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REVIEW ARTICLE

Lactoferrin in Aquaculture: A Holistic Review of its Health Benefits and Functional Feed Application

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ABSTRACT

Agriculture sector plays an important role in addressing both economic and food security challenges worldwide. This sector has the potential to make a substantial contribution in meeting the growing demand of healthy and eco-friendly nutrients for humans and animals. Lactoferrin (LF) is a functional glycoprotein found in several biological secretions including saliva, milk, tears, mucus and pancreatic juice. Recently, wide array of research has been conducted on LF owing to its multifunctional properties and could be used as a potential substitute of antibiotics and as a therapeutic remedy to cure the infectious disorders. These characteristics are attributable to its renowned antibacterial, antiparasitic, anti-inflammatory, anti-allergic, antiviral, immunostimulatory and antioxidant properties via activating the immune system and triggering the production of chemokines, cytokines and immunoglobulins. Lactoferrin not only kills multidrug-resistant *E. coli* but also enhances feed intake, immune performance, pathogens resistance and growth rate in aquatic species. It also reduces the colonization of pathogens in fish and shrimp by improving gut health and reducing the incidence of diseases. The recommended dietary requirement of LF for aquatic animals varies from 200 to 800 mg/kg, however it is influenced by several factors such as age, species size, health status and source of LF used for supplementation. This review aimed to summarize the recent research evidence regarding the health beneficial applications of LF in aquatic animals. Additionally, this review encourages the commercial utilization of LF as a functional feed additive to replace antibiotics in aquaculture industry.

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INTRODUCTION

The European Union imposed a ban in 2006 on the utilization of antibiotics as growth promoter in animal feed to promote the sustainable and responsible farming practices. This regulatory measure was taken to address the challenges regarding the development of antibiotic resistance and ensuring the safety of animal based food products (Arain *et al.*, 2022). Natural feed additives typically driven from the plants, herbs and other organic materials offer several benefits and plays an important role to promote health and growth performance of animals (Saeed *et al.*, 2021). The aquaculture industry has been using antibiotics for various purposes, including increasing production, promoting growth and protecting aquatic organisms from bacterial infections (Nabi *et al.*, 2020). The use of natural products in animal feed has indeed shown promising approach to boost immune functions, improve animal health and overall production efficiency (Rafeeq *et al.*, 2022).

Investigations on several potential antibiotic substitutes, including probiotics, prebiotics, postbiotics and plant extracts (Hussain *et al.*, 2021; Arain *et al.*, 2022), have shown promising therapeutic and nutraceutical benefits in a number of animal species. As a consequence of this, there is an urgent need to discover new antibiotic alternatives that may be utilized in aquaculture in order to enhance the disease resistance of fish and shrimps (Abdel-Tawwab *et al.,* 2022). The immunological responses of fish can be stimulated by a number of feed additives that are classified as immunostimulants (Abdel-Latif *et al.,* 2020). In the field of aquaculture, numerous immunostimulants have been investigated in aquatic studies, including chitin, glucans, phyto-biotics, herbs and their derivatives. These immunostimulants have been shown to enhance the overall immune functions and contributed to control the pathological disorders. It is true that the expansion of aquaculture farming, including shellfish, fish and aquatic plants, has the significant potential to achieve the sustainable developmental goals (SDGs) by the year 2030 (FAO, 2012).

Lactoferrin (LF) is a glycoprotein found in several exocrine secretions including mucus, saliva, tears and breast milk. It is multifunctional, single polypeptide of 690 amino acids with a molecular weight of 80 kDa (Legrand *et al.,* 2008). Numerous studies have suggested that milk derived proteins and their bioactive peptides have the potential to improve health status and can be used as a prophylactic treatment option against various pathophysiological conditions (Ashraf *et al.,* 2024). Dietary LF improves the iron absorption capability at cellular level, eliminates the toxic substances of reactive oxygen species (ROS) and modulates the cell growth (Song *et al.,* 2022). For these reasons, LF has been included in a wide variety of consumer goods, from infant formula to cosmetics, toothpaste and cosmetic powders (Tomita *et al.,* 2009). Earlier studies have also demonstrated that LF possesses numerous medicinal properties, such as antimicrobial, antioxidant and immunomodulation, which regulate iron absorption and stimulate the transcription process (Mulder *et al.,* 2008). The cationic properties of LF exhibit the bactericidal and bacteriostatic effects, leading to improve the mucosal immune defense. Moreover, it was suggested that LF has the capability to damage the external membrane of gram-negative bacteria. It improves immune status via inhibiting the secretion of several chemokines, including IL-1, IL-2 and TNF-α, and stimulates the cytotoxic potential of monocytes (Mulder *et al.,* 2008).

Lactoferrin supplementation suppresses the absorbency of intestinal mucosal cells and eliminates the inflammatory condition, leading to the improved nutrient metabolism and absorption and enhanced overall performance of aquatic animals. According to Song *et al.* (2022), the relationship between the growth performance and LF intake showed that the optimal supplementation level of LF for fish was about 5.8 g/kg body weight. Dietary LF significantly improved the growth performance of zebrafish by inhibiting the growth of pathogenic bacteria, increasing the population of beneficial bacteria, preventing diarrhea and improving the morphological integrity of digestive tract (Wang *et al.,* 2007; Ulloa *et al.,* 2016). A recent study indicated that LF exhibited superior immunological properties alongside the antimicrobial, antioxidant and anti-inflammatory characteristics (Zhang *et al.,* 2021). In addition to its medicinal properties, LF was also used as a biomarker tool for the diagnosis of numerous pathological disorders, such as Alzheimer's disease, inflammatory bowel disease and dry eye disease (Kane *et al.,* 2003).

Previously published literature covers a wide range of subjects, including livestock, poultry, human and other animal species. Despite the known biological properties of LF, further research is needed on various aquatic animals to validate the health boosting potential of this glycoprotein. Therefore, this review aims to highlight the uses and practical applications of LF in the aquaculture industry.

Structure and sources of lactoferrin: The molecular structure and amino acid profile of this protein was first identified in 1940, and was found to show 60% similarity with serum transferrin family, due to which it was grouped within the transferrin family. Molecular studies have revealed that LF comprises 703 amino acids, with two globular lobes known as polypeptides, connected by α-helix at carboxy (C) and amino (N) terminals regions, as shown in Fig. 1. Each individual lobe contains single iron binding site and two regens including N1, N2, C1 and C2 (Legrand *et al.,* 2008).

