

REVIEW ARTICLE

Short-chain fatty acids as feed supplements for sustainable aquaculture: an updated view

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Abstract

Restriction or ban on antibiotic administration in aquaculture encourages the development of environment-friendly feed additives as immunostimulants. Short-chain fatty acids (SCFAs) and their salts are 'Generally Regarded as Safe' and are often used as antimicrobials in the livestock feed industry. Formate, acetate, propionate, butyrate and their salts are among the most studied SCFAs in aquaculture. These SCFAs affect the host performance as well as physiological response upon three ways: either through effects of the feeds that are being administered, through effects on the gastrointestinal tract of the animal or through direct effects on metabolism. To date, most of the available data are focused on the effects of SCFAs on growth performance and feed utilization. Despite extensive research studies on the effects of the different type of SCFAs and their salts on growth performance and feed utilization, the effects of these feed additives on the health of aquatic organisms have only been receiving attention recently. The results of the studies demonstrated beneficial effects of SCFAs as promising feed additives in aquaculture. The present review article summarizes and discusses the topic of dietary administration of SCFAs and their salts in aquaculture with a closer look at the recent findings regarding the effects of SCFAs on growth performance and health status of fish and shellfish. Furthermore,

this review identifies the gaps of existing knowledge regarding the roles of SCFAs in the growth and health status of aquatic animals and suggests research areas that merit further investigations.

Keywords: short-chain fatty acids, sustainable aquaculture, growth performance, immune response

Introduction

Reducing the impact of stress and disease are among the most important factors which determine the success of aquaculture practice (Subramanian, Jang, Kim, Kang & Heo 2013; Faggio, Fazio, Marafioti, Arfuso & Piccione 2015). To meet these factors, precautionary administration of chemicals such as antibiotics has been routinely practiced in aquaculture (Wongsasak, Chaijamrus, Kumkhong & Boonanuntanasarn 2015). However, considering several drawbacks raised by overuse of antibiotics such as emergence of resistant pathogenic strains, administration of antibiotics in aquaculture is strictly restricted or banned. This encouraged researchers to investigate non-antibiotic growth promoters alternative that improve the health and performance of aquatic organisms (Carbone & Faggio 2016; da Silva, Vieira, Mourinho, Bolivar & Seiffert 2016). Short-chain fatty acids (SCFAs) and their salts are 'Generally Regarded as Safe' compounds and often used as antimicrobials

in the livestock feed industry (Defoirdt, Boon, Sorgeloos, Verstraete & Bossier 2009) and present a promising alternative as feed additive in farmed animals including fish. The organic acids are low molecular weight aldehyde-containing compounds with one or more carboxyl groups (Jones 1998). These SCFAs modulate the physiological status of the host in three ways: either through the effects of the feeds that are being administered, through effects in the gastrointestinal tract of the animal or through direct effects on metabolism (Freitag 2007). Generally, most of the SCFAs are capable of reducing the pH of the feed, thus inhibiting microbial growth. The modes of action of SCFAs in the intestinal tract include reduction of the pH level in the stomach and in the small intestines and also inhibition of the growth of Gram-negative bacteria through the dissociation of the acids and production of anions in bacterial cells.

Bioavailability of dietary minerals is greatly influenced by acidification through the use of SCFAs in several ways. Firstly, SCFAs modify the mineral transport mechanism by altering the acidity in the gut. Secondly, the ability of elements to chelate and form complexes (Cross, Debiec & Peterlik 1989; Ravindran & Kornegay 1993) is affected by the inclusion of these SCFAs in diet. Chelation of these acids with calcium (Ca) ions reduces precipitation and co-precipitation between Ca and phosphates or trace elements at the intestinal brush border. Thus, it increases the absorption of phosphorus (P) and other trace elements (Sugiura, Dong & Hardy 1998) in the intestines. Thirdly, these SCFAs enhance the proliferation of epithelial cells in the gastrointestinal mucosa, thus increasing the absorption area for minerals (Baruah, Sahu, Pal, Jain, Debnath & Mukherjee 2007). In addition, these SCFAs have high gross energy values (Freitag 2007) and are utilized in various metabolic processes for energy generation including the production of ATP in the citric acid cycle (Diebold & Eidelsburger 2006) and also used as substrates in intermediary metabolism (Shah, Afzal, Khan, Hussain & Zeeshan 2015). Furthermore, it has been hypothesized that production of SCFAs following microbial fermentation of prebiotics (*Ingredient of the not digestible diet that is beneficial to the host for stimulating selectively the growth and/or the activity of one or more intestinal bacteria*) can be considered as the probable mode of action of prebiotics on fish immune response, performance and disease

resistance (Song, Beck, Kim, Park, Kim, Kim & Ringø 2014; Hoseinifar, Esteban, Cuesta & Sun 2015; Dawood & Koshio 2016; Guerreiro, Couto, Machado, Castro, Pousão-Ferreira, Oliva-Teles & Enes 2016; Hoseinifar, Ringø, Shenavar Masouleh & Esteban 2016a).

