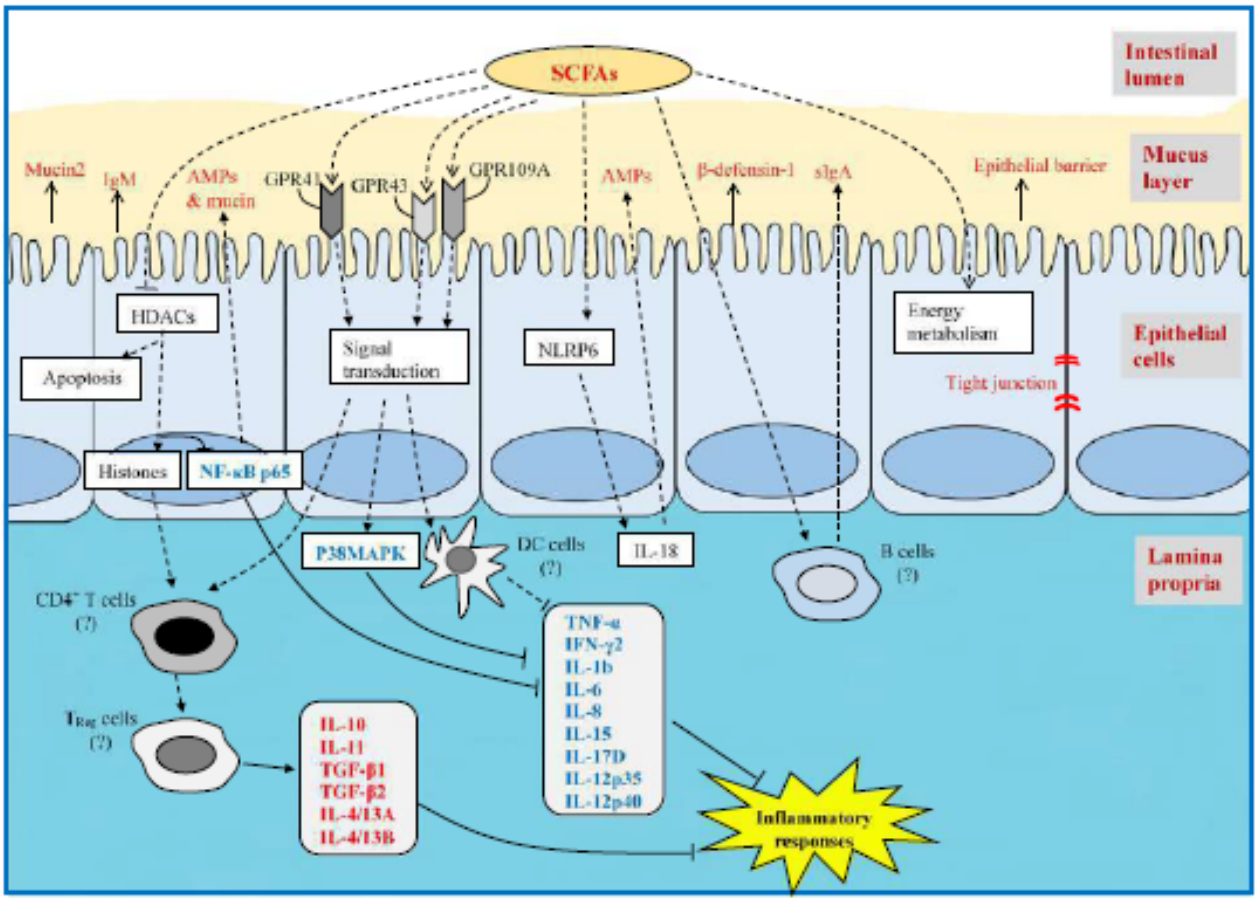

Figure 2 An overview of the proposed effects of butyrate on fish intestinal immune system. The solid arrows indicate the evidence of the mechanism; the dashed arrows indicate the hypothetical evidence; the flathead arrows indicate the inhibition. Red and blue characters indicate the increases and decreases in production of the subjects respectively. The question marks indicate the hypothetical evidence (Louis *et al.* 2014; Rooks & Garrett 2016; Levy *et al.* 2017; Tian *et al.* 2017). Abbreviations: GPR41 (or 43 or 109A), G-protein-coupled receptor 41 (or 43 or 109A); NLRP6, NOD-like receptor family pyrin domain containing 6; AMP, antimicrobial peptide; IgM, immunoglobulin M; sIgA, secretory immunoglobulin A; HDACs, histone deacetylases; T_{Reg} cells, Regulatory T cells; DC cells, dendritic cells; IL, interleukin; TGF, transforming growth factor; TNF, tumour necrosis factor; IFN, interferon; NF-κB, nuclear factor kappa B; p38MAPK, p38 mitogen-activated protein kinase.

sodium formate and butyrate with a ratio of 2:1) revealed that a negative effect on the feed conversion ratio (FCR) in fish fed the organic acid salt blend supplemented diet compared to controls, with a non-significant difference in the growth performance of the fish among treatments (Gao *et al.* 2011). The authors noted that supplementing diets with a sodium formate and butyrate blend neither had benefits in growth rate nor feed utilization of rainbow trout. A

Table 2 Summary of the effects of dietary short-chain fatty acids (SCFAs) on physiological responses of aquatic animals

Host species	SCFAs	Effects on host	References
Arctic charr (*Salvelinus alpinus*)	Na-lactate Na-propionate	Na-lactate (1%) increased growth rate, decreased amounts of water, energy, lipid, protein, free amino acids and triacylglycerols (in the diets) in gut content, and was able to prevent diarrhoea Na-propionate (1%) depressed the growth rate and absorption of the chemical contents	Ringø (1991)
Rainbow trout (*Oncorhynchus mykiss*)	A mixture of sodium formate and butyrate (2:1)	The diet with a mixture of sodium formate and butyrate (1%) did not improve growth rate or feed utilization of rainbow trout	Gao *et al.* (2011)