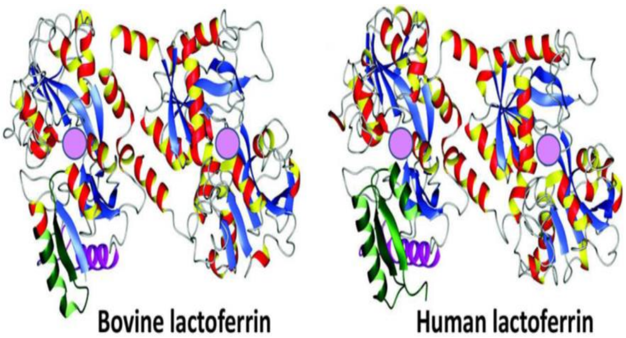

Fig. 1: Showing the crystal structure of human lactoferrin (1B0L) and bovine lactoferrin (PBD code=1BLF). Adopted from Vogel (2012): Pink spheres represent the ferric ion (Fe^{+3}) binding sites.

Among the three different variants of LF, lactoferrin-α is known for its iron binding potential. Conversely, lactoferrin-β and lactoferrin-γ exhibit the ribonuclease activity, but lack the ability to bind with iron (Kell *et al.,* 2020). Human milk and colostrum contain substantially higher concentrations of LF than other exocrine secretions of the body. On the other hand, it was also found in several other secretions such as saliva, tears, mucus and pancreatic juice (Kell *et al.,* 2020). The amount of LF in milk is influenced by several factors including species differences, stage of lactation, heath status and nutritional cogitations. Milk contains approximately 2-4 g/L of LF, however in human colostrum the concentration can reach up to 5 g/L (Artym and Zimecki, 2005). The approximate concentrations and major sources of LF are depicted in Table 1.

Bioactive properties of lactoferrin

Anti-bacterial properties: Lactoferrin has high affinity for iron and is known as iron binding glycoprotein. By binding to iron, LF can limit the availability of this crucial nutrient to bacteria, making it harder for them to thrive and reproduce. This is a broad-spectrum antibacterial mechanism because many different types of bacteria require iron for their growth. In addition, it has been demonstrated that the iron-binding sites of LF attach with the cell wall of bacteria, compromising their structural

Table 1: Major sources with different concentrations of lactoferrin

Biological fluids	Concentration (mg/ml)	References
Human colostrum	5.80±4.30	Montagne *et al.* (2001)
Camel colostrum	0.81±0.31	Konuspayeva *et al.* (2007)
Bovine colostrum	0.82±0.54	Kehoe *et al.* (2007)
Human milk	2.00-3.30	Montagne *et al.* (2001)
Camel milk	0.06-0.89	Konuspayeva *et al.* (2007)
Bovine milk	0.03-0.49	Cheng *et al.* (2008)
Goat milk	0.17-0.59	Chen *et al.* (2004)
Human tears	1.13±0.29	Balasubramanian *et al.* (2012)

integrity and ultimately leading to the death of the bacteria (Yen *et al.*, 2011). The direct interaction between the highly cationic N-terminal of LF and the negatively charged lipopolysaccharide (LPS) of Gram-negative bacteria is principally responsible for the bactericidal effects of LF. This interaction causes damage to the cell membranes of Gram-negative bacteria. Several studies have proved antibacterial efficacy of LF against a wide variety of bacteria, including *K. pneumoniae, Proteus sp., S. agalactiae, S. canis, S. pyogenes, S. zooepidemicus* and *Y. pestis* (Kutila *et al.*, 2003). Moreover, LF is reported to stop the growth and multiplication of numerous bacteria, including *S. mutans*, *S. epidermidis*, *E. coli* and others (Niaz *et al.*, 2019). Additionally, LF can be cleaved or hydrolyzed to produce smaller fragments, including antimicrobial peptides. These peptides, like lactoferricin, have potent antimicrobial properties and can disrupt the integrity of bacterial cell membranes, making them difficult to survive and form bacterial colonies (Roseanu *et al.*, 2010). Lactoferrin exhibits both inhibitory and lethal effects on numerous bacterial species that are dependent on iron, such as *Bacillus subtilis, Listeria monocytogenes, E. coli* and *Salmonella.* Owing to the antimicrobial potential of LF, it was extensively used as preservative agent in beverages, dairy products, meat, cosmetics and food products to improve the shelf life to increase the safety and health concerns of consumers. Meanwhile it could be used to restore the several pathological ailments including respiratory disorders, hepatitis, cancer and foodborne illnesses across the animals and humans, irrespective to the age of individual (Niaz *et al.*, 2019). Lactoferrin induces antibacterial effects by sequestering the zinc, and making it unavailable for the growth of bacteria (Sohnle *et al.*, 2000). The potential bioactive functions of LF are summarized in Table 2.

Currently, the nanocarriers obtained from LF (LF-based nanocarriers) are used as a promising approach in drug delivery system, particularly for the encapsulation of hydrophobic drugs. These nanocarriers offer several advantages that make them potential replacement for conventional formulations containing organic solvents or surfactants. This protein also exhibits the ability to destroy germs through a mechanism that is not dependent on iron in which LF directly interacts with cell membrane of bacteria and disturbs their morphological integrity (Hung *et al.*, 2010). It has also been observed that bovine LF has the ability to cure *E. coli* infection in cattle (Kieckens *et al.*, 2018). The promising beneficial effects of lactoferrin are shown in Fig. 2.

Antiviral activity: The antiviral potentials of LF against enveloped and non-enveloped viruses are primarily attributed to its ability via direct binding with virus particles or limit the iron availability and prevent the attachment with host cells. Moreover, LF also disrupts the replication cycle of viruses through the attachment with RNA or DNA, leading to cease the multiplication within the host cells (Chen *et al.*, 2008). Furthermore, LF showed the ability to modulate the capase-3 response and down-regulated the intensity of influenza induced apoptosis (Pietrantoni *et al.*, 2010).

The iron chelating property of LF can restrict the bioavailability of this nutrient in the surrounding environment, which is crucial for growth and multiplication, thereby impairing the proliferation of pathogens (Yen *et al.*, 2011). On the other hand, LF in combination with glycosaminoglycan, prevents or blocks the entry of virus particles into the host cell, which aids to early control of the viral infections. Mostly, the antiviral potential of LF has been attributed to its attachment with the host cells or viral particles, as well as nuclear localization within the cell to boost the mucosal immune response against a series of viral infections. Owing to the strong antiviral affinity, LF could be used as a valuable treatment option against viral diseases (Duarte *et al.*, 2022). It has been established that LF not only inhibits the microbial infections, but also improves the immune functions against viral diseases, and this has drawn reasonable attention in both medical and food industries. In January 2002, the USDA (United States Department of Agriculture) approved the utilization of activated LF as a functional ingredient in meat processing industry to improve the safety and shelf life of processed meat.

