Effects of dietary acidification and acid source on fish growth and feed efficiency (Review)

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Abstract. The human population has increased considerably worldwide, including in the Philippines. Aquaculture is one of the main food sectors that provides a cheap source of protein in the Philippines. Changes in diet composition in aquaculture have brought about concerns regarding certain negative effects at the gastrointestinal levels. The replacement of fish meal with a plant protein source in a considerable proportion in the diet of the majority of cultured fish species has led to proliferative and inflammatory responses in the intestines of various (functionally) monogastric animals. In aquaculture feed, the dietary supplementation of organic acids and their salts as growth promoters has been established. The use of acidifiers in aquafeed requires a different approach due to diversified feeding habits and the wide variation in the digestive system structure and physiological function. Dietary organic acids can increase pancreatic enzyme production, decrease stomach pH levels, inhibit pathogens, provide energy, improve mineral utilization and improve nutrient digestibility, all of which improve fish development performance. Acidifiers are currently widely used in animal feed, including aquafeed, and several manufacturers have created next-generation acidifiers with additional benefits. The present review article discusses the acidifiers, their mechanisms of action, growth, feed efficiency, immunity and future research opportunities. The fish growth rate and feed utilization efficiency are also reviewed as regards dietary acid sources, such as acetic acid, citric acid, hydrochloric acid and control-no acid. In addition, the attractability of the diets for the fish at different pH levels and dietary acid sources was determined. The survival rates of cultured fishes were determined based on the various dietary acids used. Any acids at an optimum pH level, e.g., pH 4.6 in the diet of tilapia fry, which increase attractability, growth and feed efficiency, warrant further attention.

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1. Introduction

Changes in diet composition in aquaculture have brought about concerns regarding some negative effects at the gastrointestinal levels. The majority of cultured fish species encounter issues when a fish meal is replaced with a plant protein-based source in a significant proportion of their diet. The proliferative and inflammatory responses in the intestines of various (functionally) monogastric animals, which include the Atlantic salmon *Salmo salar* (1), common carp *Cyprinus carpio* (2), and rainbow trout *Oncorhynchus mykiss* (3) are among the several concerns which have arisen.

The importance of dietary acid supplementation to fish and feed production supports expanding fish production for sustainable feed production. Products commonly contain plant by-products, oilseeds, legumes, pulses, lupins and cereal to replace fishmeal, including an exogenous enzyme applied to enhance the utilization of plant nutrients in aquaculture diets (4).

The concentration of hydrochloric acid in the stomach decreases with meal consumption, increasing pH levels. However, this increase in pH levels exerts a detrimental effect on the activation of pepsin and pancreatic enzyme secretions, decreasing the digestive capacity and affecting growth performance. As a result, acidifiers, such as organic acids and their salts provide a viable option. As a result, these have attracted increasing attention as a potential antibiotic substitute for improving fish development and health. Furthermore, organic acids are involved in several energy-generating metabolic pathways (5).

The present review article focuses on the effects of acidifiers on growth performance and feed efficiency in different mechanisms, such as acid source in the gastrointestinal tract (GI tract), its effect on the metabolism and the physiological strategy of acidification, and specifically on the effects of dietary pH levels.

2. Physiology of fish

Digestive morphology. Fish rely on a broad array of food sources in nature. Carnivorous fish that eat meat and other more digestible feeds vary in their physical and behavioral functions from herbivorous fish that eat fibrous meals, such as phytoplankton and macrophytes. Carnivorous fish have a short and simple stomach with a thick mucosa for absorption. Herbivorous fish have an additional masticatory apparatus or other physiological adaptation to aid in the breakdown of plant cell walls before digestion begins and a long, thin stomach to extend gut retention time and improve digestion and absorption.

Plant elements in diets can expose fish to a cascade of anti-nutritional agents, culminating in pathological disorders later in life (6). Soybean meal has exhibited substantial negative alterations in the intestinal architecture of Nile tilapia, such as the expansion of the submucosa (SM) and lamina propia (LP), and an increase in the number of goblet cells, as compared to other studied ingredients. Soybean meal has been reported to include anti-nutritional chemicals that lead to digestive issues in Atlantic salmon (*Salmo salar*) and summer flounder (*Paralichthys dentatus*) (7). The observed changes in the intestinal morphology of the Nile tilapia were less severe than those in salmonids and were mostly located in the proximal region. In addition, the mucosa of the proximal part of the intestinal tract is longer in the tilapia. It also possesses more branched villi than the middle and distal regions in the tilapia (8), suggesting more prone to intestinal disorders.

The digestive tract of *Oreochromis niloticus* (*O. niloticus*; Nile tilapia) is characterized by a sequence of loops arranged in a consistent and detailed pattern that is both unique among species and one of the most complex patterns ever observed in fish. The intestine leaves the stomach and enters the spiral portion of the intestine, following the elongated margins of the liver. The spiral intestine consists of two primary coils (proximal and distal) with a centripetal and centrifugal loop in each. Between two large coils, a short gastric loop is inserted. Finally, the terminal segment of the intestine leaves the spiral region and follows a straight path to the anus (9). The possession of an intestine with a length far surpassing that of the body cavity, as well as the ability to arrange the elongated gut into loops or coils of some type, as *O. niloticus*, in general, are found in adult herbivorous fish. With a total intestinal length of 0.8 to 15 times the body length, the Nile tilapia is on the shorter end of the range of herbivorous fish (8). This trait may be due to the adaptable fish diet, which can easily be changed from its standard diet.

The digestive mechanisms of the Nile tilapia differ from those of carnivores and herbivores. It can eat a wide variety of foods. A series of loops and coils comprise the macroscopic morphology of the digestive tract. The hepatic loop, proximal major coil, gastric loop, distal major coil, distal major coil and terminal segment are all found in the caudal stomach (9). The proximal gut has received increasing attention in morphological and functional responses to fasting and famine; no variations have been found between Tilapia intestinal sections and those of higher vertebrates (10,11). The Nile tilapia has a well-developed GI tract linked to its feeding behaviors and food preferences. The distal intestines of the Nile tilapia are known to have active microbial fermentation and short-chain fatty acid absorption (12,13). When the tilapia is fed a low-protein diet, intestinal bacteria release a greater amount of necessary amino acids (14).

The gastrointestinal system (GI tract) is the site of food digestion and nutritional absorption in fish and the first line of defense against hazardous chemicals (1,15). Different feed components have been shown to alter intestinal morphology (2,16). Fish are exposed to several foreign components, such as carbohydrates and anti-nutritional factors when plant-based foods substitute animal protein sources in their diet. These foreign components can interfere with the normal processes occurring in the gut (17). Some researchers have replaced fishmeal with plant protein-based diets for the tilapia (18-20). The intestinal morphology of the Nile tilapia is negatively affected by soybean meal when paired with an environmental influence, according to Tran-Ngoc *et al* (21). However, there is still a scarcity of data available on the mechanisms through which other plant-based substances alter the intestinal shape.

Histology of the GI tract. The GI tracts of cultivated omnivore fish, such as the Nile tilapia are well-developed due to their feeding patterns and food types. The esophagus, Y-shaped stomach and lengthy intestine comprise the GI tract of the Nile tilapia. The GI tract wall has a variety of cell types that are related to the anatomy and physiology of each fish. The mucosa, SM, muscularis propria and serosa are the four layers of the gut wall that comprise the basic histological anatomy of the GI tract in vertebrates (22). These were examined using optical and electron microscopes based on histological structures (23).

