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Review

Application of Organic Acid Salts as Feed Additives in Some Aquatic Organisms: Potassium Diformate

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Abstract: Antibiotics are the primary measures employed in the prevention and treatment of diseases in aquaculture. However, the frequent utilization of antibiotics can significantly impact the growth and reproduction of aquatic organisms, resulting in water pollution. The European Union (EU) has prohibited antibiotic additives in animal feed. Potassium diformate (KDF) represents the first non-antibiotic feed additive approved by the EU as a viable alternative to antibiotics. Its application in animal nutrition has been validated, demonstrating beneficial health effects. This article reviews the physicochemical properties, biological functions, synthesis conditions, and applications of KDF in aquaculture and looks toward to its future potential. It also provides insights into improving the utilization of KDF in aquaculture practices.

Keywords: dietary additives; potassium diformate; aquaculture; disease resistance; intestinal environment

Key Contribution: Potassium diformate (KDF) is the first feed additive approved by the European Unionin 2001 to replace antibiotics for growth promotion, presenting a distinct efficacy compared to that of other feed additives. There is more than one review related to the use of organic acids and KDF in livestock and poultry, the use of KDF in aquaculture is booming, and we believe that analyzing and organizing the application of KDF in the field of aquaculture can provide useful references for many people in need.

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1. Introduction

Aquatic animal products constitute an indispensable component of the human diet, with the global demand surpassing that of beef, pork, and poultry products [1]. Fish, in particular, stands out as an optimal source of nutrition for humans due to its rich content of easily digestible high-quality proteins, essential fats (such as long-chain omega-3 fatty acids), vitamins (D, A, and B), and minerals [2]. According to data from the Food and Agriculture Organization (FAO), the proportion of global fish production designated for direct human consumption has markedly increased from 67% in the 1960s to 87% in 2014, surpassing 146 million tons [3]. With the advancement of modern aquaculture, aquaculture models have shifted from extensive farming systems to semi-intensive and intensive farming systems. However, the intensification of aquaculture has escalated the incidence of disease outbreaks in cultured species, resulting in significant mortality and economic losses [4]. Under these circumstances, the primary challenge for aquaculturists is to minimize disease outbreaks, while enhancing the growth rate of aquatic organisms [5].

The incorporation of antibiotics has been the primary measure for the prevention and treatment of diseases in aquaculture. Nevertheless, the excessive use of antibiotics can lead to antibiotic residues, water pollution, and the emergence of antibiotic-resistant bacteria. This is a significant concern for environmental health and the longevity of effective disease control strategies [6]. On 1 January 2006, the European Union (EU) instituted a comprehensive ban on the use of antibiotic additives in animal feed. Potassium diformate (KDF) represents the first non-antibiotic feed additive authorized for substituting antibiotic additives [7]. This additive possesses many functions, including pathogen inhibition, the promotion of animal growth, the modulation of animals' gut microbiota, and the enhancement of animal digestibility [8]. The use of KDF in animals is safe, devoid of residue concerns, and does not give rise to the issues related to antibiotic resistance. KDF, as a feed additive in livestock and poultry, finds widespread application due to its unique biological activity and antibacterial properties, playing a crucial role in livestock and poultry production [9]. Research indicates that the appropriate addition of KDF can significantly enhance the growth rate and feed efficiency of livestock and poultry. Simultaneously, it exhibits inhibitory effects on certain pathogenic bacteria, reducing the risk of disease outbreaks [10]. Furthermore, KDF positively influences the digestive systems of animals, contributing to the maintenance of intestinal microecological balance and the improvement of digestive absorption efficiency [9–12]. As a feed additive serving as an alternative to antibiotics, KDF demonstrates potential effects in promoting health and preventing diseases in livestock and poultry production. KDF already has a certain application foundation in poultry and livestock; however, further research is needed on the application of KDF in aquaculture. This article reviews the current research on KDF in aquaculture, explaining its physicochemical properties, mechanism of action, application effects, and potential issues, aiming to provide a reference for the future application of KDF in the field of aquaculture.