Antioxidant activity: Antioxidants are commonly used in commercial firming to mitigate the detrimental effects of oxidative stress. Dietary supplementation of bovine LF (1200 mg/kg) effectively improved their ability to withstand against air induced stress and concurrently eliminated the salinity stress in orange-spotted grouper fish (Esmaeili *et al.*, 2019). Lactoferrin and chitosan nanoparticles synergistically boosted the antioxidant defense in Nile tilapia fish by elevating the circulating level of antioxidant enzymes, including catalase, superoxide dismutase and glutathione S-transferase (Abdel-Wahab *et al.*, 2021). The remarkable iron scavenging or binding capabilities of LF also play a significant role in controlling the generation of hydroxyl radicals and reactive oxygen species (ROS) through the Fenton reaction, thereby reduce the intensity of oxidative stress (Esmaeili *et al.*, 2019). Diet supplementation with LF drastically reduces the circulatory concentration of H_2O_2 and ROS. Evidence also suggested that various formulations of LF effectively activated the immune functions via stimulating the antioxidant defense (Mulder *et al.*, 2008). The long term use of LF in the diet substantially reduces the blood pressure, improves antioxidant capacity and controls the ROS generation, leading to reduced oxidative stress in Dex-induced hypertension condition (Safaeian and Zabolian, 2014). According to Wang *et al.* (2007), dietary addition of LF as a exogenous source of antioxidant to piglets significantly boosted the levels of antioxidant enzyme and mRNA, as well as improved the growth performance of the piglets. Contrarily, yellow fish bream fed diet containing bovine LF did not show any improvement in productive

Table 2: The listed biological effects of lactoferrin from different studies

Sr. No	Dose/concentration (mg/kg diet)	Fish/shrimp species	Biological effects	References
1	Lactoferrin, supplemented at 75 mg/kg	rainbow trout (*Oncorhynchus mykiss*)	Improved the immune status and overall performance of farmed fish	Rahimnejad *et al.* (2012)
2	Bovine lactoferrin @ 0, 200, 400, 800, or 1600 mg/kg diet	Different fish (rainbow trout, red sea bream and Nile tilapia)	Improved the resistance of bacterial infections in different fish	Sakai *et al.* (1993)
3	Diet fed @ 0, 100, 200, 400, 800 and 1600 mg LF kg^{-1} diet for 8 weeks	fish (*Siberian sturgeon*)	Significantly improved some physiological and immunological parameters	Eslamloo *et al.* (2012)
4	Supplemented diets @ 400, 800 and 1200 mg LF kg−1 diet	Yellowfin sea bream, *Acanthopagrus latus*	Improved fish growth health status	Esmaeili *et al.* (2019)
5	480 g/kg protein	*Epinephelus coioides*	Reduced total cholesterol level	Song *et al.* (2022)
6	400 or 800 mg/ kg^{-1}	silvery-black porgy (*Sparidentex hasta*)	Improve innate immune parameters, serum albumin value and serum glucose concentration	Morshedi *et al.* (2020)
7	600 mg/kg	Silver Carp (*Hypophthalmichthys molitrix)*	Improved the activity of non-specific immune parameters such as lymphocytes and monocytes	Soliman *et al.* (2022)
8	800 and 1200 mg/kg	Nile tilapia (*Oreochromis niloticus*)	Enhance specific inflammatory markers such as IL-10, IFN-γ, IL1b, TLR9, TNF-α, IL-21, and IL-6	Hashem *et al.* (2022)
9	200, 400, and 600 mg/kg	*Sparidentex hasta*	Increase digestive enzyme activity, including total protease and amylase	Morshedi *et al.* (2016)
10	200, 400 and 600 mg/kg diet	Nile tilapia (*Oreochromis niloticus*),	Raised immune function	Badawy and Al-Kenawy (2013)
11	0 (control), 50, 100 or 200 mg kg−1 diet	Gilthead seabream (*Sparus auratus L.)*	Immunostimulant	Esteban *et al.* (2005)
12	140 mg bovine lactoferrin (Lf) kg−1feed	Atlantic salmon (*Salmo salar*)	Not seen any effects on non-specific immunity or disease resistance	Lygren *et al.* (1999)
13	0 (control), 800, and 1200 mg LF kg1 diets were administered.	Silvery black porgy (*Sparidentex hasta*)	Enhanced lysozyme activity, somatic growth	Pagheh *et al.* (2018)
14	Dietary bovine lactoferrin (BLf) at 0.1%) and 1%.	Juvenile rainbow trout	Triggered the body's humoral defenses,	Khuyen *et al.* (2017)
15	0 mg/kg of diet, 100, 200, 400, 800, and 1600 mg/kg	Siberian sturgeon (*Acipenser baerii*)	Reduce the sensitivity against stress	Falahatkar *et al.* (2014)
16	100 mg kg^{-1}	*Giant freshwater prawn (Macrobrachium rosenbergii)*	Boost *M. rosenbergii's* resistance to *A. hydrophila* and nitrite stress.	Chand *et al.* (2006)

IL-10=Interleukin 10; IL-1β=Interleukin 1 beta; IL-21=Interleukin 21; and IL6=Interleukin 6

performance and antioxidant status (Esmaeili *et al.,* 2019). Bovine LF, when administered orally at doses of 30, 100 or 300 mg/kg, or subcutaneously at a dose of 30 µg/kg per day, significantly increased antioxidant capacity, simultaneously reducing both the production of ROS and blood pressure in patients with dexamethasone-induced hypertension (Safaeian and Zabolian, 2014).