Common carp (*Cyprinus carpio*)	Microencapsulated sodium butyrate (MSB)	MSB-supplemented diet (300 mg/kg) increased weight gain, reduced FCR, enhanced the expressions of HSP70, IL-1β and TNF-α and TGF-β within each gut segment, excepting HSP70 in the distal gut and IL-1β in the foregut, whereas it did not affect the gut microbiota	Liu *et al.* (2014)
Sea bream (*Sparus aurata*)	Butyrate (Gustor Aqua BP70®)	Butyrate increased weight gain, the availability of several essential amino acids and nucleotide derivatives, energy provision for enteric cells, and transmethylation activity of the fish	Robles *et al.* (2013)
	Sodium butyrate (SB) (BP-70 ®Norel)	SB increased growth performance and did not change significantly VSI and HSI, but the GI was significantly higher in fish fed the diet with the highest butyrate dose (0.8%) Plasma glucose and cortisol increased with SB supplementation (0.4%) SB (0.8%) increased the abundance of mucosal folding, infiltration of lymphocytes, number of granulocytes in the submucosa along the intestine and accumulation of glycogen in hepatocytes, and stimulated the expression of PCNA and ALPI through the intestinal tract	Estensoro *et al.* (2016)
	Butyrate	Butyrate (0.8%) slightly increased survival after bacterial challenge, avoided growth retardation in parasitized fish (after challenge with *Photobacterium damselae* subsp. *piscicida* (*Phdp*)), increased intestinal microbiota diversity with a higher representation of butyrate-producing bacteria and reversed most vegetable diet-induced changes in the gut proteome	Piazzon *et al.* (2017)
	SILOhealth 108Z	SILOhealth 108Z (0.5%) increased the number of lactic acid bacteria (*Lactobacillus*) and reduced Gammaproteobacteria	Rimoldi *et al.* (2018)
European sea bass (*Dicentrarchus labrax*)	SB	No change in growth between fish fed diet control and diet 0.2% SB SB stimulated the expressions of IL-1β, IL-6, IL-8, IL-10 and TNFα genes; however, only TNFα was significantly upregulated in the distal intestine and changed the intestinal morphology	Rimoldi *et al.* (2016)
Zebrafish (*Danio rerio*)	Sodium propionate (SP)	SP supplementation increased the expression of TNF-α, IL-1β and lysozyme genes in the intestine, whereas decreased that of SOD, CAT and HSP70 genes in the liver of the fish	Safari *et al.* (2016)