2. Chemical Properties and Mechanisms of Action

KDF is an organic acid salt. It is a dimer formed through hydrogen bonding between one molecule of formic acid and one molecule of potassium formate [13]. The chemical formula of KDF is $HCOOH \cdot HCOOK$, with a molecular weight of 130.14. It is a white or slightly yellow crystalline powder with no discernible pungent odor [14]. KDF dissolves in water and exhibits a pronounced hygroscopic nature. Its aqueous solution is acidic and remains stable under acidic conditions, while it decomposes into formate and formic acid under neutral or slightly alkaline conditions. The anti-mold performance of KDF in animal feed has been studied using the plate counting method. Compared with formic acid, KDF overcomes the irritability, corrosiveness, and instability of formic acid. Therefore, KDF is a more suitable additive in feed, providing a safer and more stable solution in maintaining the balance of microbial communities in aquatic animals [15]. Compared with the widely used mold inhibitor sodium diacetate (SDA), KDF exhibits better mold resistance in animal feed [15].

The key reasons for KDF serving as a growth promoter are its safety and antimicrobial properties, which stem from its simple and unique molecular structure. Generally speaking, the chemical structure of KDF ($HCOOH \cdot HCOOK$) contains formic acid and potassium formate groups. Naturally existing in nature and in the intestines of animals, it eventually decomposes into CO_2 and water, exhibiting complete biodegradability [16]. The unique antimicrobial functionality is based on the combined action of formic acid and formate salts.

KDF dissociates gently into potassium formate and formic acid, releasing H^+ ions, thereby reducing the pH of the digestive tract. The primary targets of free formic acid and its salts in the digestive system are the stomach. The gastric environment plays a suppressive role in bacterial selection and acts as an ecological filter. The production of non-dissociated formic acid can penetrate bacterial cell walls and release protons into the cytoplasm. Consequently, bacteria consume a significant amount of ATP to excrete protons, attempting to maintain the balance of the intracellular pH [16]. This results in cellular energy depletion and ultimately leads to cell death (Figure 1).