GI tract epithelial cells, goblet cells and certain gland cells comprise the mucous cells in the fish GI tract (24,25). Mucous cells in the stomach epithelium appear as compacted columnar mucous cells (26). The mucosal layer has piqued the interest of several researchers as it is a mucous membrane containing mucous cells that are crucial for lubrication, absorption and the transportation of macromolecules, increasing digestive capacity, and preventing acidity and bacteria at the epithelial level (27,28). Mucous-secreting cells, termed goblet cells, are compacted in the esophageal epithelium and are scattered in the intestinal epithelium. The mucous in the GI tract comprises various mucosubstances or mucins, such as neutral mucin and acid mucin; however, the mucins in the GI tracts of fish vary depending on the species, age and location (29). Neutral mucin is abundant in all three regions of the intestines of the Nile tilapia, crucial for enzymatic digestion and absorption. The Nile tilapia has little acid mucin in the early section of the gut and no sulfated acid mucin. As a result, acid mucin does not appear well in the intestine of the tilapia, which excretes soft meal remnants. According to histochemical research, the esophagus mucus of the Nile tilapia contains neutral and acid mucin. The epithelium mucus of the stomach of the tilapia on the other hand, has a large quantity of neutral mucin and low content of acid mucin (26). Acid mucin has been detected in the produced mucus from the gastric glands, found in the stomach epithelium of herbivorous fish, such as the tilapia. Using acidic lysis, plant cell walls have been successfully disrupted and triturated (30). This histochemical analysis of the Nile tilapia is critical to the understanding of the GI tract for the formulation of sustainable feed. Concerning this literature, the addition of organic acids or decreasing the dietary pH of the diet have significantly improved the digestibility and intestinal morphology of the tilapia. Hence, histological analysis is crucial for determining which cells are increased. In the study by Huan *et al* (31), the cross-section of the intestinal mucosa morphology of the tilapia was measured, such as villus height and width, to determine the growth and digestibility based on the intestinal structure. Some of the following terms are important for determining the different parts of histological cells: i) Enterocytes are simple columnar epithelial cells that line the inner surface of small and large intestines; ii) eosinophilic granulocytes are inflammatory cells that occasionally migrate into the LP; goblet cells are unicellular intraepithelial mucin-secreting glands scattered within the simple epithelium, such as cuboidal, columnar and pseudostratified cells; goblet cells are mucus-secreting, and they are compacted in the esophageal epithelium and dispersed in the intestinal epithelium (26); iii) the LP is the thin and delicate core of connective tissue in simple folds; iv) the lumen is the inner space of a tubular structure, such as an artery or intestine; v) microvilli are found on the top of villi; vi) the SM is a thin layer of connective tissue between the base of folds and the stratum compactum; vii) villi (height/width) are small, finger-like structures in the small intestine; viii) intestine.

The intestinal functional physiology of fish is influenced by various factors and differs by species. The function of the fish intestine is critical for ensuring cost-effective production and low waste output (32). The presence of potentially toxic components in food, such as anti-nutritional substances (33) and oxidized components (34), as well as production techniques, such as feeding regimes (35) and diet composition (36), can alter digestive functions. As a result, it is critical to continually assess the effects of acid on digestive physiology to guarantee that the raw material is safe and effective.

Tilapia fed supplemented organic acids, such as potassium diformate and calcium butyrate diets exhibit more pronounced improvements in intestinal morphology under hypoxic conditions than normal conditions. In the distal intestine, the tilapia have a thinner SM and LP, and fewer goblet cells, which is considered an improvement in the intestinal epithelium (37). The capacity of organic acid to strengthen intestinal morphology is strongly dependent on the conditions of upbringing (38). Butyric acid and butyrate also affect cellular functions that are crucial for intestinal health, such as reducing mucosal inflammation and oxidative stress and increasing the barrier function of the intestinal epithelia (39). Protease and organic acid salts in combination improve nutrient digestion and intestinal architecture (31). Different proximo-distal gradients of distinct digestive enzymes may differ within the same fish species. Lipid membrane hydrolysis, for example, occurs primarily in the proximal regions of the intestine. The hydrolysis of carbohydrates and protein components, on the other hand, appears in the medial and distal regions of the intestine (40).

While gastric glands are found in the front section of the fish stomach (41), the stomach of the Nile tilapia contains three regions (cardiac, fundic and pyloric); gastric glands are found in the cardiac and fundic regions. The fish stomach can be split into two main parts histologically: The anterior cardiac and fundic region with gastric glands and the posterior pyloric region without gastric glands (42). In addition, tubular and acinar mucous glands have been discovered in the posterior region of the stomach of the tilapia (26).

Digestive enzyme regulation. Based on the weight gain results from a previous study, it appears that acidifiers, particularly citric acid, at a dose of 1.5% of the meal, increase the activity of digestive enzymes in the red drum. When organic acids were added to the diet, pepsin activity, pancreatic enzyme activities (trypsin, lipase, and amylase) and intestinal enzymes increased (43).

i) Stomach enzymes. Hydrochloric acid concentrations in the stomach are decreased during periods of high-feed consumption when the animals are young or the meals are high in protein, for example. This reduction negatively affects pepsin activation and pancreatic enzyme secretion and impairs digestion. Therefore, acidifiers have been added to the feed to address this issue and aid feed digestion. In addition, organic acids have been found to aid in the hydrolysis of proteins (44).

The mechanism of dietary acid to feed is to lower the pH level of the feed for the increase in pepsin levels. Pepsin is an important acidic aspartic protease widely applied in protein hydrolysis. It is found in fish viscera, primarily in the gastric juice of the stomach lumen (45-48), constituting 5% of fish weight (49). The peptide linkages are easily broken, allowing proteins to degrade in acidic environments (50). It is termed pepsinogen as it is generated and released in an inactive condition in the stomach membrane (SM). This SM is stable in neutral and weak alkaline settings and contains 44 amino acids. When exposed to the hydrochloric acid (HCl) present in gastric juice (pH 1.5-2.0), the 44 amino acids are proteolytically eliminated in an autocatalytic method, resulting in the activation of pepsin (51), the pepsin activity reported in *Sparus aurata* has only been found in the stomach (52).

The main product of peptic cells is pepsinogen, and it is found in this form in the gastric mucosa (53), blood, urine and other body fluids (54,55). Pepsinogen can only be measured after its irreversible conversion to the active enzyme, pepsin, by an autocatalytic process at pH <6.04[6], following its secretion into the stomach. Pepsinogen rapidly converted to pepsin at pH 2.0, but extremely slowly at pH 5.0 to 6.0. Furthermore, pepsin functions optimally in an acidic environment with a pH of ≤2.0, whereas it functions at a slow rate at pH 5.0 to 6.0. Furthermore, pepsin functions optimally in an acidic environment (pH 2.0 to 3.5) and rapidly degrades above this pH (56). As a result, the optimal pH (the pH value that provides the highest enzymatic activity) and pH stability (the pH range that provides adequate enzyme stability) substantially affect fish pepsin activity. Pepsin activity diminishes when the pH decreases below optimal levels (57). In the study by Castillo *et al* (43), the pepsin activity was shown to be greater in the homogenized stomachs of juvenile red drum from 1.5% citric acid, 0.75 potassium diformate and 1.5% potassium diformate treatment at 2 h after feeding. However, only treatment with 0.75% potassium diformate treatment led to statistically significant results. Since the optimum pH significantly affects the activity of fish pepsin (57), it would be of interest to determine whether the pH of the stomach contents is connected to protein digestibility.