Table 2 (continued)

Host species	SCFAs	Effects on host	References
Grass carp (*Ctenopharyngodon idella*)	SB	SB (0.1%) improved the fish growth performance and intestinal growth and function, increased lysozyme and acid phosphatase activities, complement (C3 and C4) and immunoglobulin M contents, and regulated the immunity-related genes SB increased the number of *Lactobacillus* and butyrate levels, while decreased *Aeromonas* and *Escherichia coli* and acetate and propionate levels in the gut of the fish	Tian *et al.* (2017)
Nile tilapia (*Oreochromis niloticus*)	SB	The lymphocytes and monocyte numbers were affected by dietary SB (1.0, 2.0 and 3.0%), while the activities of serum enzymes alanine aminotransferase, aspartate aminotransferase and phagocytes were not affected Dietary SB (2% and 3%) changed the structures of liver and kidney, but not of gills of the fish	Ali *et al.* (2018)
Red hybrid tilapia (*Oreochromis* sp.)	SB Sodium acetate, SP Sodium formate	The supplemented salts (2% for each of the salts) did not affect the fish growth or feeding efficiencies, decrease in muscle lipid peroxidation and intestinal SCFAs, and induction of stress and compromised health	Ebrahimi *et al.* (2017)
Whiteleg shrimp (*Litopenaeus vannamei*)	Fumarate Succinate Butyrate Propionate	The highest final weights (53%, 46%, 38% and 29%) were gain in shrimp fed with fumarate, succinate, butyrate, and propionate, respectively, in comparison to the controls. Both acetate and propionate stimulated the activities of trypsin and chymotrypsin, whereas lactate and citrate inhibited. Both fumarate and succinate are potential for increased protein digestibility	Silva *et al.* (2016a)
	Butyrate Propionate	Both butyrate and propionate (0.5%, 1% and 2%) increased the final weight, feed efficiency, nitrogen retention, protein efficiency rate, survival, yield and the level of serum agglutination units of administered shrimps The butyrate-supplemented diet showed lower counts of *Vibrio* sp. in the intestine	Silva *et al.* (2016b)
	Propionic acid	Propionic acid (0.5%) did not enhance the growth performance, but increased the expression of prophenoloxidase, lysozyme, penaeidin-3a and crustin genes in the hepatopancreas of shrimps	Pourmozaffar *et al.* (2017)
	SB SB+*Lactobacillus plantarum*	Administration of SB alone or a combination with *L. plantarum* did not affect the growth performance, immunological parameters or *Vibrio* spp., and total heterotrophic bacteria counts in the intestine *L. plantarum* and SB enhanced resistance against *Vibrio alginolyticus* in shrimp	Ramírez *et al.* (2017)