Studies in fish have shown the positive effects of using these SCFAs and their salts (reviewed by (Lückstädt 2006, 2008; Ng & Koh 2016). Dietary SCFAs have demonstrated effectiveness in growth performance and bioavailability of minerals in fish. For example, dietary inclusion of citric acid/formic acid enhances the bioavailability of minerals, including P, magnesium, Ca and iron in salmonids (Vielma & Lall 1997). Due to the importance of these SCFAs in fish nutrition, research efforts have been directed to carefully examine the roles and effects of SCFAs and their salts on animal production over the past several years (Liu, Yang, Zhang, Gatlin, Ringø & Zhou 2014). To the best of our knowledge, the earliest studies on the use of these SCFAs in fish nutrition have been conducted by Rungruangsak and Utne (1981) on the effects of formic or sulphuric acid on growth performance and pepsin activity in digestive tract of rainbow trout. Thereafter, Fauconneau (1988) evaluated the possibility of substitution of protein with SCFA (citric acid). Also, Ringø (1991, 1992) and Ringø, Olsen and Castell (1994) studied the effects of dietary lactate, acetate and propionate supplementation on growth, composition of the digesta and chemical composition of the fish. Since then, several studies have addressed the effects of SCFAs and their salts on feed digestibility, growth response and gut microbiota in various aquaculture species (Liu *et al.* 2014; Ng & Koh 2016). These SCFAs are generally absorbed through the intestinal epithelia by passive diffusion, providing energy for renewing the intestinal epithelia and maintaining the gut health (Vielma & Lall 1997). Considering the importance of SCFAs as beneficial feed additive with growth-promoting and immunostimulating effects, the present review was performed to provide a comprehensive view on topic of dietary administration of SCFAs and their salts in aquaculture with a closer look at the recent findings regarding the effects of SCFAs on growth performance and health status of fish and shellfish. Furthermore, this study highlights the gaps of existing knowledge and suggests research areas that merit further investigations.

SCFAs and their salts effects on growth performance and physiology

Formate and its salt

Formate is the anion of formic acid which received attention [specially its salt, potassium diformate (KDF)] as beneficial dietary supplement in aquaculture (Fig. 1). It has been extensively reported that dietary KDF could improve growth performance and feed efficiency in aquatic animals (Lückstädt 2006; Lim, Klesius & Luckstadat 2010; Morken, Kraugerud, Barrows, Sørensen, Storebakken & Øverland 2011; Morken, Kraugerud, Sørensen, Storebakken, Hillestad, Christiansen & Øverland 2012a; Morken, Moyano, Márquez, Sørensen, Mydland & Øverland 2012b; Elala & Ragaa 2015). Atlantic salmon fed a fishmeal enriched with 8 and 14 g kg⁻¹ KDF showed a significantly better feed efficiency and improved uniformity within fish groups (Christiansen & Luckstadt 2008). Lim *et al.* (2010) observed that graded levels of dietary KDF up to 10 g kg⁻¹ tended to improve the weight gain and feed efficiency in tilapia (*Oreochromis niloticus*). In rainbow trout (*Oncorhynchus mykiss*), dietary supplementation with 10 g kg⁻¹ sodium diformate enhanced the digestibility of some nutrients, such as proteins, lipids and amino acids (Morken *et al.* 2011). Liebert, Mohamed and Lückstädt (2010) showed that supplementation of diet with 3 g kg⁻¹ sodium diformate significantly improved the protein efficiency ratio (PER) and protein retention in tilapia (*O. niloticus*) fingerlings. Also, an *in vitro* study also demonstrated that sodium diformate improved the bioavailability of amino acid using crude enzyme extracts from Atlantic salmon (*Salmo salar*) (Morken *et al.* 2012b).

Formate and other SCFAs have been shown to reduce the gut pH of fish (Lückstädt 2008b; Ng, Koh, Sudesh & Siti-Zahrah 2009). For example, Ng *et al.* (2009) reported that dietary KDF at 2 g kg⁻¹ decreased the diet pH and reduced the gut digesta pH of red hybrid tilapia, *Oreochromis* sp. This low digesta pH may benefit the digestibility of certain nutrients, such as minerals and protein. Vielma

and Lall (1997) found that the apparent digestibility of P increased significantly in rainbow trout fed a diet containing 10 mL kg⁻¹ formic acid. In addition, the lower gastric pH was associated with a higher pepsin activity and contributed to the enhanced protein digestibility and nitrogen retention. This can be considered as the primary reason for improving the growth performance and feed efficiency in fish (Yúfera, Moyano, Astola, Pousão-Ferreira & Martínez-Rodríguez 2012).