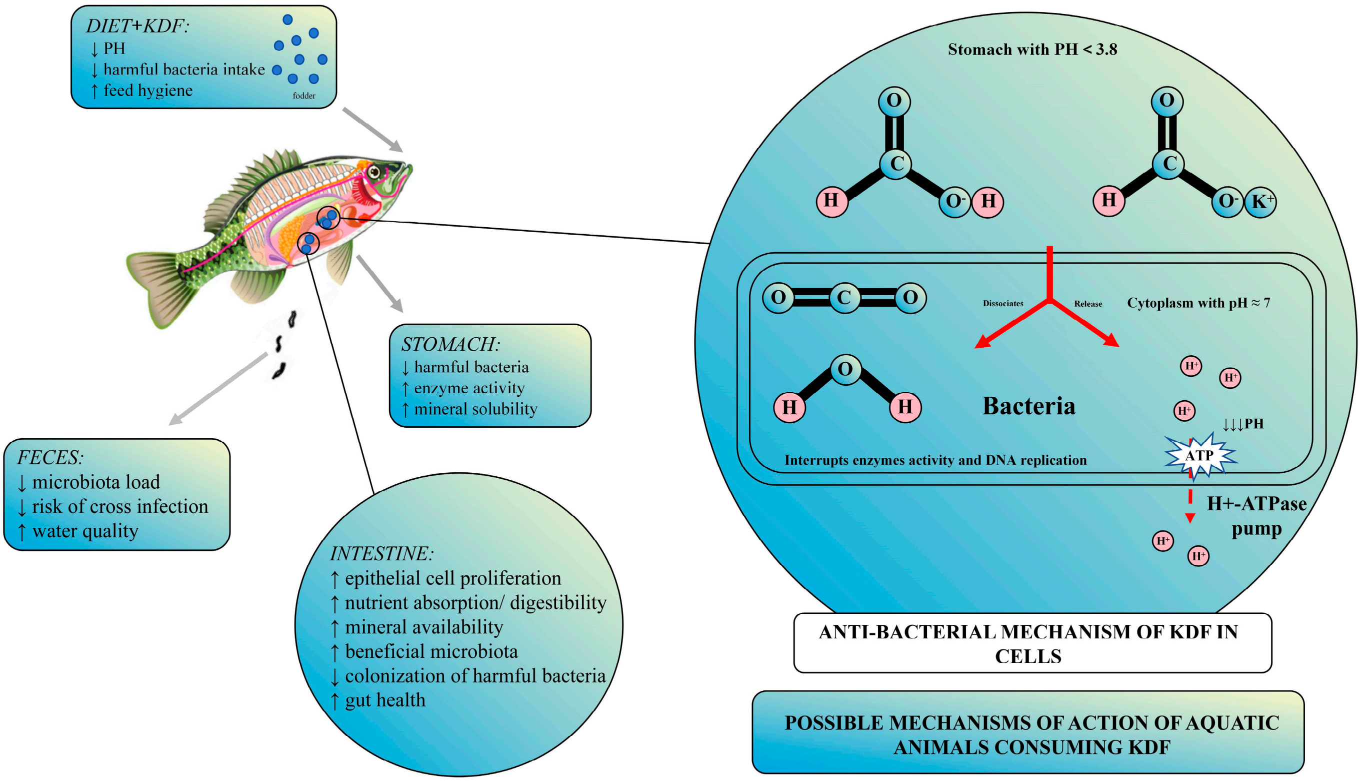

Figure 1. Mode of action of KDF against microorganisms. KDF is composed of potassium formate and formic acid, releasing H^+ to reduce the pH in the digestive tract.

3. Production and Market Conditions

KDF has always been an inevitable subject of discussion. The production of KDF is orchestrated through a chemical reaction between potassium hydroxide and formic acid, necessitating the precise oversight of the production parameters [17]. The reaction is highly exothermic, demanding rigorous temperature control. Furthermore, the raw materials are readily accessible. Transitioning to its global market dynamics, significant impacts on this industry have been noted due to regulatory changes, as observed in the European and Australasian markets. For example, the anticipation of the EU's prohibition on growth-promoting antibiotics in animal feed stimulated an increased demand and research focus on alternative products, including KDF [18]. So far, the use of potassium diformate (KDF) is less frequently reported in industrially underdeveloped areas. It is speculated that the global promotion of KDF may be limited by the production conditions and transportation costs. The simplification of the production process of KDF may promote its widespread dissemination [19].

Considering the costs associated with the utilization of KDF in farming, its usage proves to be highly economical [19]. The cost of 98% concentrated KDF specifically for farming purposes is just about USD 1.65–1.80 per kg in some markets in the United States, while on Chinese platforms, it ranges between about USD 3.06 and 4.17 per kg. Considering the relatively small rate of addition in production, only a very minimal increase in the overall production cost is incurred. In sum, the global farming industry is steadily steering towards environmentally friendly and sustainable practices. The market for KDF is projected to expand, fueled by the growing demand for healthy alternatives to antibiotics in animal feed [20].

4. The Application of KDF in Aquaculture

KDF can enhance the growth performance, improve the feed efficiency, heighten immunomodulation, increase the disease resistance, and regulate the intestinal microbiota balance of aquatic organisms [21] (Figure 2, Table 1).