Exocytosis releases pepsinogen as a proenzyme (zymogen) from main cells. The acidic stomach juice reversibly activates some pepsinogen molecules. These activated pepsinogen molecules permanently activate themselves and the remaining inactive pepsinogen molecules to create Pepsin by intramolecular and intermolecular cleavage. Catalysis exposes the catalytic domain by removing an autoinhibitory region (activation peptide) (Fig. 1).

ii) Intestinal enzymes. Enzyme activities in the digestive tract of gilthead sea bream were investigated by Deguara *et al* (52). The results of their study revealed that pepsin activity was found exclusively in the stomach. By contrast, other enzymes such as trypsin, chymotrypsin, carboxypeptidase A, carboxypeptidase B and amylase were found in all gut regions, including the stomach. Pepsin activity has been found to be absent in all other sections of the digestive system save the stomach in white sturgeon and striped snakehead (58). The activity of other enzymes, such as trypsin and amylase, was much lower in the stomach (52). Trypsin, chymotrypsin, carboxypeptidases A and B, and amylase have all been found in the stomach of fish (59-61). Chakrabarti *et al* (58) hypothesized that fish intestines are still at an evolutionary stage when most areas can generate all of the major enzymes before the emergence of site-specific enzyme synthesis found in higher invertebrates.

Inorganic acid inclusion, trypsin activity, lipase and amylase levels have been shown to be higher in juvenile red drum fed acidified diets, such as citric acid and potassium diformate. Increasing secretin levels leads to a lower pH, possibly stimulating pancreatic secretions (43). In addition, digestive enzymes, such as leucine-aminopeptidases and phosphatases increase the activity of the intestine. As acid and alkaline phosphatases are involved in the hydrolysis of phosphorus, the stimulation of digestive enzymes by organic acids may be one of the reasons for enhanced mineral digestion by fish. However, organic acids may indirectly affect the activity of the intestine digesting enzymes.

Haemato-immunological responses. Malic acid (5 g/kg) added to the Nile tilapia diet has been shown to increase the hematological values (62). On the other hand, a positive result on

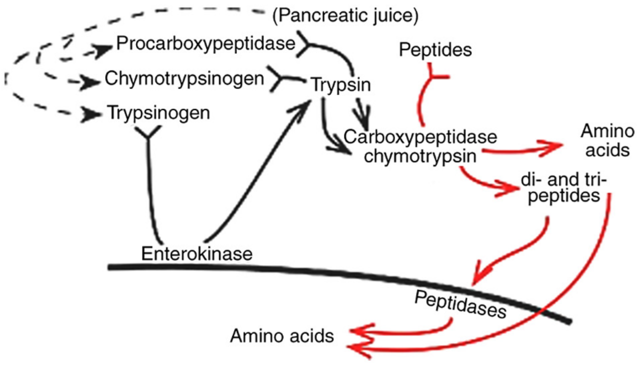

Figure 1. Pathways of activation of pepsinogen to pepsin. Two-step activation is shown as an example for multiple-step activation.

organic acid basal meal enriched with commercial formic and propionic acids at a 1 or 2 g/kg feed dosage was fed to Nile tilapia fingerlings. This improved growth, hemogram parameters, white blood cell counts, lymphocyte and neutrophil counts, and the body chemical composition, and also reduced microbial contamination in intestine in a dose-dependent manner in the Nile tilapia fingerlings (63). The amount of uric acid in the blood has also been shown to be decreased when an organic acid is added to the diet (64), which results in the improved utilization of amino acid digestibility and proteins due to the protein metabolism in the urea as the major end product.

Gut microbiota and blood parameter. With the supplementation of acid to fish, the microbial balance and proper pH in the digestive tract eliminate pathogenic microorganisms and maintain fish health satisfactorily. Therefore, the use of organic acids is a preventive alternative in maintaining the health of cultured fish. Its actions in the GI tract inhibit the growth of pathogenic bacteria, mainly Gram-negative bacteria, aid in the digestion and absorption of nutrients, as well as exert beneficial effects on animal performance (65). However, some organic acids, particularly citric, metacetonic and acetic acids, are added to pellets for storage effects or the improvement of feed utilization, or both (22). Other studies have not found a growth-promoting effect of organic acids (66,67). This is dependent on the fish species, type of organic acid and dosage used (5). As a result, it is critical to recognize that the potential benefits of organic acids may vary depending on the species and dose used.

Acidifiers improve feed performance; they decrease the uptake of pathogenic organisms and toxic metabolites. In the intestinal tract, the acidifiers reduce the pH level in the stomach, particularly in the small intestine, through the delivery of H^+ ions. On the other hand, they inhibit the growth of gram-negative bacteria by dissociating the acids and producing anions inside bacterial cells (68).

At a pH <5, the growth of several Gram-negative bacteria is reduced. A low pH also creates a natural barrier against germs rising from the ileum and large intestine. Low molecular weight acids are lipophilic and penetrate past the cell membrane of Gram-negative bacteria. Organic acid supplementation has been shown in several trials to reduce pathogenic bacterial counts in the digestive tract, while increasing the amount of acid-tolerant, 'good' bacteria, such as Lactobacilli (5).

Another effect of the supplementation of organic acids is antimicrobial activity. Owen *et al* (69) demonstrated that the proportion of Gram-positive bacteria in *Clarias gariepinus* treated with sodium butyrate tended to increase. Using organic acids/salts in aquafeeds for commercial purposes can improve growth and disease management and exert antimicrobial effects as it releases protons into the cytoplasm and penetrates the cell wall of Gram-negative bacteria (68). The bacteria require a large amount of adenosine triphosphate to maintain a balanced intracellular pH, resulting in cellular energy depletion with eventual cell death (65). Lactic acid bacteria is one of the most common probiotic bacteria used in aquafeed (70). Lowering the gut pH with dietary potassium diformate exerts a eubiotic effect on the allochthonous, beneficial lactic acid bacteria. Lactic acid bacteria can grow at a relatively low pH, exhibiting further resistance to organic acids and salts than Gram-negative bacteria. These indigenous probiotic bacteria can colonize the intestinal surface and form a barrier, serving as the first defense to limit the direct attachment or interaction of pathogenic fish bacteria to the gut mucosa (70). Organic acids reduce the pH level of the gastrointestinal content in the red hybrid tilapia. They could lead to an enhanced reproduction of beneficial bacteria, the inhibition of pathogenic microorganisms and subsequently, in an improved micro-ecosystem (71,72).

The improvement of protein digestibility and nitrogen retention is contributed to the lower gastric pH associated with a higher pepsin activity (73). For example, in red hybrid tilapia, dietary potassium diformate at 2 g/kg was shown to reduce the diet and digesta pH of the fish stomach and gut; it also markedly decreased the total bacterial counts in the feces (67).

As low molecular weight lipophilic organic acids can infiltrate through the cell membrane of Gram-negative bacteria, the acidification of their metabolisms can cause bacterial cell death. In the hybrid tilapia, dietary potassium diformate promotes the colonization of certain gut bacteria, while inhibiting the growth of others (74). Another explanation for improved growth performance could be the spread of indigenous probionts. These Gram-positive bacteria aid in fermenting some non-digestible carbohydrates, hence increasing nutritional availability (70). The acid anions of the dissociated organic acids are counteracted by the high intracellular potassium concentrations in Gram-positive bacteria (75). The organic acid can acidify the cytoplasm of Gram-negative bacteria, resulting in eventual cell death. Potassium diformate can modify microbial communities in tilapia guts, accounting for its ability to initiate an immune response. The continuous vitalization provided by the endemic intestinal flora affects the quality and quantity of immune cells in the gut mucosa (76). Elala and Ragaa (77) examined the eubiotic effect of a dietary acidifier (potassium diformate) on the health status of cultured *O. niloticus*. Tilapia were fed graded levels of potassium diformate (control diet, 0%; 0.1, 0.2 and 0.3%) for 60 days at an initial body weight of 6.15 g. The addition of potassium diformate to the diet improved growth and apparent protein digestibility. It also exerted a eubiotic effect on the proliferation of indigenous bacteria, which is crucial for stimulating the immunological response to diseases.