Secondly, formate and other SCFAs can improve the general health status of cultured aquatic animals by its stronger antimicrobial effect towards pathogens (Ramli, Heindl & Sunanto 2005; Lückstädt 2008b; Defoirdt *et al.* 2009; Elala & Ragaa 2015). It was reported that the total faecal bacteria reduced significantly in tilapia fed with an organic acid blend and KDF diets (Ng *et al.* 2009), and dietary KDF stimulates the colonization of certain bacteria and inhibits the growth of others in the gut of hybrid tilapia (*O. niloticus* × *O. aureus*) (Zhou, Liu, He, Shi, Gao, Yao & Ringø 2009). In addition, dietary administration of 3 and 6 g kg⁻¹ formic acid could decrease intestinal *Vibrio* spp. and total bacterial counts and enhance the survival rate of *Vibrio parahaemolyticus*-infected shrimp in laboratory conditions (Chuchird, Rorkwiree & Rairat 2015). da Silva, do Nascimento Vieira, Mouriño, Ferreira and Seiffert (2013) demonstrated that propionate, butyrate and acetate salts exhibited strong inhibitory capacity against *Vibrio* species in marine shrimp, *Litopenaeus vannamei* (da Silva *et al.* 2013). This microbiota modulation and gut health improvement may be another reason for improving the growth performance and feed efficiency in fish.

However, red hybrid tilapia fed with diets supplemented with KDF or organic acids/salts blend showed no significant difference in the apparent digestibility of dry matter, protein, lipids and P (Ng *et al.* 2009; Storebakken, Berge, Øverland, Shearer, Hillestad & Krogdahl 2010; Castillo, Rosales, Pohlenz & Gatlin 2014; Elala & Ragaa 2015). Zhou *et al.* (2009) observed no significant improvement in the growth performance of tilapia fed with KDF containing diet. Various factors, such

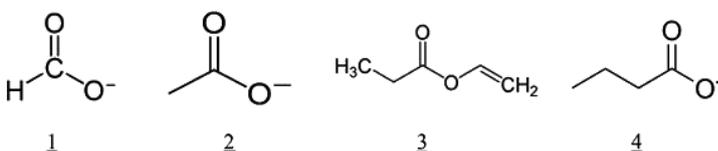


Figure 1 Chemical structure of different type of short-chain fatty acids (1: Formate; 2: Acetate; 3: Propionate; 4: Butyrate).

as species and the physiological age of the experimental fish, the type and level of SCFAs, the diet composition and the culture conditions, may all influence the manifestation of the potential growth-promoting effects of dietary SCFAs in aquaculture.

Acetate and its salt

Acetate is the anion of acetic acids which received limited attention compared to salt of other SCFAs (Fig. 1). Ringø (1992) reported that 1 g kg^{-1} sodium acetate as additive promoted the growth of Arctic charr, *Salvelinus alpinus* (L.), reared in brackish water, while 10 g kg^{-1} sodium formate gave only a non-significant improvement compared with negative control. The stimulated growth of fish fed sodium acetate to some extent may be explained by the higher feed intake, but enhanced digestibility of dietary components might also contribute to the enhanced growth. da Silva *et al.* (2013) indicated that acetate salt exhibits high inhibitory capacity against *Vibrio* species in marine shrimp (*L. vannamei*), and this may benefit the health of aquatic animal and therefore improve the growth performance.

Propionate and its salt

Sodium and potassium propionate are the salts of propionic acid which received increasing attention during last years as beneficial dietary supplement (Fig. 1). Sodium propionate has been demonstrated to decrease the *Vibrio* species concentration in the intestinal tract of marine shrimp (*L. vannamei*) and increase the coefficient of apparent digestibility of energy and P (da Silva *et al.* 2013). Subsequently, the same group showed that dietary supplementation with sodium propionate in different dietary concentrations (5 , 10 and 20 g kg^{-1}) modified the intestinal microbiota and enhanced the growth of *L. vannamei* (da Silva *et al.* 2016). Hassaan, Wafa, Soltan, Goda and Mogheth (2014) tested Ca propionate as a feed additive in Nile tilapia fingerling, and the results showed that 10 g kg^{-1} Ca propionate improved the growth rate, and 5 , 10 and 15 g kg^{-1} Ca propionate improved the feed efficiency, PER and protein productive values. On the contrast, Ringø (1991) reported that dietary inclusion of 10 g kg^{-1} sodium propionate had a growth-depressing effect in Arctic charr in brackish water compared to the

control. However, on the contrary with their finding, in a 8-week study on Caspian Sea white fish, Hoseinifar, Zoheiri and Caipang (2016b) observed notable improved growth performance, feed utilization as well as non-specific immune response in white fish fed different levels of dietary sodium propionate (2.5 , 5 , 10 and 20 g kg^{-1}) compared to fish fed the control diet. Furthermore, inclusion of sodium propionate in zebra fish diets increased expression of immune and antioxidant growth-related genes (Safari, Hoseinifar & Kavandi 2016).