similar scenario was found in European sea bass (*D. labrax*) fed with a butyrate-supplemented diet (Rimoldi *et al.* 2016), where the specific growth rate and FCR were not significantly different between fish fed a butyrate-supplemented and controlled diet. On the contrary, it has been reported that the addition of butyrate (0.21%, Gustor Aqua BP70®) to diets for sea bream (*Sparus aurata*) increased the weight of fish (Robles *et al.* 2013). Furthermore, the authors reported that butyrate enhanced the availability of essential amino acids, nucleotide derivatives, energy provision for enteric cells and transmethylation activity in the fish (Robles *et al.* 2013). Increase in weight gain and a reduction in FCR have also been observed in common carp fed an MSB diet (Liu *et al.* 2014), while the potential beneficial effects of an SB (BP-70 ®Norel) supplemented diet on the growth performance of gilthead sea bream (*S. aurata*) have been observed (Estensoro *et al.* 2016). Although both viscerosomatic and hepatosomatic indexes were not significantly altered, the gut index was significantly higher in fish fed on SB-supplemented diet (0.8% BP-70) compared to the other groups, the SB (0.4%) resulted in an increase in the plasma glucose and cortisol levels in gilthead sea bream

(Estensoro *et al.* 2016). Recently, Tian *et al.* (2017) reported that SB (0.1%) appears to have beneficial effects in young grass carp, as it improved the growth performance (weight gain, specific growth rate, feed intake and feed efficiency), growth and function of intestine (including weight, length, somatic index, folds height, trypsin, chymotrypsin, lipase and amylase activities) and activities of lysozyme and acid phosphatase, while decreasing the concentration of both acetate and propionate in intestine. However, in red hybrid tilapia (Ebrahimi *et al.* 2017), 2% of sodium butyrate, acetate, propionate or formate was shown to have no effect on the growth or feeding efficiency of the fish, a decrease in muscle lipid peroxidation and intestinal SCFAs, and induction of stress and compromised health was observed.

In shrimp culture, diets supplemented with propionate and butyrate enhanced the final weight of whiteleg shrimp (Silva *et al.* 2016b). A diet containing 2% butyrate resulted in higher feed efficiency, nitrogen retention, protein efficiency rate, survival and yield of shrimp compared to controls (Silva *et al.* 2016b). Sodium salts of fumarate, succinate, butyrate and propionate added at 73 mM kg^{-1} to commercial diet, and one salt per diet, was demonstrated to act as growth promoters in whiteleg shrimp (Silva *et al.* 2016a). The highest final weights of 53%, 46%, 38% and 29% were observed in shrimp fed with fumarate, succinate, butyrate and propionate, respectively, compared to the controls. Moreover, both acetate and propionate were observed to be stimulators of trypsin and chymotrypsin activities, whereas lactate and citrate were inhibitors. Also, both fumarate and succinate had potential benefits on protein digestibility (Silva *et al.* 2016a). However, the growth performance of whiteleg shrimp fed propionic acid (0.5%) had no significant difference compared with control shrimp after a 60-day culture (Pourmozaffar *et al.* 2017). Taken together, the aforementioned studies suggest important roles of supplemented SCFAs on the growth performance and digestion of aquaculture species.