Anti-inflammatory potential: Despite the several medicinal properties, LF plays an important role to modulate the immune function by regulating the inflammatory stimuli and enhance the mucosal defense mechanism within the organs such as stomach, vagina and ocular organs. This protein reverses the inflammatory condition by limiting the production of inflammatory cytokines and chemokines, as well as prevents the attachment of lipopolysaccharide (endotoxin) to the inflammatory cytokines, thereby mitigated the detrimental effects of inflammation (Conneely, 2001). It has been proved that LF effectively reduces the severity of localized inflammatory reactions by blocking the allergens, and stimulates the protective activities of immune system. According to Conneely (2001), LF serves as a potent anti-inflammatory protein to control the inflammatory reactions in the localized areas of digestive, reproductive and respiratory systems. It is well known that LF has been widely used as a therapeutic remedy to control or treat the numbers of pathophysiological conditions associated with the inflammation, including inflammatory bowel disease. Additionally, this protein is also used to cure diverse range of other disorders like inflammatory anemia, premature birth, type-2 diabetes, Alzheimer disease and oxidative stress caused by infections (Lepanto *et al.,* 2019). In addition, LF effectively suppresses the secretion of neutrophil extracellular traps (NETs) (Okubo *et al.,* 2016). These neutrophil extracellular traps are involved in the developmental inflammatory thrombosis conditions associated with the microbial infections. In LPS-induced acute abdominal inflammatory condition, LF reduces the circulating level of C-C Motif Chemokine Ligand 5 (CCL5) and C-C Motif Chemokine Ligand 2 (CCL2), which are macrophage - related chemokines. This action intensifies the activation of p65 signaling in mice, inhibiting NF-KB signaling and amplifying the expression of CCL5 and CCL2. As a result, LF deficiency exhibits the inflammatory conditions and encourages the localized migration of macrophages (Okubo *et al.,* 2016).

Immune modulating properties: Besides the numerous therapeutic potentials, iron-free LF serves as a vital component of the secondary cytoplasmic granules present in the neutrophils and contributes to the primary defense mechanism of the body (Mishra *et al.,* 2018). Inflammatory condition accelerates the formation and release of LF from 0.4-2.0 g/ml to 200 g/ml at the site of inflammation. The rising trend of LF secretion in response to inflammation validates the notion of anti-inflammatory properties of this glycoprotein (Yen *et al.,* 2011). Lactoferrin induces anti-inflammatory effects through its interaction with epithelial cells involved in the attachment of specific receptors including IL-1 and low-density lipoprotein receptor-related protein-1. These interactions modulate the several cellular processes, such as cell differentiation, cell proliferation and even down-regulate the inflammatory processes (Legrand *et al.,* 2008).

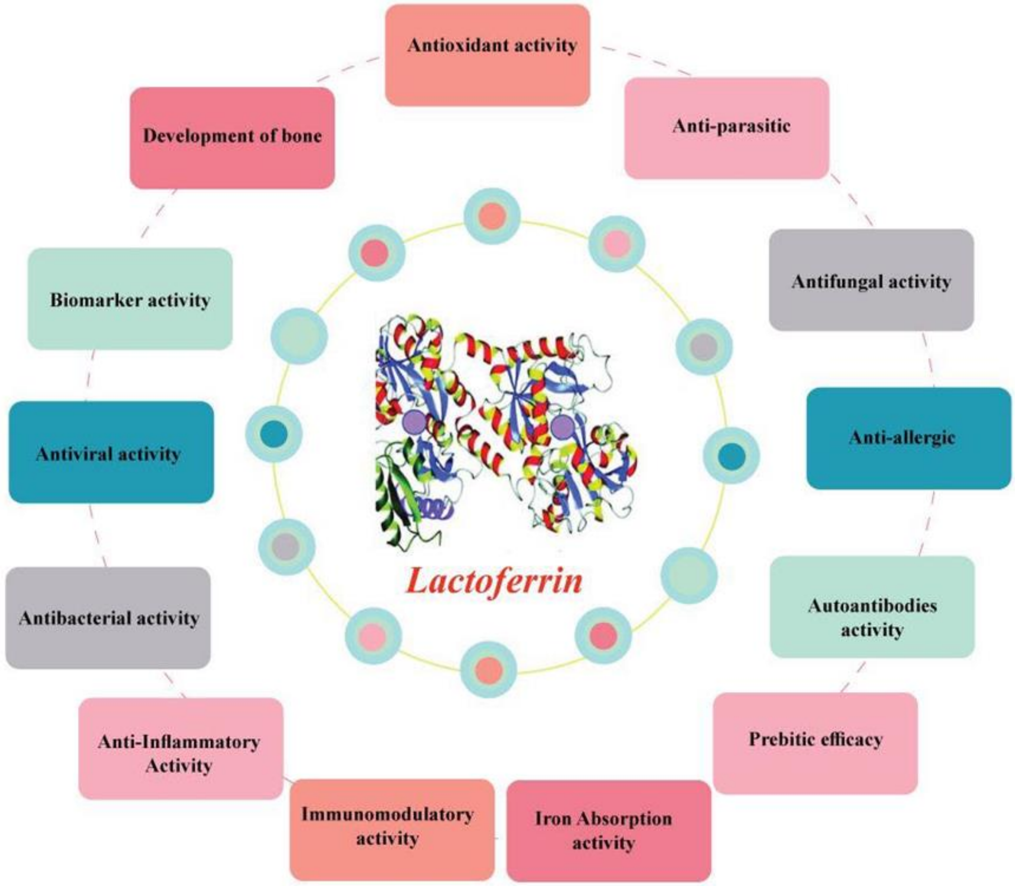

Fig. 2: Promising biological activities of lactoferrin (Saeed *et al.,* 2023; Luna-Castro *et al.,* 2022).

Moreover, LF exhibits the immunomodulatory effects via suppressing the oxidative stress and stimulates the antioxidant defense (Mulder *et al.,* 2008). Bovine LF appears to have a considerable impact on the activity of chicken leukocytes when they are cultured *in vitro*. Chicken granulocytes were able to achieve a higher respiratory burst capacity after being treated with LF. Yen *et al.* (2011) reported that dietary supplementation of LF boosted the immune performance of birds via activating the secretion of IgA and lysozyme that may limit gastrointestinal pathogen invasion. Lactoferrin could also work with lysozymes or macrophages to kill *E. coli in vivo*. Furthermore, LF boosted the antibody response by stimulating antigen-presenting cells (B-lymphocytes), thereby interacted with T-lymphocytes and encouraged the maturation of T cell precursors into competent helper cells. This process was triggered by the interaction between LF and T-lymphocytes (Hung *et al.,* 2010). The LF is a vital part of the host's first line of defense because it is vital to both innate and adaptive immune responses (Sabra and Agwa, 2020), as shown in Fig. 3.