Butyrate and its salt

Butyrate (also known as butanoate) is the traditional name of butyric acid (or butanoic acid) anion (Fig. 1). It has been extensively reported that sodium butyrate could improve the growth performance and feed efficiency of aquatic animals (Owen, Waines, Bradley & Davies 2006; Nhan, Wille, De Schryver, Defoirdt, Bossier & Sorgeloos 2010; Robles, Lozano, Sevilla, Márquez, Nuez-Ortín & Moyano 2013). Owen *et al.* (2006) observed slightly improved growth and a concomitant reduction in feed conversion ratio (FCR) of catfish (*Clarias gariepinus*) fed the fishmeal diet supplemented with 2 g kg^{-1} sodium butyrate, while fish receiving defatted soya together with 2 g kg^{-1} sodium butyrate showed no improvement. Sodium butyrate supplementation also appeared to increase the proportion of Gram-positive bacteria in the hindgut of *C. gariepinus*, although this increase was not statistically significant. Similarly, dietary supplementation with butyrate in different concentration modulated the intestinal microbiota and enhanced the growth of *L. vannamei* (da Silva *et al.* 2013, 2016). De Schryver, Sinha, Kunwar, Baruah, Verstraete, Boon, De Boeck and Bossier (2010) reported that diets supplemented with 20 – 100 g kg^{-1} poly- β -hydroxy butyrate modulated the intestinal microbiota of European seabass (*Dicentrarchus labrax*). Liu *et al.* (2014) revealed that dietary supplementation of microencapsulated sodium butyrate (MSB) could stimulate immune responses and improve the intestinal condition of the common carp (*Cyprinus carpio*). Particularly, the function of improving the intestinal condition was more outstanding when the fish were pre-fed with oxidized oil. Recently, Robles *et al.* (2013) represent an advance in the knowledge of the metabolic consequences of using butyrate as feed additive in fish. They observed a significant increase

in the weight of sea bream (*Sparus aurata*) receiving butyrate, while metabolomics provided some clues on the suggested effects of this feed additive. It seems that butyrate increased the availability of several essential amino acids and nucleotide derivatives. Also, the energy provision for enteric cells might have been enhanced by a decrease in glucose and amino acid oxidation related to the use of butyrate as fuel. Additionally, butyrate might have increased transmethylation activity.

Nhan *et al.* (2010) reported that feeding the giant freshwater prawn (*Macrobrachium rosenbergii*) larvae with poly β -hydroxybutyrate (PHB)-containing *Artemia nauplii* significantly increased survival and development of the larvae. Sui, Liu, Sun, Wille, Bossier and Schryver (2014) reported that bio-encapsulation of PHB into rotifers and *Artemia* was an efficient approach to deliver PHB to the Chinese mitten crab (*Eriocheir sinensis*) larvae and resulted in a significantly enhanced survival, development rate and osmotic tolerance. The addition of commercial PHB particles (average diameter 30 μm) or PHB-containing bacteria protects *Artemia franciscana* from pathogenic *Vibrio campbellii* (Defoirdt, Halet, Vervaeren, Boon, Van de Wiele, Sorgeloos, Bossier & Verstraete 2007; Halet *et al.* 2007), and this may be one of the important reasons for improving the growth performance and survival of larvae.

Organic acid blend

Sherif and Doaa (2013) reported that feeding Nile tilapia (*O. niloticus*) fingerlings on 2, 3 and 4 g kg^{-1} SCFA blend (formic acid 47.1%, phosphoric acid 23.0%, citric acid 5.8%, acetic acid 10.1% and copper sulphate 1.1%) resulted in remarkably higher SGR and feed efficiency compared to the negative control group (Sherif & Doaa 2013). On the contrast, Bjerkgeng, Storebakken and Wathne (1999) did not observe significant effects of a mixture of SCFAs [sodium salts of acetic, propionic and butyric acids (5:5:2, by weight), 5 and 20 g kg^{-1}] on growth, organ indices including the HSI and WIL, feed utilization and apparent digestibility coefficients of Atlantic salmon (*S. salar* L.). Similarly, Gao, Storebakken, Shearer, Penn and Øverland (2011) reported that dietary supplement of 10 $\text{g acid moiety kg}^{-1}$ of sodium formate and butyrate blend (ratio 2:1 on the acid moiety weight basis) failed to improve the growth rate or feed utilization of rainbow trout

and did not significantly alter the mucosal folds of the distal intestine.