Nutrients and mineral absorption. Acidifiers improve feed performance by enhancing nutrient absorption, the proliferation of cells in the mucosal epithelium of the intestine, preventing aquatic pollution and reducing phosphorus discharge in water (78). For example, a 0.922 g/kg organic acid blend (calcium propionate, calcium formate and sodium acetate) supplemented protease in meat and bone meal diet has been shown to improve nutrient digestibility and nutrient retention in the Nile tilapia (31). In addition, supplementing diets with organic acid and their salts has been found to exert beneficial effects on mineral absorption (79) and nutrient digestibility (80).

Organic acid supplementation decreases duodenal pH levels, improves nitrogen retention and improves nutritional digestibility (81). In aquaculture, dietary acidification using organic acids or their salts in aquatic animals has been used (82). Furthermore, organic acids or their salts inclusions in the diet of aquatic animals have been observed to increase the nutritional value of the diets of aquatic animals and their growth (82). Several studies have been performed on various aquatic species, including carnivorous species, such as rainbow trout, Atlantic salmon and Arctic charr, herbivorous filter feeders, such as carp and tilapia, and omnivorous species, such as catfish and shrimp (5,83-89). In addition, organic acids improve phosphorus absorption (90). Thus, organic acids, salts, or mixtures are promising feed additives for aquatic animals to enhance some fish species' growth performance and feed utilization. Furthermore, they inhibit bacteria and contribute to nutrition, which is involved in several metabolic pathways for generating energy and improving major nutrient digestibility (82).

The addition of 0.2% formic and propionic acid/salt to the diet of the Nile tilapia has been found to increase the amount of retained protein and fat. In addition, supplementation with acidifiers improves nutrient digestibility (91). A high dose of formic and propionic acid/salt is required to improve the immune status in tilapia feed. Fish extracts and other aquatic organisms are rich in organic acids (92). Organic acid compounds improve weight gain and the food conversion ratio by improving dry matter, crude protein digestibility and mineral absorption (67,73,93).

RNA upregulation. In the study by Busti *et al* (94), the effects of dietary organic acids on the immune response of European sea bass resulted in the upregulation of target genes, such as IL-8, IL-10 and TGF-β. Thus, the organic acids exhibit prebiotic properties in the gut microbiota, promoting beneficial bacteria taxa, such as *Lactobacillus*, *Leuconostoc* and *Bacillus*. Furthermore, these acids appear to induce a potential functional reconfiguration of the gut microbiota, enabling a significant decrease in several inflammation-promoting and homeostatic functions. Again, for the first time in the seabass study, the exposure to suboptimal rearing conditions was shown to modify the gut microbiota structure, reducing lactic acid bacteria and increasing proteobacteria. These findings were consistent with the inflammatory process observed at the mRNA level (94).

The feeding frequency in the two feeding trials in the juvenile red drum (*Sciaenops occelatus*) revealed a balance in the timeframe of feeding, such as 08:00, 12:00, 16:00, to digest the eaten feeds from the stomach and intestine in an appropriate, timely manner. It was reiterated in the illumination pattern, and feeding time is involved at a different level in regulating the secretion of digestive juices. There is a significant change in stomach pH levels after feeding (95). The addition of acid to lower the dietary pH in the red hybrid Tilapia and rainbow Trout (*Oncorhynchus mykiss*) studies may increase the pepsin activation and mineral absorption (67,96). It may explain why growth and feed efficiency have improved. The concentration of hydrochloric acid in the stomach is reduced during periods of high-feed intake, and the pH level increases. The increase in pH levels negatively affects pepsin activation, potentially attenuating protein digestion in the stomach (5,57). This may be the reason why the pH 8.00 diet-initiated mortality indicates that growth is declining in Nile tilapia fry. The dietary pH adjustment is a major solution that best fits the required dosage level to the Nile tilapia dietary acidification. The stomach converts pepsinogen to pepsin rapidly at pH 2.0 to 4.0; however, this conversion is attenuated at pH 5.0 to 6.0 and declines rapidly above this pH (56); this coincides with the red hybrid Tilapia and rainbow Trout (*Oncorhynchus mykiss*) (67,96). In the case of pigs, the optimum pH value has the highest enzymatic activity, significantly affecting pepsin activity. Pepsin activity decreases when the pH levels fall below the ideal level (97). Thus, perhaps the pH 4.6 diet significantly affects pepsin activity.

3. Dietary acidification

Acidifiers present a promising alternative for improving the performance and health of livestock. The effects of dietary acidification and acid sources on the growth performance of fish and feed efficiency are presented in Table I. Acidifiers can also improve feed performance; they can improve growth, feed utilization and disease resistance in fish (5), improve the shelf life of pellets (98), and improvement in pig growth performance as a result of increased food consumption due to increased diet palatability, a more efficient conversion of the food to live weight due to a reduction in bacterial competition for nutrients, or increased enzymic activity due to a reduction in gastric pH due to the acidification of the diet (99). These organic acids, such as acetic, butyric, citric and malic acids, and their salts are found in plant and animal tissue. They can be used as acidifiers in the livestock feed industry, including aquaculture. When added in sufficient amounts, acidifiers and their salts can stimulate growth and feed efficiency and enhance feed quality. The acids in the aquaculture diet should be standardized to respond to gastric and agastric species (78).

The use of acids to preserve fish and fish viscera in the preparation of fish silage is a popular procedure with widespread use in fish feed and recorded benefits (100,101). According to Batista (102), fish silage production was initiated in the 1930s, initially with sulphuric and the hydrochloric acid preservation of fish waste. The advantages of acid-preserved products have drawn the attention of the scientific community, leading to the investigation of the effects of these short-chain acids on fish feed.

Inorganic acid. In all monogastric species, stomach acid is involved, such as fishes are hydrochloric acid, a very strong inorganic acid produced by gastric glands. This acid reduces the pH of the stomach to a level of 2 to 3, depending on the species and diet. Hydrochloric acid generation is minimal at birth/hatching, but increases as animals mature. The pH of the stomach decreases as more acid is formed. Therefore, pepsin, a proteolytic enzyme required for protein digestion, must be activated at a low pH. Pepsin activity is optimal at a pH of 2. Its effectiveness is greatly limited at higher levels (103).

Organic acid. Organic acids are commonly used as an additive as they function as chelating agents to bind cations along the gut resulting in improved mineral absorption (104). In aquaculture species, organic acids are common acidifiers. Organic acids have been found to exert positive mineral absorption effects (90) and nutrient digestibility (80) by reducing the pH levels in the digestive tract, specifically in the stomach and small intestine, through H^+ ion deposition (5).

Romano *et al* (105) (Table I) demonstrated that when the citric acid level was increased by 2%, there were leukocyte infiltrations and more excessive necrosis and hemorrhaging that affected the growth of tilapia. Citric acid and its salts, and formic acid and its salts are the most extensively studied organic acids in aquaculture, according to the review article by Ng and Koh (106). These short-chain organic acids are ingested primarily by passive diffusion through the intestinal epithelia, supplying energy for intestinal epithelia renewal and gut health (93). In addition, citric and lactic effectively enhance feeding behavior when applied individually or with other extractive compounds (107).

There is an improvement in growth and feed efficiency in the induced optimization of intestinal pH by malic acid in the Nile tilapia (108). However, no clear association has been found between the pH of the diets and pepsin activity, implying that other factors may be involved in the synthesis of the enzyme. A recent study determined the optimum levels of acid supplementation at 6.25 g/kg (sodium butyrate), providing growth performance immunity against *Aeromonas hydrophila* (109). Furthermore, these lactic acid bacteria have increased digestive enzyme activities in fish (110).