Iron absorption activity: Fish, like other animals, can absorb nutrients through various mechanisms, however the LF stimulates the mucosal secretions that are more beneficial for maintaining the functional integrity of GIT (Embleton and Berrington, 2020). Iron is an essential mineral required for the development and growth of living organisms, and also necessary for the regulation of numerous physiological processes within the body. This mineral is vital for the formation of several enzymes required for important cellular processes and metabolic pathways. Insufficient supply of iron has adverse effects on immunity and other physiological processes, however surplus iron triggers the chemical reactions to produce excessive free radicals of ROS, leading to threat the cellular homeostasis (Phaniendra *et al.,* 2015). This mineral is essential for all kinds of organisms, including fish and other vertebrates, and plays a pivotal role in several physiological functions, including cellular respiration and oxygen transportation (Eslamloo *et al.,* 2012). The LF possesses 300-folds higher capability to bind iron under diverse range of pH in comparison to the serum transferrin protein. Increasing the iron export from the stomach improved the iron storage in ferritin; it can also affect iron homeostasis (Yen *et al.,* 2011). The iron absorption of Siberian sturgeon (*Acipenser baerii*) was shown to be greatly influenced by bovine LF, as demonstrated by a significant drop in plasma iron contents in all bovine LF treatment groups as compared to the control group (Eslamloo *et al.,* 2012). The human body requires minute quantity of iron (1-2 mg/day) to maintain the recycling activity because of reuse of the iron via metabolic processes (Camaschella, 2015). In the iron recycling activity, LF plays a crucial role in maintaining the iron balance within the normal range. Consequently, iron overload and deficiency are avoided. According to Wang *et al.* (2019), incorporation of bovine LF into the diets of pregnant women can help to prevent the iron deficiency anemia. Consequently, it is probable that individuals suffering from iron deficiency could consider LF as a valuable iron source. Moreover, bovine LF has been added to functional drinks to help professional sportsmen replace the iron they lose through perspiration during training and competition (Yen *et al.,* 2011).

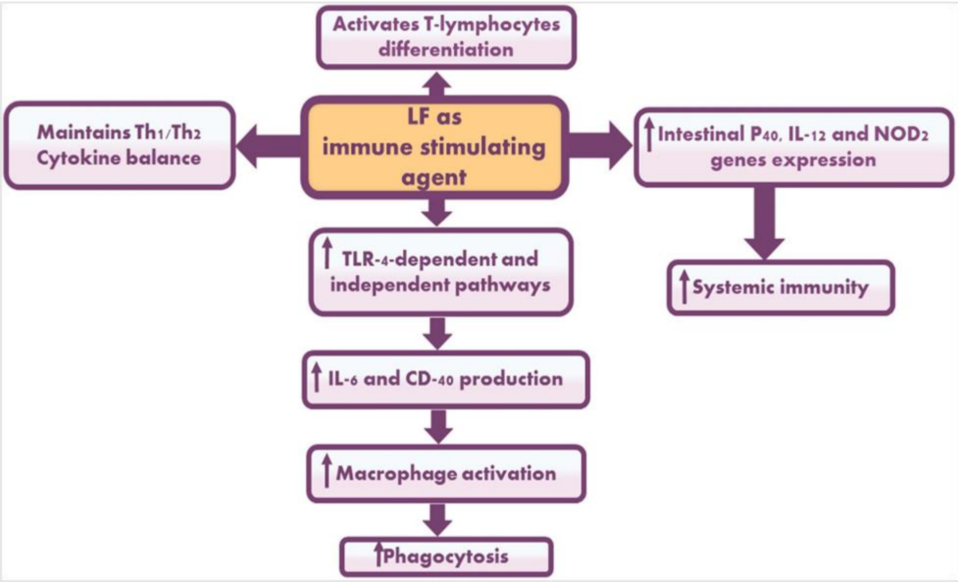

Fig. 3: A schematic diagram demonstrating the immune stimulating mechanisms of lactoferrin protein (Sabra and Agwa, 2020).

Biomarker activity: The right biomarker is essential to achieve early-stage diagnosis and personalized disease management. Biomarkers can be described as a signal or marker that reflects regular biological functions, pathological processes, or reactions to a stimulus or treatment. It was reported that LF may serve as a biomarker for a number of disorders (Zhang *et al.*, 2021). This protein is categorized as a protective or safe biomarker for diagnosis, prognosis, monitoring and pharmacodynamics purposes. These characteristics make the clear understanding regarding the progression and cause of disease, diagnosis and prediction of infection, and regression or treatment outcome of pathological elements.

Lactoferrin and nanotechnology in aquaculture industry: Nanotechnology has the potential to make significant contributions to both the aquaculture and nano-feed sectors, benefiting the overall sustainability, efficiency and productivity of the aquaculture industry. Nanotechnology has great potential because of its capability to influence matter at the nanoscale, which includes structures and materials at the atomic and molecular level, often ranging in size from 1 to 100 nanometers. The use of nanotechnology has the potential to facilitate the development of sophisticated packaging materials that effectively safeguard feed against spoiling and contamination (Sabra and Agwa, 2020). Nanotechnology can play a significant role in various aspects of aquaculture, including improving water quality, application as cleaning material for fish tank and enhanced fish and prawn production (Sabra and Agwa, 2020). The physical characteristics of food pellets can be improved by the use of even very modest amounts of nanoparticles. For example, adding single-walled carbon nanotubes to trout diets results in the formation of a thick pellet that maintains its shape in the water. This is essential to reduce food waste and pollution in aquaculture systems because improper buoyancy, inadequate food stability, or pellet texture result in significant losses for the industry. Thus, there has been an increasing focus in recent years within the industry on the creation of nano-formulations.

For instance, DNA based nano-medicines are used to boost the immune systems of fish. In the same way, iron nanoparticles can be used to enhance the growth efficiency of fish (Mohammadi and Tukmechi, 2015). Superoxide dismutase, catalase and glutathione S-transferase levels in Nile tilapia were increased following dietary administration of chitosan nanoparticles and bovine LF (Abdel-Wahab *et al.*, 2021). After an experimental infection with *Aeromonas hydrophila*, Nile tilapia that were fed diets with increasing concentrations of bovine LF, either singly or in combination with chitosan nanoparticles, demonstrated significantly greater relative percentage survival rates than the control group (Abdel-Wahab *et al.*, 2021). It is crucial to understand that the combination of nanoparticles with LF is not authentic to develop the potent drug. Conversely, this approach could be used to develop new supplements, pharmaceuticals and functional foods (Duarte *et al.*, 2022).