In a study with Western white leg shrimp, Romano, Koh & Ng (2015) observed that supplementation of diet with 20 g kg^{-1} SCFA blend (including formic, lactic, malic and citric acids) improved growth performance, and dry matter and P digestibility in shrimp (*L. vannamei*). Similar results have been reported using the same levels of above-mentioned SCFA blend in tiger shrimp (*Penaeus monodon*) diet (Ng, Koh, Teoh & Romano 2015). In addition, both shrimps fed SCFA blend containing diets showed higher survival after challenged with pathogenic *V. harveyi*.

Goosen, Görgens, De Wet and Chenia (2011) investigated SCFAs and SCFAs salts as growth promoters in cultured South African abalone (*Haliotis midae*) when incorporated as feed additives in formulated feed. The results showed that SCFA blend (1% acetic acid+1% formic acid) significantly improved mass growth rate of *H. midae*. The mode of growth enhancement could not be established; it could not be shown that the acid and salt treatments had any effects on intestinal microflora, but possible energy effects of the acids were speculated as potential mechanism.

Furthermore, there is a report available about the possible effects of Biotronic[®], a blend of SCFAs and their salts on fish (Moradi 2015). Common carp fry fed different levels of Biotronic[®] (10, 20 and 30 g kg^{-1}) and the results revealed remarkable improvement of growth performance and feed utilization. Also, evaluation of intestinal microbiota showed significant elevation of lactic acid bacteria level (LAB), while total bacterial count remained unaffected.

Moreover, Zhu and colleagues (Zhu, Qiu, Ding, Duan & Wang 2014) in a 8-week study investigated the effects of SCFA blend (fumaric acid and benzoic acid, and 2-hydroxy-4-(methylthio)butanoate acid) on growth performance, feed utilization and the blood reactive oxygen species level, serum superoxide dismutase (SOD) activity and catalase (CAT) activity of juvenile yellow catfish (*Pelteobagrus fulvidraco*). The results showed that SCFA blend had no remarkable effects on growth performance, body composition and diet utilization. Also, juvenile yellow catfish fed SCFA blend showed decreased CAT and SOD enzyme activity. Zhu *et al.* (2014) suggested that this shows SCFAs could protect the fish body from cell oxidative damage.

Immunomodulatory effects of acidifiers and their salts on fish and shrimp

Beside the extensive researches on the effects of different types of SCFAs and their salt on growth performance and feed utilization, the effects of these feed additives on the immune response and disease resistance of aquatic organisms have been receiving attention recently. Indeed, the hypothesis suggested regarding the mechanism of action prebiotics on fish health (i.e. production of SCFA following fermentation of prebiotic by beneficial bacteria in intestinal microbiota) motivated the researchers to investigate the effects of various SCFAs and their salts on fish and shellfish health status, immune response as well as disease resistance.

An understanding of the immune system of the farmed aquatic species is crucial in aquaculture nutrition most especially in the use of feed additives in the diet (Ajiboye, Yakubu & Adams 2012). It is for this reason that in the course of developing the suitable feed additives for aquatic species, researchers have to gather sufficient data on how these feed additives affect the health status of the fish, because both health condition and growth of the aquatic species go hand in hand in ensuring maximum yields during harvest. The subsequent discussion focuses on the various studies that had been conducted on how these SCFAs modulate the immune responses of both fish and shellfish and the corresponding manifestations of these aquatic organisms when they are exposed to pathogenic agents.

Haematological parameters including red blood cell (RBC) count, haemoglobin (Hb) and white blood cell (WBC) count are considered valuable indices to assess fish health (Hoseinifar, Mirvaghefi, Merrifield, Amiri, Yelghi & Bastami 2011). In beluga, *Huso huso*, supplementation of 30 g kg⁻¹ citric acid in the diets had no significant effect on RBC and WBC counts, indicating that diet acidification does not likely cause any stress or dietary imbalance, which would result in the increase in WBC counts. Similarly, mean cell volume, mean cell Hb, serum glucose and total proteins were not affected by citric acid inclusion which may indicate that acidification does not cause any metabolic stress. Furthermore, this species of fish also showed increased Hb against diet acidification (Khajepour & Hosseini 2012). Increased haematocrit (Ht) values by 10 and