Dietary pH levels. The resulting pH of the diet due to a combination of ingredients in the formulation also affects gastrointestinal pH levels with related consequences on protein digestion and gut microbiota in monogastric vertebrates (111). A high proportion of fish meal with a high acid-binding capacity in the diet ensures the nutrition of fish. Compared to most alternative vegetable aquafeed components, including sunflower, soybean, rapeseed and gluten, fish meal has one of the greatest buffering capabilities (112). Decreasing the fish meal content in modern aquaculture diets may affect the ideal gastrointestinal pH for digestive enzyme action and gut bacterial community (111). These observations also suggest that fish nutritionists could manipulate the final dietary pH to elicit positive growth performance, ultimately increasing profitability in the aquaculture industry. A limited number of studies have addressed the effects of dietary pH on the conditions of aquatic animals, e.g., shrimp (83) and channel catfish (84).

The pH value of animal feed is crucial as it can affect digestion following ingestion. For example, the increase in pH levels in the stomach negatively affects the activation of pepsin

Table I. Effects of dietary acidification and acid sources on different fish growth performances and feed efficiency.

Fish species	Acid/acid salt sources	Dose (%) pH	Parameters increased in SGR (%), FCR, and WG (g)	(Refs.)
Nile tilapia (*Oreochromis niloticus*)	Hydrochloric acid	pH 4.60	WG: 2.6; FCR: 1.3; SGR: 4.9	(86)
Common carp (*Labeo rohita*)	Citric acid	3.00%	FCR: 1.09; SGR:1.58	(87)
Red drum (*Sciaenops ocellatus*)		1.50%	WG: 1547; FCR: 0.98	(43)
Tilapia (*Oreochromis sp*.)		2.00%	WG: 193.05; FCR:1.82; SGR:2.10	(105)
			WG: 1060.2 FCR:1.30 SGR: 2.35	(67)
Nile tilapia (*Oreochromis niloticus*)		pH 4.60	WG: 2.6; FCR: 1.5; SGR: 4.7	(86)
Beluga *(Huso huso)*		3.00%	WG: 688.8 FCR: 1.08 SGR:3.75	(90)
Rohu (*Labeo rohita)*		3.00%		(140)
Carp (*Cyprinus carpio)*		3.00%	WG: 112.56 FCR: 1.17 SGR:1.20	(141)
Sea bream *Pagrus major*		1.00%	WG: 69.32 FCR: 1.00 SGR:2.20	(146)
Nile tilapia (*Oreochromis niloticus*)	Acetic acid	pH 4.60	WG: 2.6; FCR: 1.5; SGR: 4.7	(86)
Nile tilapia (*Oreochromis niloticus*)	Malic acid	0.800 or 3.20%	WG:94.10 FCR: 1.48	(139)
Nile tilapia (*Oreochromis niloticus*)		5.00 or 10.0 g/kg	WG:48.5 FCR: 1.4 SGR: 2.40	(108)
	Formic and diformic	1.00 or 2.00 g/kg	WG: 15.33 FCR: 5.20 SGR: 0.70	(63)
Nile tilapia (*Oreochromis niloticus*)	Calcium propionate, calcium formate and sodium acetate	0.922 g/kg	WG: 945.2; FCR: 1.20	(31)
Red drum (*Sparus aurata*)	Sodium butyrate	6.25 g/kg	WG: 9.83%; FCR: 1.18 SGR: 1.67	(135)
Nile tilapia (*Oreochromis niloticus*)	Potassium diformate	0.3%	WG:24.0 FCR: 1.45 SGR: 2.07	(113)
Nile tilapia (*Oreochromis niloticus*)		2 g/kg	WG: 1060.2; FCR: 1.30; SGR: 2.35	(67)
		0.2 or 0.3%	6.75% increased apparent protein digestibility (APR)	(77)

WG, weight gain; SGR (%), specific growth rate; FCR, feed conversion ratio.

and possibly decreases the protein digestion capability in fish (57). Therefore, supplementing diets with organic acid and their salts exerts beneficial effects on the growth performance of fish (113). On the other hand, it decreases the digestive pH levels of the GI tract by accumulating H^+ ions, thus reducing the pH levels in the stomach and stimulating the activation of pepsinogen to pepsin, increasing protein digestibility and reducing gastric emptying rate. In addition, the acids lower the pH of the stomach and foregut, which stimulates pepsin activity and improves protein digestibility and mineral absorption (114). Furthermore, the acids further enhance protein digestion by increasing the rate of proteolysis of large protein molecules (115).

The buffering capacity of feed ingredients plays a main role in reducing pH levels in the feed and stomach. Animal protein (e.g., fishmeal) has a 15-fold higher buffering capacity than cereals, which is why it is widely used in aquaculture diets. Due to the low hydrochloric acid output of young animals, these effects are of particular importance (116); the concentration of hydrochloric acid in the stomach decreases during food consumption, increasing pH levels. The activation of pepsin and pancreatic enzyme secretions are inhibited by this increase in pH levels, reducing the digestive capacity. Munilla-Morán and Saborido-Rey (117) determined that the pH optima for pepsin activity in the stomach of *Sparus aurata* were 2.0. Similar to all enzymes, the digestive enzymes markedly affect pH levels. Distinct enzymes have different pH optimums when activity is at its peak; activity decreases on either side of this optimum rapidly and significantly. A pH adjustment can produce a 50% reduction in inactivity in certain enzymes. As a result, it significantly affects the rate and scope of digestion. Normal digestive processes can explain the changes in pH levels in the stomach of fish. The pH levels decrease as acids are secreted in response to feed entering the stomach, followed by increases in pH levels as acid secretion is terminated and digestion is evacuated. The acidity decreases somewhat in the upper intestine before increasing when bicarbonate ions are released into the gut lumen. The effect of bicarbonate secretion becomes evident when going down the length of the intestine. There is an ever-increasing trend in the average pH from 6.8 in the upper intestine to 7.9 in the lower intestine. The optimum pH in the different gut regions would affect the digestibility of some dietary ingredients, leading to better feeding and the performance of fish (117).

Decreasing the pH value in feed leads to a lower buffer capacity. It thus promotes digestion in the animal since less hydrochloric acid has to be produced in the stomach to activate pepsin, and therefore, optimal protein digestion is ensured (118). Furthermore, the acid anion is complex with calcium (Ca), phosphorus (P), magnesium (Mg), and zinc (Zn) results in the improved digestibility of these minerals (119). On the other hand, different acidifiers do not affect the contents of the intestine 6 h following ingestion. Therefore, organic acids are probably mostly metabolized by that time. Furthermore, pancreatic secretions may act as a buffer against the effects of the acidifier (43).

In another study by Yúfera *et al* (120), the pH levels in the stomach of juvenile gilthead seabream were measured after they were fed via three different strategies: Once, twice, or continuously. Feeding was performed at the time points of 09:00, 09:00 and 17:00, or continuously between 9:00 or 17:00 and 21:00. Under the three feeding regimes tested, the gastric pH levels exhibited significant daily rhythms. The constant supply feeding regimen ensured that the stomach pH levels remained in the optimal pepsin range for a long period of time. It may be one of the reasons for the significantly greater weight of the fish following the feeding regimen. It may also be explained by higher gastric activity in fish with a gastric pH <4.5, as described by Márquez *et al* (57).