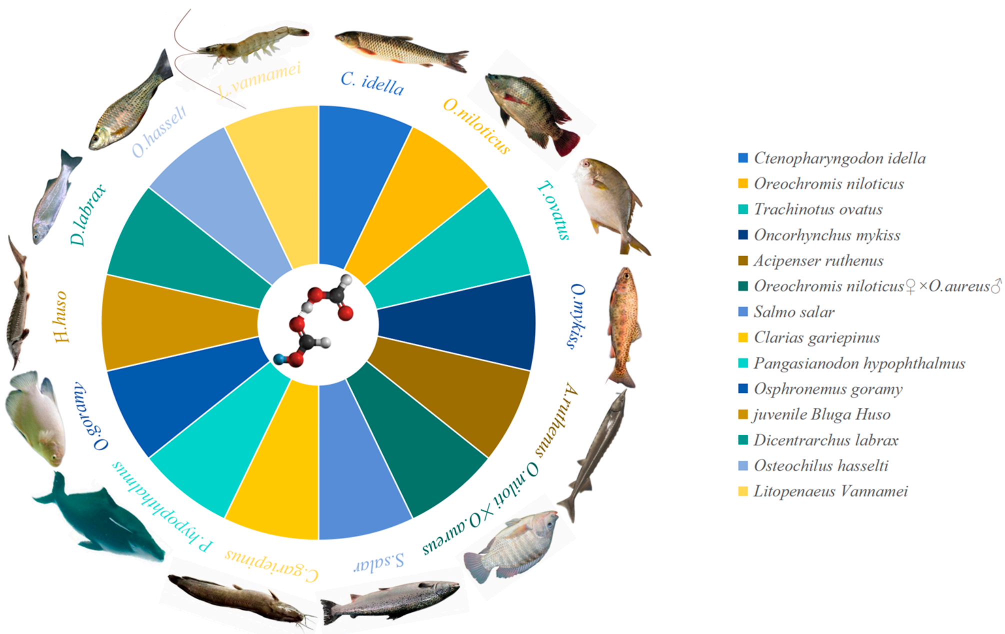

Figure 2. Addition of potassium diformate to aquaculture organisms. In chemical structures, black represents carbon atoms, red represents oxygen atoms, grey signifies hydrogen atoms, and blue symbolizes potassium atoms.

Table 1. Application of KDF in selected aquatic organisms.

Species	Dosage	Route of Administration	Influence	References
Ctenopharyngodon idella	3 g/kg	Feed for 70 days	Enhancing growth performance, pancreatic protease and lipase activities, lowering intestinal pH, and modulating gut microbiota.	[22]
Oreochromis niloticus	2–3 g/kg	Feed for 60 days	Enhancing growth performance and feed efficiency, reducing intestinal pH, and enhancing immune response.	[23]
Trachinotus ovatus	6.58 g/kg	Feed for 56 days	Enhancing growth performance, muscle elasticity, antioxidant enzyme activities, and improving intestinal morphology.	[24]
Oncorhynchus mykiss	12 g/kg	Feed for 56 days	Enhancing growth performance, lipase, protease, and amylase activities, and reducing glucose and cortisol levels.	[25]
Acipenser ruthenus	8.48–8.83 g/kg	Feed for 70 days	Enhancing growth performance, protein content, immunity, intestinal villus length, and width.	[26]
Oreochromis niloticus ♀× *O. aureus* ♂	3 g/kg	Feed for 56 days	Enhancing growth performance and feed conversion efficiency, and modulating gut microbiota.	[27]
Salmo salar	13.5 g/kg	Feed for 80 days	Enhancing growth performance and digestibility	[28]
Clarias gariepinus	5 g/kg	Feed for 40 days	Increasing survival rate and immunity.	[29]

Table 1. *Cont.*

Species	Dosage	Route of Administration	Influence	References
Pangasianodon hypophthalmus	5 g/kg	Feed for 14 days	Enhancing immune response and disease resistance.	[30]
Osphronemus goramy	3–5 g/kg	Feed for 40 days	Enhancing growth performance, feed efficiency, survival rate, and reducing intestinal pH.	[31]
Bluga huso	1.5–2 g/kg	Feed for 60 days	Enhancing growth performance, digestive enzyme activity, gene expression levels, antioxidant activity, and gut microbiota modulation.	[32]
Dicentrarchus labrax	2–3 g/kg	Feed for 90 days	Enhancing growth performance, hemoglobin levels, and immunity.	[33]
Osteochilus hasselti	1–5 g/kg	Feed for 56 days	Enhancing disease resistance.	[34]
Litopenaeus vannamei	2 g/kg	Feed for 70 days	Enhancing growth performance, disease resistance, immunity, and gut microbiota modulation.	[35]