Enhancement of immune responses and disease resistance

Supplementation of SCFAs in diets has shown beneficial effects by enhancing the immune systems and disease resistance in many aquatic animals. A diet supplemented with MSB enhanced and changed the immune response of common carp associated with the expressions of gut heat shock protein-70 (HSP70), pro-inflammatory cytokines (such as IL-1β and TNF-α) and anti-inflammatory cytokines (TGF-β) within each gut segment, except HSP70 in the distal gut and IL-1β in the foregut (Liu *et al.* 2014). In gilthead sea bream, Estensoro *et al.* (2016) observed that the two genes, proliferating cell nuclear antigen (PCNA) and intestinal FA-binding protein (FABP2), were expressed in the intestinal tract; however, only PCNA had significantly low expression in the fish fed on 0.8% butyrate-supplemented diet. Similarly, administration of butyrate (0.8% in the diet) for 10 weeks decreased the cumulative mortality of gilthead sea bream after challenge with *Photobacterium damselae* subsp. *piscicida* (*Phdp*) (Piazzon *et al.* 2017). However, the authors noted that butyrate supplementation has no benefits in preventing the bacterial infection, but rather reduced inflammation in the challenged fish and restored the gut integrity and function through an increase in the diversity of gut microbiota (i.e. butyrate-producing bacteria) and reversing alterations in the gut proteome (Piazzon *et al.* 2017). In the case of sea bass, it has been shown that butyrate (0.2%) stimulated the expression of pro-inflammatory interleukin (i.e. IL-1β, IL-6, IL-8), anti-inflammatory interleukin (IL-10) and TNFα genes, but with only the expression of TNFα significantly upregulated in the distal intestine (Rimoldi *et al.* 2016), suggesting butyrate had a promoting effect by increasing cellular turnover. In addition, SB was found to be effective in stimulating the intestinal immune system of grass carp by increasing the contents of complements (C3 and C4), immunoglobulin M and modulates the expression of immune-related genes, including inflammatory cytokines (Tian *et al.* 2017). Also, the fish fed the SB diet have shown a lower enteritis morbidity than the controls after challenge with *Aeromonas hydrophila* ($P < 0.05$) (Tian *et al.* 2017). Furthermore, SB increased the numbers of lymphocytes and monocytes in the bloodstream of Nile tilapia (*O. niloticus*) fingerlings, whereas the activities of the serum enzymes alanine aminotransferase, aspartate aminotransferase and the phagocytes of fish fed on SB-added diets were not significantly affected (Ali *et al.* 2018). Addition to butyrate, sodium propionate (with 5, 10 and 20 g kg^{-1} feed) was observed to stimulate the expression of inflammatory response genes (such as TNF-α, IL-1β and lysozyme), antioxidant enzyme genes (SOD and CAT) and HSP70 gene in zebrafish (*Danio rerio*) (Safari *et al.* 2016). The upregulation of inflammatory response genes was dose dependent, with the highest expression observed in fish fed 20 g kg^{-1} feed of sodium propionate.

Silva *et al.* (2016b) observed that whiteleg shrimp fed diets supplemented with either butyrate or propionate had a significantly higher serum agglutination titre compared with those in the controls, while the total haemocytes count, serum phenoloxidase activity and serum antimicrobial titre against Gram-negative bacteria (*Vibrio alginolyticus*) had no significant difference between treatment groups. In another study, 60 days of propionic acid-supplemented diet administration in whiteleg shrimp resulted in an increase in the expression of prophenoloxidase, lysozyme, penaeidin-3a and crustin genes in the hepatopancreas (Pourmozaffar *et al.* 2017). Additionally, administration of SB alone or in

combination with *Lactobacillus plantarum* enhanced the resistance of *L. vannamei* to *V. alginolyticus* infection but did not affect performance, immunological parameters and the number of *Vibrio* spp. or total heterotrophic bacteria in the intestine (Ramírez *et al.* 2017).

So far, available data indicate that butyrate, propionate and propionic acid may enhance the immune response of aquatic animals by increasing the levels of immune components and regulating the expression of immune-related genes. However, further mechanistic studies are needed to provide a better understanding of the mode of action of SCFAs on the immune system of aquatic animals.

Impact on the intestinal structure and function

In general, there have been few studies on changes in the structure and function of intestine under the influence of SCFAs in aquatic animals. Recently, MSB has been shown to be a protector of the gut mucosa (increasing in the microvillus density) of common carp fed with oxidized soybean oil diets (Liu *et al.* 2014). Moreover, well-developed microvillus of the intestine expanded tubulovesicular system of the apical cytoplasm and characteristic vacuoles with an irregular shape and heterogeneous content of the supranuclear cytoplasm of European sea bass fed on SB have previously been reported (Rimoldi *et al.* 2016). A diet supplemented with 0.8% butyrate increased the abundance of mucosal folding, infiltration of lymphocytes through the epithelial base, number of granulocytes in the submucosa along with the intestine and accumulation of glycogen in hepatocytes (Estensoro *et al.* 2016).