Yúfera *et al* (120) also investigated the association between stomach pH levels and pepsin activity in juvenile marine fish. Fish were fed either a single meal, twice, or the same diet continuously at the same time. The stomach pH of fish fed only once was around 4.5, and the highest pepsin activity was reported before the feeding, with 30 pepsin activity units per fish. The fish fed continuously had a stomach pH of 5.25 and a pepsin activity of almost 280 units per fish in the late afternoon. This demonstrates how low pH levels may affect pepsin activation. Ringø *et al* (85) examined the effects of 1% sodium lactate on the growth of Arctic charr fish and discovered a significant increase. On the other hand, these results were not observed in another study in Atlantic salmon using the same dosage (66).

Fabay *et al* (86) (Table I) focused on the contribution of dietary pH influencing the gut pH levels, which presumably affects the capability and efficiency to utilize dietary nutrients that convert to the flesh (i.e., growth and feed efficiency). Therefore, the evaluation of the growth rates and feed efficiency of the Nile tilapia as regards dietary pH in that study was an indirect comparison of the general physiological conditions in which the total digestive enzyme activities operate on their corresponding substrates, stomach and intestine. The inclusion of hydrochloric acid in the diet was more effective in the tilapia fry with a pH range of 4.6.

4. Acid sources of fish

The condition that possibly affects the growth performance and feed utilization may be the gastric or gastrointestinal pH mechanisms. The gastrointestinal luminal digestive disorders are very important in optimizing the utilization of modern aquafeeds. Gut pH seems to be influenced by the pH of the diet (121). The pH of the stomach and intestine creates a medium in which the adequate digestion of dietary proteins and lipids is affected by an optimal environment for the activation and activity of the digestive enzymes. A suitable pH level in the intestine may also be the perfect habitat for some gut flora and fauna to thrive, while others do not survive.

Stomach acid and intestinal acid. The majority of fish have a low acid secretion in their lumen compared with mammals. The inclusion of dietary acidifiers reduces the pH levels in the GI tract, increases phytic acid breakdown and eliminates GI pathogenic microorganisms. It also decreases the emptying time of the GI tract, improves nitrogen retention, increases nutrient digestibility, and improves mineral absorption and transportation (81,122). The stomach acid secretion of vertebrates, such as fish exhibits continuous acid secretion and low gastric pH levels during fasting. The presence acid is maintained neutral gastric pH during fasting hydrochloric acid released only after a meal's ingestion. Fish such as tilapia and catfish can digest feeds due to the presence of a stomach and intestine. It is important to acknowledge that dietary acids may provide beneficial effects. The optimal pH level in the gut has a significant effect on the activity of fish pepsin (57).

The main reason for the addition of acid to the diet is to lower the pH level of feeds, which may benefit the digestive functions of the Nile tilapia towards an increased growth rate and better feed efficiency. The H^+ ion from the acid stimulates the activation of pepsinogen to pepsin in the stomach, thus improving protein digestibility (94). In addition, during feed intake, the hydrochloric acid concentration in the gut is reduced. In the study by Moriarty (123), the stomach pH level of tilapia was found to be ≤1.6, enabling it to digest a high protein content (~49%), lysing in a high concentration of amino acid as the feed remains for longer periods of time in the stomach.

Similarly, in that study, the protein composition of the diet was 47-49%, and the diet with a pH 4.6 led to maximal growth rate and an optimal feed efficiency. The stomach does not secrete acid when it is empty; thus, acid secretion begins upon ingestion between pH 2.0 to 3.0 (123). Therefore, if the acid requirement of the tilapia was at pH 1.6, it required a pH 0.4-1.5 to convert it into pH 2.0-3.0, which meant that approximately pH 1.0 was sufficient to maintain the acid requirement of the tilapia. The pH of the control diet was ~5.7, which requires pH 1.0 to convert it into 4.6; the acid requirement is sufficient for in the stomach to digest the high protein content. In another study on Indian carp fed a diet supplemented with 3.0% citric acid, Baruah *et al* (87) (Table I) reported a decrease in the pH of feed from 5.87 to 4.85, with a subsequent reduction in the pH of gut digesta from 6.62 to 5.65, increasing growth; their results directly agree with dietary pH study (86).

Chyme pH and buffering capacity. The pH value influences the digestion and availability of nutritional matters in the digestive tract of animals in the gut (116). The digestion of feeds creates a dynamic alteration in the resting pH of each GI tract section which influences the dissolution and precipitation of the dietary ions. In addition, the chemical characteristics of the chyme are also altered during digestion, as it passes along the GI tract with protein and carbohydrate degradation occurring in the stomach and intestine that affect the binding of ions to the solid phase (124).

Buffer capacity is defined as the HCl secretion in ml or mmol required to lower the pH levels to pH 3.0 in the stomach following feed intake. Different nutrients in animal feed increase the buffer capacity of the feed, which is crucial for fish. A feed with a high buffer capacity leads to a higher mortality than a feed with a lower buffer capacity. Therefore, it is important to consider the buffer capacity of the feed for the dosage rate. The concentration of hydrochloric acid in the stomach is reduced during periods of high feed intake, and the pH rises. The increase in pH negatively affects pepsin activation, potentially reducing the ability of the stomach to digest proteins (57). The stomach pH of fish given only one feed decreased significantly to 2.6 after 8 h in a study by Deguara *et al* (52); however, when fish receive two feeds, the pH drops to 2.5 immediately after feeding and increases to 5.1 after 12 h.

Plant ingredients, such as extracted sunflower (ESF), pea protein concentrate and soy protein concentrate exert various and differing effects on the digestive physiology and metabolism of the Atlantic salmon (1). This involves a decrease in chyme-associated leucine aminopeptidases in fish fed plant ingredients compared to fishmeal (88).

In addition, cellular sloughing has been observed in at least some intestinal regions, which could be due to lower enterocyte turnover and leucine aminopeptidase activity in rainbow trout (*Oncorhynchus mykiss*) fed at least some plant ingredients. When fed soybean protein, the fish have been found to exhibit an increase in the height and thickness of their intestinal villi (125).

ESF does not affect the brush border membrane-leucine aminopeptidase activities, resulting in a significant increase in the nitrogen concentration in all intestinal compartments. Therefore, this suggests a reduced protein digestibility compared to the fishmeal diets. In salmon and other fish species, ESF has a relatively high digestibility (126). The high levels of nitrogen in chyme in the ESF diet, on the other hand, suggest that the protein digestibility of the ESF is low due to high inclusion levels (20%). The high nitrogen levels indicate that the stomach secretion of pepsin or other proteins is stimulated, as evidenced by the 30% increase in nitrogen in the stomach, which is significantly higher than that of salmon fed the other alternative feed ingredients. Furthermore, the ESF significantly increases the dry matter content of chyme in the mid-intestine, suggesting that other components other than pancreatic enzymes and bile acids may be secreted or present in the intestinal chyme, such as the higher number of bacteria found in this diet group (1).

The optimum dietary pH in the study by Fabay *et al* (86) (Table I) was slightly lower than that observed another study in the stomach chyme of the Atlantic salmon at pH 4.8 (89) and that in rainbow trout (*Oncorhynchus mykiss*) at pH 4.9 (125). The effects of dietary pH on chyme pH vary between fish species. Nikolopoulou *et al* (127) demonstrated that 2 h after feeding, the sea bass (*Dicentrarchus labrax* L.) and gilthead seabream (*Sparus aurata*) pH levels in the stomach were 4.7 and 5.7, respectively. The former exhibited dietary effects on stomach pH, while only the latter exhibited changes in the chyme of the proximal and mid-intestine. The study by Fabay *et al* (86) indicated indirect evidence that there was an effect on chyme pH. This effect was manifested in the growth and feed efficiency performance of the Nile tilapia.