20 g kg⁻¹ citric acid in red sea bream (Sarker, Satoh & Kiron 2007) and by 30 g kg⁻¹ citric acid in common carp have also been observed (Khajepour, Hosseini & Imanpour 2012). The addition of citric acid might cause maximum liberation of iron, copper, Ca and P ions from the phytic acid complex, which resulted in increased Hb and Ht values (Khajepour, Hosseini & Hoseini 2011). Other types of organic salts, Ca lactate and Ca propionate, were included in the diets of tilapia, *O. niloticus* (Hassaan *et al.* 2014). Results showed an increase in Hb content, Ht values and total protein of the fed fish. However, the authors observed a decrease in aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in fish fed 10 g kg⁻¹ Ca lactate. The lower levels of the liver enzymes of the fish fed 10 g kg⁻¹ Ca lactate could be an indication of a healthy liver for the fish. Furthermore, in a most recent article, the effects of different levels of dietary sodium propionate on serum and mucosal non-specific immune parameters of Caspian Sea white fish were assessed (Hoseinifar *et al.* 2016b). The results revealed feeding fish with 10 g kg⁻¹ galactooligosaccharide (GOS) remarkably increased skin mucus total immunoglobulin (Ig) level and lysozyme activity. Also, inclusion of sodium propionate in diet significantly elevated serum total Ig level and lysozyme and alternative haemolytic complement activity (ACH50). The authors concluded that sodium propionate can be considered as a beneficial immunostimulants for Caspian Sea white fish.

On the other hand, incorporation of KDF at 3 g kg⁻¹ of the diet of tilapia, *O. niloticus*, stimulated the growth of beneficial intestinal flora as well as the activation of cellular and humoral innate immunity including phagocytic activity/index, nitroblue tetrazolium reduction test and serum/gut mucous lysozyme activity (Elala & Ragaa 2015). The cumulative mortality of the fish groups fed on KDF and challenged orally with *Aeromonas hydrophila* was lower than that of the control group. The resistance against diseases increased with dietary KDF in a dose-dependent manner (Ramli *et al.* 2005).

In a study by Liu *et al.* (2014), using MSB in combination with oxidized soya oil in the diets of common carp, *C. carpio* showed the interaction between the pre-fed diets and the sustained-release times of dietary MSB was significant for the relative expression levels of gut heat-shock protein-70 (HSP70), pro-inflammatory cytokines (IL-1 β and

TNF- α) and anti-inflammatory cytokines (transforming growth factor- β) within the different segments of the gut. They also observed increased microvilli density in the MSB-supplemented groups indicating the role of sodium butyrate in protecting the gut from damage and helping in cellular and tissue repair when incorporating oxidized soya oil in the diets of the fish. Moreover, in a most recent with the same species, Safari and Hoseinifar (unpublished) observed remarkable increase in the expression of immune-related genes (TNF-alpha, IL1B, LYZ) of sodium propionate fed fish. Also, feeding common carp with sodium propionate improved skin mucus as well as serum immune parameters.

Determination of possible effects on SCFA on fish health through molecular approaches has been investigated in another recent study (Safari *et al.* 2016). Zebra fish (*Danio rerio*) (0.42 ± 0.06 g) was fed experimental diet containing varying levels of sodium propionate (0, 5, 10 and 20 g kg^{-1}) for 8 weeks and possible effects of these experimental diets on mucosal immune response (TNF α , IL β , Lyz), antioxidant defence (SOD, CAT) as well as heat-shock protein 70 (HSP70)-related gene expression were studied. The results showed remarkable elevation of mucosal immune-related genes (include TNF α , IL β , Lyz) in a dose-dependent manner; the highest up-regulation was observed in fish fed 20 g kg^{-1} sodium propionate. As reported in case of mammals, SCFAs can be used by the immune cells of the gut-associated lymphoid tissue (GALT) (Bach Knudsen, Serena, Canibe & Juntunen 2003). Furthermore, recent findings revealed that SCFAs alter the immune response through binding to G protein-coupled receptor, GPR43, which is expressed mainly on innate immune response and inflammatory cells (Maslowski & Mackay 2012). Although the presence of such a SCFA receptor has not been confirmed in fish, it seems that elevation of fish mucosal immune response can be attributed to the above-mentioned mechanisms. Furthermore, Safari *et al.* (2016) observed down-regulation of the HSP70 and antioxidant enzyme (SOD, CAT) gene expression in liver of zebra fish following dietary administration of sodium propionate. The authors speculated that down-regulation of HSP70 in sodium propionate fed zebra fish occurred due to elevated tolerance towards usual stress during culture, such as stresses caused by sampling for monitoring water quality, fish biometry or other unwanted stresses. Also, they have suggested that

down-regulation of antioxidant enzyme gene expression is due to the protection of the fish body from cell oxidative damage which was provided by dietary supplementation of sodium propionate. However, it should be mentioned that up-regulation of genes during dietary experiments needs to be interpreted with caution and not all up-regulation is a positive indication.