Several investigations show that nanoparticles may absorb LF while preserving the structure of the protein (Sabra and Agwa, 2020). This suggests that utilizing nano-scaled LF could serve as a promising foundation for creating advanced formulations of metronidazole-loaded LF nanoparticles with excellent therapeutic effects against trichomoniasis (Tabari *et al.*, 2021). Recent investigations have demonstrated that the incorporation of compounds into nanoparticles improves the antimicrobial potential of that compound. Stronger interaction with anionic microbial membranes are strengthened by nano-aggregation, which makes it easier for cationic charges to be distributed more uniformly throughout the polymer's surface (Duarte *et al.*, 2022). One approach to achieve this is by combining LF with olive oil in an aqueous solution. The distinctiveness of these types of conjugates stems from their exceptional loading capacity, stronger ligand attachment and compatibility with biological systems. In contrast, Magnetic Resonance Imaging (MRI) and LF-coated (low-frequency coated) magnetic nanocarriers are two different approaches in the field of medicine and nanotechnology, that could be applied in the treatment of cancer (Duarte *et al.*, 2022). These workers proposed that the gellan gum complexes prepared with LF may be useful in treating *S. aureus* infections. Previous research indicates a knowledge gap about the real-world implementation of novel carriers or novel formulations. In the realm of aquaculture, nanotechnology offers innovative opportunities for improving growth and boosting production performance. Nanotechnology has the potential to revolutionize various aspects of aquaculture. Consequently, the industry is actively investigating nano-formulations, as depicted in Fig. 4.

Nano-aggregation can also improve LF characteristics; when combined with inorganic nanoparticles, LF offers significant advantages. These combinations stand out due to their increased capacity for holding substances, ease of attachment to molecules and compatibility with biological systems. Recently, feed supplements like nanoparticle-based LF have proven to be valuable additions to aquaculture feeds. However, further detailed research is required to guarantee the effectiveness and safety of LF in order to avoid potential harm to aquaculture and the environment.

Biological effects of lactoferrin in different animal species: Lactoferrin plays multiple roles in the body, including the regulation of iron uptake in the digestive system in different animal species. Furthermore, it exhibits antioxidant and anti-inflammatory properties, alongside its well-established antimicrobial activity, which has drawn the greatest attention from researchers (Giansanti *et al.*, 2016). It is discovered that incorporating LF into bird feed enhanced the performance of their immune system. It is estimated that dairy industry produces about 20 to 30 tons of LF through the conversion of milk into various dairy products such as skim milk or cheese. It has been suggested that bioactive LF acts as a potent antibacterial compound in poultry. Based on the research findings, LF (67.5 g/egg) can be supplemented to the layer breeder eggs via *in vivo* route during incubation period, which significantly improved the bone strength and post hatch productive performance of laying hens (Saki and Mahmoudi, 2015). However, dietary supplementation of LF positively strengthened the beneficial microflora, lymphocyte count and overall performance of broiler chicken by reducing the population of pathogenic bacteria. It was demonstrated that porcine LF boosted the cell mediate immunity and reduced the incidence of infectious bursal disease via improving the serum level of IgG and interleukin-2 (IL-2) in broiler chicken. These findings proved that LF has the potential to improve the protective immunity against infectious disorders in avian birds (Hung *et al.*, 2010). The LF also showed antibacterial properties against the multidrug resistant *E. coli* and improved the productive performance by improving the feed efficiency and weight gain in broiler chickens (Kieckens *et al.*, 2018). It was suggested that feed supplementation of LF effectively recover the diseased birds by elevating the mRNA expression of immunomodulatory chemokines and improving the blood concentration of IgA and IgG (Humphrey *et al.*, 2002). Another study verified that activated-LF prevented the multiplication and spread of pathogens via blocking the cell or tissue attachment sites (Naidu, 2002).

Several investigations have shown that the milk derived proteins, fermented dairy products and their hydrolysate exhibited diverse nutraceutical effects including antimicrobial, immunomodulatory, opioid and growth promotion (Nongonierma and FitzGerald, 2015). The hydrolysate derived from LF has shown strong affinity against several pathological agents and could be used as effective treatment option in animals and humans (Niaz et al., 2019).

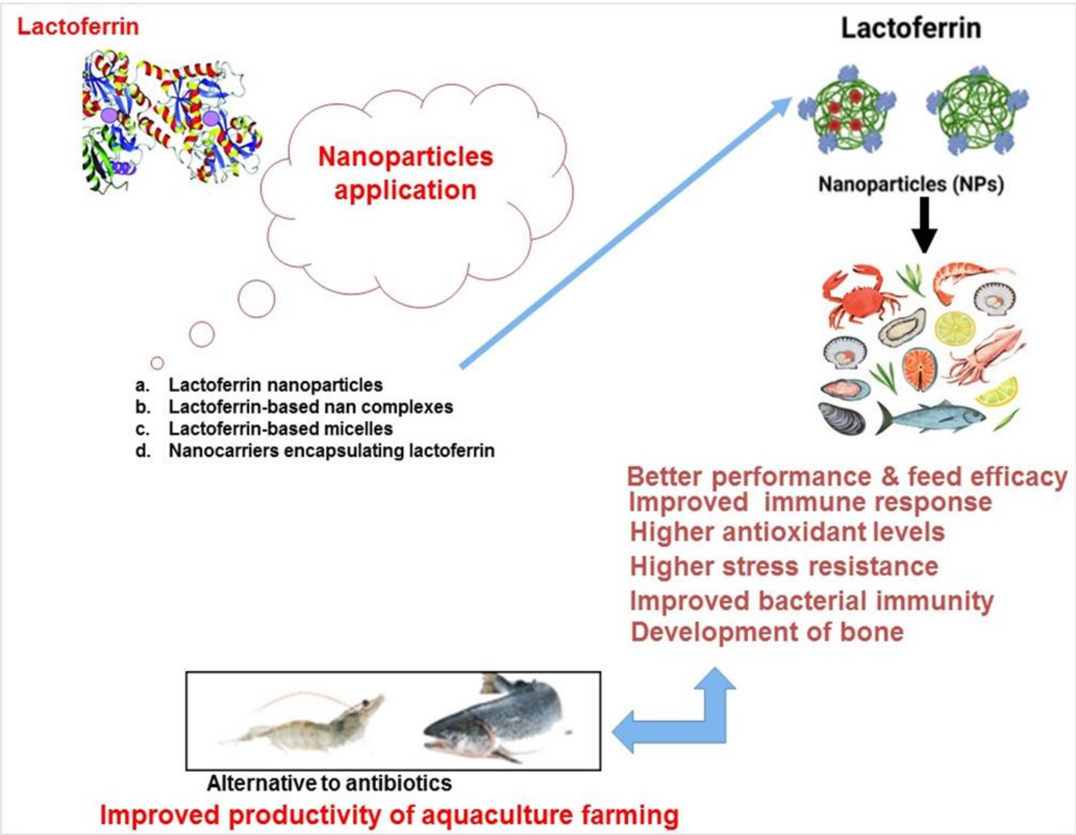

Fig. 4: Nanotechnology and its promising effects in aquaculture industry.