The adjustment of the pH level to 4.6 in diets may have induced some enzymes. For example, carboxypeptidases A and B levels in stomach peptic digestion cannot increase without acid in the diet (123). Citric, hydrochloric and acetic acid directly lead to a lower dietary pH. This in turn presumably results in a lower gut pH level in fish. This may also activate and enhance pepsin and other digestive enzyme activities, and improve the solubility of minerals. The stomach is an organ that requires high acid levels, while intestine pH levels vary. In the case of dietary treatments with a higher dietary pH, diets with 7.0 and 8.0 may have different digestive physiology. They may result in the alkalinization of the chyme entering from the stomach. Alkalinization is necessary for the intestine to maintain intestinal epithelial integrity and pancreatic and intestinal activity (128). This innate alkalinization may not be sufficient to increase digestion efficiency; thus, this suggests a lower growth performance than the diet with a pH 4.6 (86).

Gastric acidification strategies. Two gastric acidification strategies have been reported in vertebrates. First, species that maintain a permanent acidic environment in the stomach are not affected by the presence or absence of acids (e.g., mammals and birds). Second, species maintain a neutral pH in the stomach lumen between meals and become slightly acidic following a meal, e.g., sharks (129).

The first strategy has been observed in cobia juveniles (121), rainbow trout (*Oncorhynchus mykiss*) (124), southern bluefin tuna (*Thunnus maccoyii*) (130), and in some Elasmobranchii species (131,132). It appears that these species are strictly carnivorous, and the observations were from studies that involved a comparison of fed and fasted fish; by contrast, the Nile tilapia is omnivorous and requires a daily feeding habit. In gilthead seabream (*Sparus aurata*), erratic daily feeding by changing the time of feed delivery at random each day may affect the daily pattern from neutral/acid alternation to permanent acidification (133). This voracious species can always activate pepsinogen to begin the hydrolysis of the ingested prey thanks to a constant acidic gastric pH. Continuously feeding Nile tilapia acidic diets of various pH resulted in irreversible lumen acidification (i.e., the first technique of gastric acidification). Thus, the atmosphere in which the production of digestive enzymes or the activation of established digestive enzymes occur is related to increased nutrient utilization.

The adjustment of dietary pH levels in feed may reflect the acid requirement in the gastrointestinal lumen of the tilapia. Studies on the effects of organic acids on the growth performance of fish have yielded conflicting results. Some researchers have not found growth-promoting effects of organic acids (66), which vary from species to species, type of organic acid and the dosage used (5). Several studies have evaluated the effects of dietary supplementation of specific organic acid on the diet of tilapia (Table I). Still, the optimal dietary pH requirement has not been directly investigated to date. Some results of studies on specific dietary acid supplementation in the tilapia include the following: Improved growth performance upon the addition of 2 g/kg of potassium diformate (67); increased activity of intestinal protease upon the supplementation of citric acid (134); increased growth performance when potassium-diformate at ≥5 g/kg was added to the diet (73); it reduced the total bacteria count in the gut, and increased body weight and improved feed utilization when the diet contained a combination of malic acid at 10 g/kg and *Bacillus subtilis* at 1.1×10^5 led to an increased in growth performance (112). In other species such as sea bream, the supplementation of coated sodium butyrate increased weight gain by 9.83% (135), supplementing 1.5% citric acids to the diet juvenile red drum (*Sciaenops ocellatus*), improving growth performance in terms of weight gain (43).

Similarly, sodium butyrate has been used to supplement the diet of *Carassius auratus* (136). The effects of different organic acids on the intestine vary substantially and depend on the dose of the respective organic acid in the feed (103). It provides clear evidence that the use of any of the three acids used to adjust the pH to 4.6 resulted in maximal growth and survival, while a markedly high (pH 8.0) and low pH (pH 2.5) may cause growth deprivation in the Nile tilapia and mortality (86). This result was supported by Romano *et al* (105) (Table I) with the dietary supplementation of citric acid to the diet of tilapia resulting in reduced growth as the acid concentration increased and significantly decreased intestinal short-chain fatty acid. Thus, at a dietary pH of 4.6, the following mechanisms may improve nutrient utilization: Reducing gastric pH levels, increasing digestive enzymes, increasing mineral solubilization during digestion processes, or altered intestinal microbial behavior, which may produce valuable nutrient contents.

5. Growth performance

The growth rate is a key indicator for determining the economic efficiency of commercial fish farming, and it is influenced by a variety of factors. Therefore, the impact of various acidifiers on the growth of a variety of fish species has been investigated. Organic acids, salts, or mixtures, according to some findings, can improve the growth and feed utilization of certain fish species (137). Nonetheless, other studies have not found growth-promoting effects of organic acids (67), which appear to depend on the fish species, type of organic acid and dosage used (5). Based on this information, the possible beneficial effects of organic acids may be species- and dosage-dependent. For example, a dose of 6.25 g/kg sodium butyrate has been shown to improve the growth performance, e.g., weight gain and specific growth rate in the tilapia (109). Likewise, a dose of 1% oxalic acid + malic acid and calcium lactate + sodium acetate has been found to enhance growth and feed utilization (138).

Studies on the effects of citric acidified diets on growth and feed performance have yielded positive results. A mixture of organic acids (acetic, lactic and citric acid) was used in a previous study to assess rainbow trout growth. Chen *et al* (139) (Table I) examined the effect of dietary L-malic acid on the growth and feed utilization of the genetically improved farmed Tilapia. Various factors, such as the experimental fish species and physiological age, the type and the level of organic acids, the diet composition, and the culture conditions, may all influence the growth-promoting effects of dietary organic acids (72).

The findings in the study of Fabay *et al* (86) re consistent with earlier findings in the literature in the used of organic acid in the aquaculture diet (Table I). Optimum levels that have been shown to elicit maximal growth performance are as follows: 1 or 2 g/kg of formic and propionic acid (63), 0.3% potassium diformate (115), 2 g/kg potassium diformate (67), and increased feed efficiency at 0.2 or 0.3% of potassium diformate (80), 0.2% formic and propionic acid (100). The observation that a dietary pH 4.6 resulted in the optimal growth among the treatments was supported by the study of Elala and Ragaa (77) (3 g/kg potassium diformate), Chen *et al* (139) (Table I) (0.8 or 3.2% L-malic acid; increased fish body weight and weight gain), Huan *et al* (31) (organic acid blend; increased weight gain), and Hassaan *et al* (108) (malic acid at 5; or 10 g/kg rose weight gain and specific growth rate).

While the use organic acid in aquaculture can improved growth rate, feed efficiency and fish health have been reported in several studies (Table I), Reda *et al* (63) also reported the supplementation of the Nile tilapia diet with a mixture of formic acid, propionic acid, and calcium propionate at concentrations of 0.1 and 0.2%. The optimal dietary pH value has been shown to be 4.6 (86), which affects the growth performance in the final average body weight. Other dietary pH values higher or lower than pH 4.6 have resulted in lower growth rates. Thus, the pH of feeds may affect fish gastrointestinal or chyme pH.