Butyrate supplementation has been shown to improve the use of vegetable diets in gilthead sea bream (Piazzon *et al.* 2017). Fish fed a diet supplemented with 0.4% butyrate had the capacity in reverting nutritionally regulated proteins (associated with digestion, transport, cell signalling and cellular morphology) to levels much closer to control diet, with high abundance of mucins and accompanying proteins that benefit intestinal function and integrity (Piazzon *et al.* 2017). Nevertheless, the effects of butyrate (2% and 3% in the diet) on liver (increasing the liver steatosis with lipid deposition within hepatocytes) and kidney (disappearing the septum between cells) in Nile tilapia have been clearly demonstrated (Ali *et al.* 2018).

Taken together, these reports suggest that butyrate might be a direct substrate that affects changes in the structure and function of the intestinal tract of aquatic animals. Moreover, butyrate might be involved in enhancing the functions of the liver and kidney. Future investigations are required to explore whether the changes in intestinal morphology and function are beneficial to the host metabolism and health as well as the effects of butyrate (and other SCFAs) on liver metabolism.

Impact on the abundance of commensal microbiota

Previous studies have demonstrated roles played by SCFAs in altering the gut microbiota of several aquaculture species. The number of *Aeromonas* was found to be higher in grass carp fed on PSB (590.3 mg SB kg^{-1} diet) compared with that fed on MSB (160.8 mg SB kg^{-1} diet) or control ($P < 0.05$) (Tian *et al.* 2017). In gilthead sea bream, fish fed on diets supplemented with butyrate (0.4%) harboured the highest diversity of intestinal microbiota compared with that fed on control diet (Piazzon *et al.* 2017). Butyrate reduced the frequency of Proteobacteria, but increased Firmicutes, Fusobacteria and Bacteroidetes in the gut of gilthead sea bream, with significant increase specifically among *Vibrio*, *Bacillus*, *Fusobacterium* and *Tannerella*, while *Photobacterium* was decreased in the intestinal mucus of butyrate-fed gilthead sea bream compared to those without supplementation (Piazzon *et al.* 2017). In addition, the intestine of shrimp fed a butyrate-supplemented diet revealed a lower number of *Vibrio* compared with the other treatments (Silva *et al.* 2016b). In a study involving gilthead sea bream, Rimoldi *et al.* (2018) reported that 0.5% SILOhealth 108Z (containing a specific combination of short- and medium-chain 1-monoglycerides) increased the number of beneficial lactic acid bacteria (i.e. *Lactobacillus*) and decreased Gammaproteobacteria (including several potential pathogenic bacteria). All these findings indicate that SCFAs can change the balance of gut microbiota by reducing the abundance of potential pathogens that invade or/and harbour the intestine of their hosts. In contrast, SB has no significant influence on the microbial communities harbouring the common carp intestine (Liu *et al.* 2014). The slight differences in the microbial communities are interpreted to be associated with alternations in the intestinal mucosal morphology and immune responses. The extent to which the mechanisms that microbiota are modulated by SCFAs in aquatic animals are not yet clear, but microbial metabolites are known to be indirectly associated with the control of intestinal microbial composition through the host's immune responses (Macia *et al.* 2015; Rooks & Garrett 2016; Levy *et al.* 2017). Activation of host's NOD-like receptor family pyrin domain containing 6 (NLRP6) inflammasomes by microbial metabolites promotes the secretion of IL-18, mucus and antimicrobial peptides, which function in the maintenance of a stable microbial community in the intestine (Levy *et al.* 2017). Moreover, SCFAs trigger B cells, crucial players in the maintenance of intestinal homoeostasis, to produce secretory immunoglobulin A (sIgA), which targets to specific bacteria and moderately alter the microbial composition in the host (Rooks & Garrett 2016; Levy *et al.* 2017).

In short, SCFAs have been demonstrated to be involved in altering the intestinal microbiota and *vice versa*.

Currently available data are controversial among species, and the relationship between SCFAs and the intestinal microbiota of the host needs further future investigation.