4.1. Enhancing Growth Performance and Feed Efficiency

Since KDF is applied as an additive in aquatic feed, researchers continuously explore its health-promoting effects on aquaculture organisms. Relative to other feed additives, KDF has a wider range of applications. Besides improving the growth performance of aquatic animals, it also benefits the preservation of feed. In a study on *Osphronemus goramy*, it was noted that the addition of KDF significantly increased their feeding rate. This phenomenon is attributed to the elevated levels of KDF in the feed, subsequently influencing the osmoregulatory system of the fish. Because KDF is an organic acid salt, the energy for growth is only available in small amounts, and the remainder is utilized to balance the osmoregulatory system of fish. Additionally, KDF has the capability to eliminate pathogenic bacterial cells in the digestive tract, increase the population of symbiotic bacteria, and enhance the absorption of nutritional substances in the feed (Figure 1). This makes the nutrients in the feed more easily absorbed, thereby improving the efficiency and value of feeding. KDF induces acidity in an animal's digestive tract. This contributes to the enhancement of assimilative enzyme activity, thereby increasing the feed intake to promote weight gain. Following the addition of 0.3% KDF, *Osphronemus goramy* achieved an average daily growth rate of 1.31%. In aquaculture experiments, a 1% daily growth rate is considered favorable for *Osphronemus goramy*, as it is a species known for its relatively slow growth [31]. Similar results were observed in a study on sea bass (*Dicentrarchus labrax*), where three treatment groups were designed. Following the addition of KDF, sea bass exhibited more pronounced increases in final body weight and weight gain compared to those of the control group. Juvenile sea bass also demonstrated higher protein efficiency ratios and protein productive values, with the highest total protein content reaching 7.42 g/dL, compared to the control group's 5.57 g/dL [33]. The incorporation of KDF in the feed enhanced mineral absorption in the intestine stimulated the secretion of certain enzymes, such as proteases, thereby promoting growth [36]. In the experiment involving juvenile Beluga (*H. huso*), Sayah et al. [32] combined the use of KDF and calcium diformate (CaDF) to assess their impact on the growth of juvenile Beluga. KDF and calcium diformate (CaDF) share similar functions, allowing for synergistic research. These experiments indicate that feeding KDF can improve the feed conversion ratio and specific growth rate in juvenile Beluga. This is attributed to the stimulation of digestive enzyme secretion upon incorporating KDF into the feed, leading to heightened feeding interest in juvenile Beluga, and consequently promoting their growth performance [37]. Overall, the aforementioned studies underscore the significant role of KDF supplementation in the growth performance and digestion of aquaculture species [38].