A 0.922 g/kg organic acid blend (calcium propionate, calcium formate and sodium acetate) supplemented protease in meat and bone meal diet has been shown to improve weight gain in the Nile tilapia (31). The optimal dietary pH value has been found to be 4.6 (86), which affects optimal growth performance, manifesting in weight gain. Other dietary pH values higher or lower than pH 4.6 result in lower growth rates. The pH of feeds may affect fish gastrointestinal or chyme pH. Citric acid (3%) has been found to induce weight gain in the Beluga (90), Rohu (140) and common carp (141). In addition, a significant increase in the final weight, weight gain and daily growth rate has been observed in a growth trial experiment on *Carassius auratus* fed a diet supplemented with apple cider vinegar (142). In another study, 1% citric acid improved weight gain in the yellowtail (143). In the case of the red drum, based on the weight gain results from the study it appears that acidifiers at a dose of 1.5% of the diet, especially citric acid, may improve growth performance (weight gain). Chen *et al* (139) (Table I) examined the effect of dietary L-malic acid and demonstrated that both 0.8 and 3.2% L-malic acid improved the weight gain compared to the control at 0.0%. A study on the red hybrid tilapia (*Oreochromis sp.*) fed a diet supplemented with 0.2% potassium diformate revealed a significant decrease in the mortality rate following a challenge with *Streptococcus* bacteria (from 58.3 to 16.6%) (74). Lim *et al* (144) also observed that graded dietary potassium diformate up to 10 k/g improved weight gain and feed efficiency in *O. niloticus*.

An increased protein digestibility has been observed with the use of 0.2 and 0.3% potassium diformate compared with the control diet by almost 6.75% (77). Furthermore, red hybrid tilapia fed diets supplemented with 2 k/g potassium diformate have been shown to exhibit a tendency towards an increased body weight, feed utilization and nutrient digestibility (67).

A previous study on the South African abalone (145) reported an increase significantly in the specific growth rate compared with the control when the abalone was fed a diet containing a mixture of sodium benzoate and sodium sorbate. Citric acid (3%) has been found to enhance the specific growth rate of the Beluga (90), Rohu (140), and common carp (141). A significant increase in the specific growth rate has also been observed in *Carassius auratus* fed a diet supplemented with apple cider vinegar (142). The best dietary pH in the Nile tilapia diet was pH 4.6 (86), which affected the best growth performance, which manifested a specific growth rate. Other dietary pH values higher or lower than pH 4.6 resulted in lower growth rates. Thus, the pH of feeds may have affected fish gastrointestinal or chyme pH.

6. Feed efficiency

In a previous study, 1% citric acid was found to improve weight and the feed conversion ratio in the red sea bream (146). A higher feed conversion ratio, total length, and feed intake were also found in an organic acid-treated group (137). Cuvin-Aralar *et al* (113) reported an improved growth and feed conversion ratio (FCR) in the juvenile Nile tilapia fed a diet supplemented with 0.3% potassium diformate compared with the control diet. The primary explanation for the improved growth efficiency and protein digestibility of fish diet supplemented with potassium diformate is the pH levels in the stomach and upper gut. In tilapia grow-out in Indonesia, Ramli *et al* (147) studied potassium diformate (potassium salt of formic acid) as a growth promoter. In this sample, fish were fed six times a day with a diet containing various percentages (0, 0.2 0.3, and 0.5) of potassium diformate over a period of 85 days. The diets included 32% crude protein, 25% carbohydrates, 6% lipids and 10% fiber. Potassium diformate markedly increased feed consumption over the whole feeding period, from day 1 to 85, and weight gain significantly improved the feed conversion ratio.

Furthermore, the protein efficiency ratio was also improved considerably due to the addition of the formic acid salt. The potassium diformate additions of 0.2 and 0.5% yielded optimal results. The authors concluded that applying the potassium diformate at 0.2% was an efficient tool to control bacterial infections in tropical cultures (74).

At the same concentration, citric acid was shown to improve feed performance in the red sea bream (148), and the protein efficiency ratio in the Beluga (90) and Rohu (140). In the study by Ryan *et al* (118), pH affects the optimal feed efficiency banifested by the protein efficiency ratio. Other dietary pH values higher or lower than pH 4.6 result in lower growth rates. Thus, the pH of feeds may affect fish gastrointestinal or chyme pH.

It has been demonstrated that the shrimp diet supplemented with acidifiers improves digestibility (149-151). However, the evaluation is limited to *Litopenaeus vannamei* diets supplemented with butyrate and propionate (149). In another study, formic, lactic, malic and citric were incorporated into shrimp diets. The results revealed that with addition of acid, increased the growth of *Litopenaeus vannamei* (148) and *Penaeus monodon* (150). The diets of *Litopenaeus vannamei* were supplemented with 0.5%, potassium diformate increased productivity over the control diet. In addition, these organic acids and their salts increase nitrogen and phosphorus retention and bioavailability of calcium and phosphorus (79,90,152). The effects of vinegar and sodium acetate on the growth performance of Pacific white shrimp (*Penaeus vannamei*) were examined by Serrano *et al* (151). The results revealed that the supplementation of organic vinegar to the diet improved the growth performance (final average body weight, weight gain and specific growth rate) and feed utilization (feed conversion ratio and protein efficiency ratio).

7. Conclusions

Feed researchers, developers and manufacturers are being encouraged to use plant protein ingredients to formulate feeds due to a global shortage of fish meals. However, the presence of anti-nutritional factors and imbalanced amino acid profiles in plant protein sources are obstacles they face. Their digestibility and growth performance are poor due to these two factors, and their use in aquafeeds is restricted. In conclusion, with the addition of acid to the diet, pepsin activation and mineral absorption increase, resulting in improved growth and feed utilization. In the dietary acidification of tilapia, the growth performance induced by the use of acetic, citric and hydrochloric acids in the feed has revealed that dietary pH and not a specific acid were the most crucial improvements in the fish stomach, specifically in monogastric species. This is the basis for vertebrates; two gastric acidification strategies have been discovered. The first are those that keep the stomach acidic at all times, regardless of whether food is present (e.g., mammals and birds). The second is to maintain the pH of the stomach lumen at a neutral level between meals and to subsequently become slightly acidic. However, the majority of studies on teleost fish studied thus far have used this second strategy. On the other hand, certain types of fish, such as cobia juveniles, rainbow trout, southern bluefin tuna and some Elasmobranchii species, have been observed using the first strategy, strictly carnivorous. Studies have compared fed and fasted fish feeding in the gilthead seabream; erratic daily feeding by changing the time of feed delivery at random can change the daily pattern from neutral/acid alternation to permanent acidification.

Following feeding in fish, pepsinogen is activated to begin the hydrolysis of the ingested prey to a constant acidic gastric pH. In the case of dietary acidification, the continuous feeding of acidic diets at various pH levels or amounts to certain types of fish, such as the tilapia and carp, may lead to a condition of permanent acidification of the lumen (i.e., the first strategy of gastric acidification). Thus, this dietary acidification follows the first strategy. This refers to a situation in which the production of digestive enzymes or the activation of existing digestive enzymes was easily achieved to improve nutrient utilization. Thus, the data on the fish growth rate and feed utilization efficiency were reviewed on dietary acid sources, such as acetic acid, citric acid hydrochloric acid. In addition, the attractability of the diets at various pH levels and dietary acid sources is another factor to determine the efficiency of the diet. The survival rates of cultured fish were increased based on the various dietary acids used that match the requirements of the fishes. Any acids at an optimum pH level, as for example pH 4.6 in the diet of the tilapia fry, which increased attractability, growth and feed efficiency warrant further attention. In general, the addition of acid to the diet, specifically in monogastric fish, such as the carp (no stomach) and tilapia (herbivorous, less acid produced in the stomach), can improve growth, and feed efficiency digestibility and mineral absorption in a culture system. Thus, the inclusion of acid in aquaculture diets may have promising results for feed manufacturers.

Furthermore, these dietary acidifications can eliminate the impact of bacterial infections to prevent diseases and result in high survival. Acids in the diet can efficiently achieve a sustainable, economic and safe fish production. Further studies are required to focus on cultured fish with different dietary pH levels in herbivorous and omnivorous species, such as milkfish and carp.

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All authors (RVF, AER, MSA and JVF) contributed to the conception and design of the study. In addition, all authors have read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

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Competing interests

The authors declare that they have no competing interests.

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