In terms of protection from bacterial challenge, Ramli *et al.* (2005) demonstrated that incorporating various levels of KDF in the diets of tilapia significantly improved survival rates of fish after the challenge with *Vibrio anguillarum* in a dose-dependent manner. Their results were in addition to improved feed intake, live weight gain, FCR and PER in the fish fed diets containing this SCFA. This led the authors to conclude that the use of KDF at 2 g kg^{-1} is an efficient method to control infections with *V. anguillarum* in tropical tilapia culture.

In shrimp culture, very few studies have been conducted to assess the effects of SCFAs and their salts on shrimp. Most research data on shrimp were obtained from studies performed on Pacific white shrimp, *Litopenaeus vannamei*. Incorporation of a commercial SCFA, acidified Ca sulphate at either 1% or 2% in the diets of Pacific white shrimp, showed better responses to stress and enhanced immune responses including hemocyte phagocytic capacity, hemolymph protein concentration, hyaline cell counts and hemolymph glucose than the control group (Anuta, Buentello, Patnaik, Lawrence, Mustafa, Hume, Gatlin & Kemp 2011). In addition, the inclusion of 20 g kg^{-1} sodium propionate in the diets of this species of shrimp decreased the *Vibrio* species concentration in the intestinal, aside from increasing the apparent digestibility of energy and P (da Silva *et al.* 2013). Later, da Silva *et al.* (2016) demonstrated that the inclusion of varying levels (5, 10 and 20 g kg^{-1}) of sodium butyrate or sodium propionate in the diets of Pacific white shrimp resulted in lower counts of *Vibrio* sp. in the intestine. The shrimp in the experimental groups had higher serum agglutination titers. Likewise, supplementation of various levels of dietary citric acid in the diets of Pacific white shrimp showed increased levels of serum phenoloxidase (PO), superoxide dismutase and lysozyme in the experimental group fed with $2\text{--}3 \text{ g kg}^{-1}$ citric acid (Su, Li, Leng, Tan, Liu, Chai & Guo 2014). Higher survival rates in the two fed groups were also observed following infection with *V. alginolyticus*.

For other species of shrimp, a study by Ng *et al.* (2015) in tiger shrimp, *P. monodon*, showed that inclusion of 20 g kg⁻¹ microencapsulated SCFA blend in the diets resulted in significantly lower total viable bacteria and presumptive *Vibrio* spp. counts in the pond and water of the fed shrimp than the control after 22 weeks of feeding. The authors also observed significantly higher survival to *V. harveyi* challenge in the fed group, and this was likely attributed to enhanced PO activity and less hepatopancreatic damage. Likewise, the use of these microencapsulated SCFA blends as feed additives for *L. vannamei* showed similar effects (Romano *et al.* 2015). The authors observed lower hepatopancreatic *V. harveyi*, higher PO activity and lesser hepatopancreatic damage in shrimp fed with either 10 or 20 g kg⁻¹ microencapsulated SCFA blend. Survival was also higher in the fed group upon challenge with pathogenic *V. harveyi*. Thus, the authors concluded that inclusion of the SCFA blend in the diet of shrimp could substantially improve productivity and resistance to pathogenic bacteria and is a viable alternative to the use of antibiotics in the culture of shrimp.

In summary, supplementation of various SCFAs and their salts in the diets of both fish

and shrimp resulted in the modulation of the immune responses of the host. Both fish (Fig. 2) and shrimp (Fig. 3) responded favourably to these feed additives resulting in the up-regulation of the beneficial immune components and at the same time a corresponding decrease in immune parameters that are detrimental to the host (*e.g.* liver AST and ALT). All these immune responses work synergistically to help the fish or the shrimp combat pathogenic agents. The increased resistance against bacterial pathogens (*e.g.* *V. anguillarum* and *V. harveyi*) as well as the lower counts of pathogenic *Vibrio* spp. in the intestines and hepatopancreas of shrimp following dietary administration of SCFAs is an indication of modulation of various components of the immune system. In spite of the benefits that these SCFAs provide to the host, few studies have been conducted at the molecular level in both fish and shrimp. It is expected that with the emergence of molecular technologies to study gene expression in fish and shrimp, these molecular tools should be used to assess the effects of these SCFAs on the immune system of cultured organisms in addition to humoral and cellular assays.