4.2. Improving Disease Resistance

In numerous aquatic organisms, the addition of KDF to feed has demonstrated beneficial effects in enhancing their immune system and disease resistance. An increased or decreased white blood cell count can serve as an indicator of an organism's disease resistance [39]. White blood cells play a crucial role in the defense system of fish against pathogenic infections [40]. They possess non-specific immune defense functions, enabling them to eliminate pathogens through phagocytosis [41]. The fish's defense system releases neutrophils triggered by external chemical stimuli or chemotaxis in the event of infection [42]. The typical white blood cell count range for freshwater fish is 2.0–15.0 $\times 10^{-4}$ cells/mm^3 [43]. In a cultivation experiment with *Osteochilus hasselti*, KDF addition to the feed enhanced the immune response of *O. hasselti* [43]. The lowest average white blood cell count was observed in the group with no KDF, while the highest increase was observed in the KDF group. KDF can disrupt the osmoregulatory system within an organism [44]. According to Lantu, an excess of salt within a fish's body can lead to the fish expending more energy to stabilize its osmoregulatory system [34]. Red blood cells contain oxygen-binding hemoglobin, and their quantity is influenced by a fish's activity, water temperature, species, age, gender, and nutritional status. The control group showed the lowest average increase in red blood cells, measuring 2.05 $\times 10^6$ cells/mm^3, while the KDF-added group (0.3%) exhibited the highest average increase, reaching 2.49 $\times 10^6$ cells/mm^3. The normal range of red blood cells in bony fish is generally between 1.00 and 3.00 $\times 10^6$ cells/mm^3. An increase in red blood cells leads to a higher concentration of hemoglobin, allowing for the binding of more oxygen. The results indicate that among all the treatments, Nilem fish exhibited a significant decrease in the red blood cell count. However, 0.1%, 0.3%, and 0.5% KDF added to the feed caused relatively higher red blood cell counts than those in the control group (0%). This may be attributed to the enhanced resistance of the organisms due to the addition of KDF, leading to an increase in white blood cell count in Nilem fish. Consequently, the groups given KDF showed lower infection levels, allowing organs such as the liver, spleen, and spinal cord to continue forming red blood cells.

KDF possesses the potential to alleviate the symptoms of infection in aquatic animals. *Aeromonas hydrophila*, a commonly found bacteria in aquatic environments, is the primary bacterial species responsible for fish mortality [45]. Infection with *A. hydrophila* results in noticeable symptoms, such as a loss of appetite, surface lesions, gill hemorrhage, edema, and ulcers, which can lead to the swelling and damage of the liver, kidneys, and spleen, resulting in death [46]. KDF can alleviate the aforementioned symptoms [47,48]. One researcher noted that fish infected with *A. hydrophila* exhibited pathological changes, such as darkening of their body color, weakness, unresponsiveness to feed, and local hemorrhaging [49]. The exotoxins released by *A. hydrophila* circulate throughout the body via the bloodstream, leading to hemolysis and ruptured blood vessels. This vascular and tissue rupture leads to visible hemorrhaging on the body surface [50]. The condition of the body's surface deteriorated between Day 3 and Day 9. Nilem (*O. hasselti*) fingerlings infected with *A. hydrophila* exhibited symptoms such as hemorrhaging, exophthalmos, ulceration, and dropsy [50]. Hemorrhaging is the initial response to *A. hydrophila* infection, resulting in tissue damage [51]. Dropsy is attributed to the secretion of the aerolysin cytotoxic enterotoxin (ACT) gene, which contributes to tissue damage. According to Noor El Deen et al., fish infected with *A. hydrophila* exhibited superficial skin lesions, leading to imbalanced swimming, a darkened body color, exophthalmia, and hemorrhaging [52]. However, administering KDF can treat the complex infection symptoms caused by *A. hydrophila* in aquatic animals within a relatively short period. Elala et al. supplemented the diet of *A. hydrophila*-infected *Oreochromis niloticus* with 0.3% KDF. Beyond the 14th day, the injuries tended to heal in response to the KDF treatment. This recovery is attributed to the potent antibacterial and antiviral activities of KDF, thereby enhancing the overall health status of the host [18]. The fish fed an organic acid blend and potassium diformate as feed additives showed a significant reduction in the total bacterial count per gram of feces. Da Silva et al. identified organic acids (butyrate, propionate, and acetate) as having the most pronounced inhibitory effects against *Vibrio* sp. in marine shrimp, *Litopenaeus vannamei* [53]. These acids can permeate the membranes of Gram-negative bacteria, releasing protons into the cytoplasm [54]. The bacteria undergo substantial ATP expenditure in their efforts to extrude protons to preserve the equilibrium of the intracellular pH [55], leading to the depletion of cellular energy resources and culminating in cellular demise (Figure 1). The serum total proteins play a key role in immune responses and serve as a fundamental indicator of the health status of fish [56]. Hussein et al. [33]. revealed that feeding with diets containing 2–3 g/kg of KDF significantly increased the sea bass's total protein content, phagocytic rate, and lysozyme activity compared to those of the control group. The inclusion of 2 g/kg of KDF in the diet can be used as an immunostimulant. KDF enhances the fish's immune system and exhibits a robust health-promoting effect [57].