Conclusion and perspective

In summary, most of the studies have provided basic information on the production, absorption and roles of SCFAs in the gut of the host. In the case of aquatic animals, the potential roles of SCFAs to increase fish and shellfish immune response have been described previously (Hoseinifar *et al.* 2017b). The available data confirm the importance of SCFAs (as feed additives) in maintaining intestinal homoeostasis, as well as, acting as energy sources, anti-inflammatory agents and growth promoters. However, there is still a lot of research that needs to be carried out through further investigations. To improve our understanding of how SCFAs are beneficial to aquatic animals, experimental designs on the life stages, trophic levels, living environments, aquaculture systems and health status would have to be taken into considerations. Furthermore, there is currently limited information on the SCFAs receptors and the routes by which SCFAs positively or negatively influence metabolic functioning in aquatic animals. SCFAs elicit their beneficial effects by controlling the levels of fatty acid oxidation and fat storage in multiple tissues, as well as, regulating glucose homoeostasis in human and mice (den Besten *et al.* 2013; Canfora *et al.* 2015; Morrison & Preston 2016; Zhou *et al.* 2016). However, such information is lacking aquatic animals and yet to be explored. While available data on the effectiveness of SCFAs on growth performance, feed utilization and immunomodulation have been reported in mammals, the regulatory mechanisms have not yet been studied in aquatic animals. These aspects, therefore, require future detail studies.

Given that microbial metabolites such as butyrate could be beneficial and serve as mediators for host metabolism (Sonnenburg & Bäckhed 2016), these metabolites could therefore be directly supplemented or used as additives in aquaculture feed or the bacteria that produce these metabolites could probably be developed and used as probiotics. Supplementation of prebiotics (Kihara 2008; Geraylou *et al.* 2012) increased the concentration of SCFAs in the intestinal contents, which indirectly enhanced the immune status of the host. Further studies on the selection of prebiotics that have the potential to stimulate the growth and activity of beneficial SCFA-producing bacteria are worthy of being explored.

Probiotics have shown beneficial effects on aquatic animals, as they act as immune modulators and activators of host defence pathways by enhancing the immune system and disease resistance as well as improving nutrient availability (Hoseinifar *et al.* 2016a). Butyrate producers are not only indicators of a diverse, healthy microbiota but also seem to be actively involved in maintaining a stable and healthy gut community (Louis *et al.* 2014). Studies relating to the isolation, identification, development and application of butyrate-producing microbes as probiotics have not being explored much in aquatic animals and therefore merit further investigations. Similarly, the interactions among SCFA producers, SCFA production and host, which reflect the colonization capacity and potential roles of probiotics in the host, should be studied in detail. In addition, the interplay between the SCFA-producing microbiota and the immune system of the host, how the immune system controls the SCFA producers and how these microbiota shape host immunity, should be considered in further studies.

Gnotobiotic approaches have been reported to have potential roles in providing a better understanding of the functions of microbiota in numerous biological processes of their host (Rawls *et al.* 2004; Fiebiger *et al.* 2016). Rawls *et al.* (2004) revealed the molecular foundations of host–microbiota interaction in the intestine of gnotobiotic zebrafish. How specific SCFA producers or/and SCFAs affect the intestinal epithelium using gnotobiotic approaches have received fewer studies in aquatic animals. Additionally, improved characterization of metabolites combined with advanced technologies and computational tools (Honda & Littman 2016) may provide insights into how microbiota-derived metabolites influence metabolisms in the hosts. Also, metagenomics, transcriptomics, proteomics and metabolomics data (Sonnenburg & Bäckhed 2016) could provide information on the metabolic and functional capabilities of the microbiota responsible for the production of metabolites in the intestine of the hosts. Such information would facilitate and lay the foundation for using microbiota and their metabolites as eco-friendly materials in the metabolic and immune modulation of farmed aquatic animals.

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