Mastitis stands out as the most important pathological ailment affecting dairy cows. This disease is caused by numerous bacteria including *S. aureus*, *S. uberis* and *S. dysgalactiae,* which usually originate from within the udder. Treatment of beta-lactam-resistant *S. aureus* in the mammary glands with LF has proved effective for control of mastitis. The synergistic effects were induced by decreasing the activity of beta-lactamase in *S. aureus* strains in response to the combined treatment of LF and penicillin G (Lacasse *et al.,* 2008). Recently, bovine LF showed *in vitro* biocidal efficacy against the Prototheca-mastitis causing algae *Prototheca zopfii* and various fungal-mastitis causing yeasts isolated from bovine mastitis cases. The *in vivo* confirmation of these encouraging *in vitro* results is, however, necessary. In particular, antimicrobial activity of antimicrobial peptides (AMPs) and lactoferricin against mastitis bacteria has sparked attention to their possible use in the management of udder infections. Kawai *et al.* (2003) conducted an *in vivo* trial on cows with subclinical mastitis caused by organisms like *E. coli* and *Staphylococci* by injecting the animals with luteinizing hormone. In a separate method, the LF-derived peptide lactoferricin was expressed in goat mammary glands as a preventive therapy by employing a plasmid vector. It was concluded based on the published literature that LF can be used as an effective bioactive feed additive for the control of pathological ailments and improve the health status and productivity of diverse animal species.

Applications of lactoferrin in aquaculture: One of the quickly expanding food production industries that have remarkably expanded and improved global food security is the aquaculture sector**.** Published data have spurred scientists to explore feed additives with unique growth-promoting, antioxidant and immunomodulatory properties that can be regularly integrated into fish diets (Abdel-Latif *et al.,* 2020). There is a dire need to find out the substitute of antibiotics that could effectively control the bacterial infestation in fish and shrimp with minimum side effects (Abdel-Tawwab *et al.,* 2022). Bovine LF could serve as a potential substitute for antibiotics in this context. Furthermore, it can be incorporated into fish and shrimp feed to enhance their nutritional value and mitigate the detrimental effects of stressful situations on these aquatic species. It is conceivable that antimicrobial, anti-inflammatory, antiparasitic and antiviral properties of LF may be associated with these beneficial effects (Luna-Castro *et al.,* 2022). According to various investigations, LF has a number of additional biological properties in addition to being an immunostimulant. Additionally, LF can strengthen the general immune system and increase resistance to numerous diseases in a variety of fish and shellfish species. Bovine LF offers several benefits in aquaculture (Luna-Castro *et al.,* 2022). For instance, Kumari *et al.* (2003) demonstrated that bovine LF could be incorporated into fish diets to increase resistance against variety of pathological conditions caused by bacteria such as *Streptococcus* species and *Vibrio anguillarum* in rainbow trout (*Oncorhynchus mykiss*) and *Aeromonas hydrophilia* in Asian catfish (*Clarias batrachus*). Furthermore, bovine LF has been shown to increase stress tolerance and growth indices in various fish species, including goldfish (*Carassius auratus*) and Japanese and Asian catfish (Kumari *et al.,* 2003), Siberian sturgeon (Eslamloo *et al.,* 2012), flounder (*Paralychthys olivaceus*) and rainbow trout (Rahimnejad *et al.,* 2012). In order to provide antibiotic-free aquaculture, bovine LF supplementation was recommended to improve the fish immunological conditions (Morshedi *et al.,* 2020). The efficacy and suitability of bovine LF in nutritional strategies can be influenced by several elements, like dose, species, culture conditions, diet, environmental circumstances and management. Luna-Castro *et al.* (2022) recently published a report about aquaculture, and how bovine LF influences stress tolerance, immunity, and bacterial disease resistance. Numerous other studies have revealed that LF might benefit the gut mucosa immune system (Mohammadabadi, 2021). Similarly, Kumari *et al.* (2003) found that adding bovine LF to the diets of Asian catfish (*Clarias batrachus*) resulted in a considerable increase in the fish ability to survive after being subjected to bacterial infection. According to Chand *et al.* (2006), the consumption of dietary LF at a dose rate of 100 mg/kg of diet for seven days considerably improved both the disease resistance against *Macrobrachium rosenbergii,* as well as the survival rates, following an *A. hydrophila* challenge. Dietary inclusion of 0.8 or 1.2 g/kg bovine LF improved the immunological variables including IgM and IgG in tilapia fish (Hashem *et al.,* 2022).

Additionally, Welker *et al.* (2007) demonstrated that supplementation of bovine LF had no effect on serum lysozyme (LYZ) levels in Nile tilapia diets. Furthermore, when comparing the LYZ activity values in seabream fed with bovine LF to those in the prebiotic and control groups, no significant changes were observed (Morshedi *et al.,* 2020). Variables like fish species, experimental conditions, water quality, and pepsin activities in fish stomach, which may influence fish ability to digest LF in the intestinal lumen and, as a result, its bioavailability in the bloodstream, could account for these discrepancies in the literature. According to Hashem *et al.* (2022), dietary supplementation of bovine LF (800 mg/kg) considerably raised the number of RBCs and total WBCs in tilapia fish. Bovine LF due to iron-binding capacity can raise iron levels in diets, potentially improving the health of the fish. Previous reports from human studies indicated that dietary LF could improve the iron status of newborns and pregnant women and could be used to treat human iron-deficiency anemia (Morton, 2019). However, the impact of bovine LF on the blood protein fractions of fish is debatable. According to Esmaeili *et al.* (2019), yellowfin seabream fed a meal containing higher level of bovine LF (1200 mg/kg diet) had increased levels of total protein and albumin. According to recent research performed by Soliman *et al.* (2022), dietary supplementation of bovine LF (600 mg/kg diet for 30 days) significantly increased the level of total protein, globulin and albumin in *Hypophthalmichthys molitrix,* the silver carp. Ulloa *et al.* (2016) demonstrated that LF exerted a dual impact on zebrafish, functioning as an anti-inflammatory drug in the digestive tract, while also boosting the fish resistance to bacterial infection (Fig. 5). Furthermore, it was discovered that dietary LF dramatically enhanced the expression of pro-inflammatory responses in shrimp and fish as well as the growth indices, feed efficiency, digestive enzymes, iron metabolism, blood metabolites, immunology, and disease resistance.