In summary, the existing data suggest that KDF may enhance the immune response of aquatic animals, and consequently improve the disease resistance of aquaculture organisms by elevating levels of immune components and modulating the expression of immune-related genes. However, the mechanistic impact of KDF on the immune system of aquatic animals requires further in-depth investigation at the molecular and cellular levels.

4.3. Modulation of the Gut Microbiota

KDF exhibits inhibitory effects on most harmful intestinal bacteria. Other studies indicate that KDF demonstrates stronger antibacterial activity against Enterobacteriaceae and Salmonella compared to Lactobacillus, enhancing the overall health of the intestinal microbiota in cultured animals. Research has shown that the addition of varying levels of KDF, specifically 1, 1.5, and 2 g per kg, to the diet of *H. huso* led to an increase in intestinal lactic acid bacteria (LAB) in *H. huso* after 60 days [32]. Organic acids have a regulatory effect on the intestinal health of *H. huso*, such as enhancing their intestinal balance and digestion, removing toxins, and improving their immune status [32]. Saliva et al. indicated that KDF as well as propionates, butyrates, and acetates had inhibitory effects on *Vibrio* species [58]. Formic acid inhibits *V. cholerae*, *V. harveyi*, *V. parahaemolyticus*, *V. vulnificus*, and *V. anguillarum* [59]. Abu-Elala and Ragaa [23] conducted a cultivation experiment with *O. niloticus* and added KDF (0, 1, 2, and 3 g/kg) to the feed. The addition of KDF to the feed improved the feed efficiency of *O. niloticus*. The feed containing KDF significantly reduced the total bacterial count in the feces, which is attributed to lipophilic organic acids being able to diffuse into the cell membranes of Gram-negative bacteria and acidifying them, leading to bacterial death. The addition of KDF to the feed enhanced the relative abundance of intestinal bacteria in *O. niloticus*. Zhou et al. [27] similarly found that the addition of KDF to the daily diet can impact the intestinal microbiota of external bacteria by selectively increasing the relative bacterial abundance (RBA, %) of the genus Bifidobacterium, such as *α-Proteobacterium* IMCC1702-like, *Streptococcus* P14-like, and three uncultured bacterial species.

L. vannamei is one of the most extensively cultured shrimp species globally, representing a significant portion of shrimp aquaculture. Investigating feed additives to enhance the production of the South American white shrimp is of paramount importance. Other researchers explored the effects of KDF, sodium formate (SF), and a combination of KDF+SF. The addition of KDF to the daily diet significantly reduced the total culturable bacterial count (TCBC) and presumptive *Vibrio* spp. count (PVC) in all the treatment groups [35]. Similarly, the total bacterial count in the feces of red hybrid tilapia (*Oreochromis* sp.) significantly decreased when using different doses of a mixture of organic acids and 0.3% KDF [60]. The addition of KDF (2%) to *L. vannamei* feed for 15 days resulted in a reduced TCBC and PVC. When varying amounts of butyrate salts were added to the feed of *L. vannamei*, the count of *Vibrio* species decreased at 27 and 47 days [58]. According to Mine and Boopathy [61], formic acetic, propionic, and butyric acids have inhibitory effects on *V. harveyi*, with formic acid showing the strongest inhibition. KDF can penetrate bacterial cells, leading to intracellular pH reduction, which inhibits bacterial metabolism, resulting in cell death, which is the primary reason for the decrease in bacterial count caused by these acids. These findings carry important implications for animal farming, particularly shrimp farming.