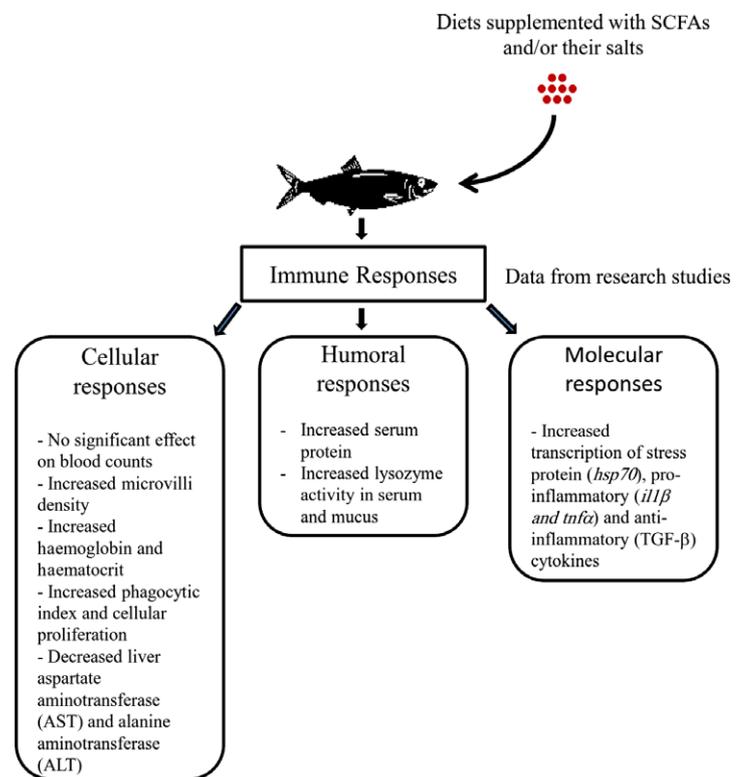


Figure 2 Modulation of the immune response in fish following dietary supplementation with various SCFAs and their salts. [Colour figure can be viewed at wileyonlinelibrary.com].

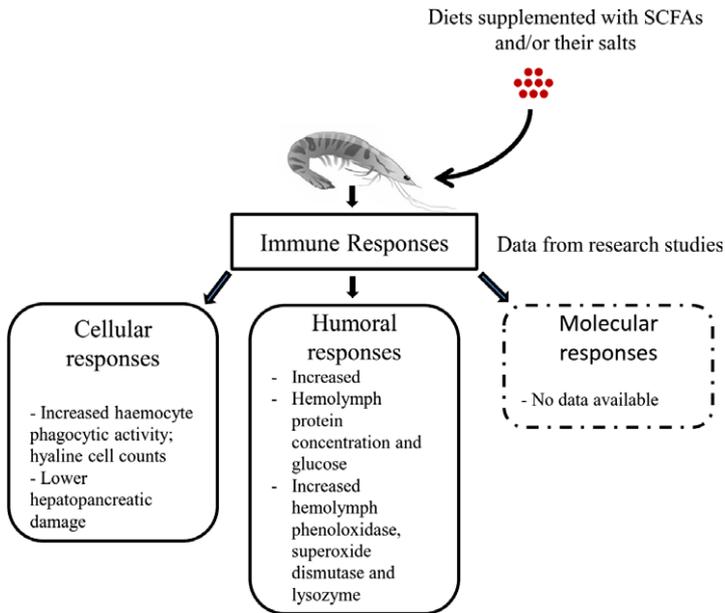


Figure 3 Modulation of the immune response in shrimp following dietary supplementation with various SCFAs and their salts. [Colour figure can be viewed at wileyonlinelibrary.com].

Research gaps and future perspectives

Considering the increasing evidence regarding the beneficial effects of different types of SCFAs and their salts on growth performance and health status of several fish and shellfish species, it can be stated that SCFAs and their salts have great potential as environment-friendly feed additive to increase fish and shellfish immune response. However, as reviewed in the present article, most of available data are regarding the possible effects of SCFAs and their salts on growth performance and feed utilization, and there is a gap in existing knowledge regarding the impact of different SCFAs and their salts on fish and shellfish antioxidant defence and innate and adaptive immune parameters especially at molecular level. Furthermore, although there is some suggested mode of action for SCFA effects on fish immune response, still there is need to determine such mechanisms in fish and shellfish. Furthermore, the study of the interaction between dietary SCFAs and gut microbiota as well as the measurement of SCFAs in fish gut is also an interesting topic for future researches. The last but not least, the application and efficiency of SCFAs and their salts in early life stage of fish and shellfish are poorly understood and merit extensive researches. As a whole, the above-mentioned facts revealed that there will be vast areas of research in this field.

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