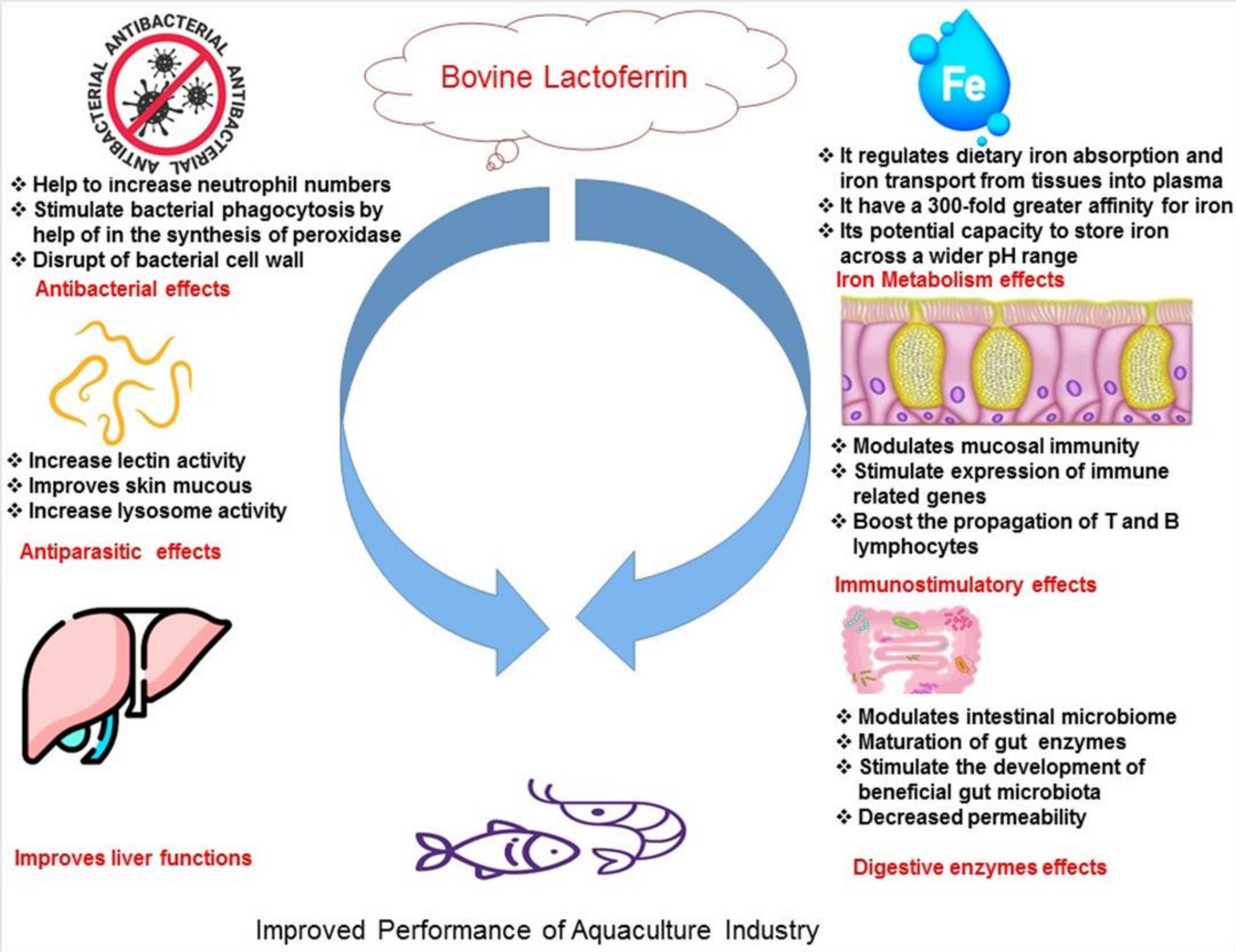

Fig. 5: The promising proposed potential of bovine lactoferrin in aquaculture industry.

Based on the reviewed research, it appears that LF could be the promising substitute of antibiotics. It also has the potential to be used as a dietary supplement for fish and shrimp to alleviate the negative impacts of stressful conditions encountered under several environmental and pathological conditions. These effects could be related to antimicrobial, anti-inflammatory, antiparasitic and antiviral properties of LF. While these biological functions of LF are significant, further investigation and analysis are required to comprehensively grasp its role in preserving or restoring the health of fish.

Conclusions: Aquaculture is of paramount importance in ensuring the provision of secure and nourishing sustenance for individuals worldwide. Lactoferrin (LF), a compound renowned for its versatility, is utilized extensively in the food and pharmaceutical sectors, where it is also incorporated into infant formula and functional beverages. Dietary supplementation of LF could expedite growth, decrease mortality rates, control iron metabolism, mitigate disease outbreaks, boost the antioxidant defense system and generally improve the health of fish and shrimp. The utilization of LF as a dietary supplement in aquaculture and other animal species has garnered significant attention due to its capacity to enhance growth performance, booster immune responses and improve intestinal health. Furthermore, LF has been demonstrated to fortify immune system of fish and enhance disease resistance when faced with bacterial challenges. However, further research is needed to better understand the potential risks and benefits and for additional information concerning the practical applications of LF in aquaculture nutrition. The insights presented here should serve as a valuable foundation for future research endeavors aimed at advancing the long-term sustainability and effectiveness of aqua feeds within the aquaculture industry.

Conflicts of interest: The authors declare no conflict of interest.

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Authors contributions: NH, JH and MS conceptualized the idea, drafted the contents and prepared the original draft. YX and MAA performed the literature search and reviewed the final draft. MFH designed and edited the figures and tables. HC was responsible for project administration and funding arrangements. All the authors also contributed in literature search, critically reviewed and approved the final manuscript.

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