4.4. Reducing Intestinal pH Levels

Under typical conditions, the gastrointestinal pH of aquatic organisms is generally maintained between 5 and 8. Proteins undergo degradation due to amino acids in this slightly acidic-to-mildly alkaline environment. The dissociation state of these amino acids can significantly influence their absorption rate [17–19,31,60]. By supplementing KDF in the feed of aquaculture organisms, a microbial balance is achieved in the digestive tract, maintaining an appropriate pH, eliminating pathogenic microorganisms, and preserving the health of the organisms. Adding KDF to the feed is a preventive alternative for maintaining the health of farmed fish. This can lower the intestinal pH [62], suppress the growth of pathogenic bacteria, mainly Gram-negative bacteria, aid in nutrient digestion and absorption, and have beneficial effects on animals' production performance [19]. KDF enhances the feed performance. Within the gastrointestinal tract, KDF can lower the pH in the small intestine by transporting H^+ ions. KDF inhibits the growth of Gram-negative bacteria by dissociating the acids in bacterial cells after entering the intestine [63]. At pH < 5, the number of several Gram-negative bacterial species decreases. A low pH forms a natural barrier against bacteria from the ileum and large intestine. Low-molecular-weight KDF is lipophilic and can penetrate the cell membranes of Gram-negative bacteria [64]. The supplementation of KDF can reduce the population of pathogenic bacteria in the gastrointestinal tract, while increasing the population of acid-resistant beneficial bacteria, such as *lactobacilli*.

Ng et al. [60] found that the pH of the feed decreased as the KDF concentration increased, leading to a reduction in chyme pH in the stomach by 0.22 and a maximum reduction of 0.53 in the intestinal chyme. Compared to the control group, the addition of KDF to the feed significantly reduced the intestinal chyme pH in red hybrid tilapia (*Oreochromis* sp.). Lowering the gastric pH activates gastric proteases, and reducing the intestinal pH may increase the mineral solubility. Baruah et al. [60] reported a decrease in the feed pH from 5.87 to 4.85, followed by a decrease in the chyme pH in the intestines. The higher the KDF content in the feed is, the lower the intestinal pH is, which has a more significant impact on the nutritional utilization and growth performance of tilapia.

The intestinal pH of *O. goramy* decreased after adding KDF to the feed. In the feed with 0.3% KDF, the initial pH was 7.01, and the final pH was 6.61. In the feed with 0.5% KDF, the initial pH was 7.01, and the final pH was 6.49. In the feed with 0.8% KDF, the initial pH was 7.11, and the final pH was 6.12. Fish receiving KDF had a lower stomach pH compared to those without KDF. The reduction in stomach pH allows for faster digestion of the feed, and a lower pH facilitates better feed breakdown, promoting fish growth [31].

5. Conclusions

KDF exhibits various effects, including antibacterial activity, growth promotion, an improved feed conversion ratio (FCR), the regulation of intestinal microbiota, and a reduction in the intestinal pH. However, the practical application of KDF faces several challenges. Its physical properties, such as its susceptibility to high-temperature decomposition and high hygroscopicity, make it sensitive to environmental factors (temperature, humidity, pH, etc.) during processing and storage. Nevertheless, extensive research is still needed through further investigations.

To enhance our understanding of how KDF benefits aquatic animals, experimental designs must consider the life stages, nutritional levels, living environments, aquaculture systems, and health conditions. The quantity used in aquaculture experiments must be clearly defined, as the excessive use of KDF can impact the digestion, absorption, and metabolism of mineral elements. Exceeding a certain amount of KDF may not produce additional benefits, or may even have adverse effects, highlighting the necessity of defining the appropriate usage levels.

Research on the application of KDF has primarily focused on livestock and poultry production, with limited studies in aquaculture. With the global implementation of "antibiotic-free feed" policies, KDF is poised to benefit from significant development opportunities. Future research should prioritize the preparation and processing techniques of KDF, expanding its application scope. Lowering the cost of KDF use and addressing practical application challenges can facilitate its adoption in aquaculture, contributing to sustainable and environmentally friendly practices in the aquaculture industry, ensuring the safety of aquatic products.

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