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Likenaz ve Laminarinaz Enzimlerini Üreten *Bacillus* Suşlarının Balık Yem Katkısı Olarak Kullanımı

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ÖZ

Hayvanlardan yüksek verim elde etmek için hayvan sağlığını korumanın yanında yemden yararlanma yeteneğini de üst düzeye çıkarmak gerekmektedir. Bu yöndeki önemli uygulamalardan biri yem katkı maddeleridir. Yem katkı maddeleri balık üretimini ve refah düzeyini yükselten, sindirimi ve sindirim sistemi mikroflorasını iyileştiren, besin maddelerinin ve yemin korunmasına katkıda bulunan, bitkisel ve hayvansal ürünler ile mikroorganizmalardır. Her ne kadar enzim üretiminde bitkisel ve hayvansal dokular kullanılsa da, enzim üretiminde mikroorganizmaların kullanımı ilk sıralardadır. Moleküler genetik tekniklerinin kullanılmaya başlamasından sonra enzim üretiminde sorumlu genler mikroorganizmalarda klonlanarak, enzimlerin endüstriyel boyutta daha saf ve daha ucuz bir şekilde üretilmeleri mümkün hale gelmiştir. Balıklarda bağışıklık sistemini desteklemek, mortaliteyi azaltmak, büyüme performansında artış ve hastalıklara karşı dirençli olmalarını sağlamak için bu enzimleri üreten *Bacillus* suşlarını yem katkısı olarak kullanmak iyi bir alternatiftir. Bu derlemede balık yemlerinde kullanılanılan likenaz ve laminarinaz enzimleri ve bu enzimleri üreten *Bacillus* şuşları ile yapılmış çalışmalar incelenmiştir.

Use of *Bacillus* Strains Producing Lichenase and Laminarinase Enzyme as Fish Feed Additives

Review Article

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ABSTRACT

To obtain high efficiency from animals, it is necessary to maximize the ability to benefit from feed and to protect animal health. One of the important applications in this direction is feed additives. Feed additives are plant and animal products and microorganisms that increase fish production and welfare, improve digestion and digestive system microflora and contribute to preserving nutrients and feed. Although plant and animal tissues are used in enzyme production, the use of microorganisms is in the first place. After using of molecular genetic techniques, the genes responsible for enzyme production were cloned in microorganisms, making it possible to produce enzymes more purely and cheaply on an industrial scale. It is a good alternative to use *Bacillus* strains that produce these enzymes as feed additives to support the immune system, reduce mortality, increase growth performance and provide resistance against diseases in fish.In this review, lichenase and laminarinase enzymes used in fish feeds and studies with Bacillus strains producing these enzymes were examined.

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Introduction

As in all animal husbandry, feed is the most critical input affecting productivity and cost in fish farming. In feeds, feed raw materials containing high protein levels are heavily added to the feed content. Fish meal is an indispensable protein source in the aquaculture feed industry because it contains high protein and balanced amino acid components. However, in recent years, due to the decrease in fish stocks in our country's seas and its use in human nutrition, fishmeal production has decreased considerably and feed producers have found the solution to import fishmeal from other countries. Therefore, fish meal prices have increased feed costs and brought the use of plant resources to the agenda.

From a cost point of view, adding enzymes to the feed brings a meager cost. The price of commercial feed enzymes is around 3-4 Euros/kg. Since enzymes are used at the level of 1-2% in feeds, it reflects a very low cost (Yiğit and Koca, 2011).

When essential nutrients such as water, oil, carbohydrates, protein, minerals and vitamins are added to the feed under normal growing conditions, it ensures that the nutrients in the feed reach the animals without deterioration and that the feed is digested more quickly and that it is absorbed from the intestines and transported to the body cells, and increases the amount and/or quality of the product, substances that increase feed efficiency, provide economic benefits and are generally referred to as "Feed Additives" are among the most discussed and researched subjects in recent years (Kutlu and Çelik, 2005).

In addition, until recently, it has become almost a necessity in the livestock industry, and antibiotics have been used in the poultry industry for many years to stimulate growth and promote development. Antibiotics; are chemical substances produced by microorganisms such as bacteria and fungi and protect their environment from the effects of harmful microorganisms. For many years, antibiotics have been used as feed additives to improve growth and feed efficiency as well as to treat infections. Animals with antibiotics added to their feed consume less feed than usual to reach the expected growth level (Chattopadhyay, 2014). Therefore, it significantly reduces the cost of feed, which constitutes a large part of animal breeding costs. Antibiotics added to the feed also enable more efficient conversion of feed into animal product.

It is observed that the daily growth rate of animals fed with antibiotic feed fortified foods increases by 1-10% compared to animals fed with feed-fortified foods without antibiotics (Hughes and Heritage, 2002). However, antibiotics used in feed prevent the growth of beneficial microorganisms along with pathogenic bacteria in the intestines. In addition, since resistance to these antibiotics will be gained over time, the effects of beneficial microorganisms will decrease. Moreover, it destroys the intestinal flora, delays its healing, and often causes pathogenic bacteria to become dominant in the intestinal mucosa (Rosen, 1995). Antibiotic residues, a common problem as a natural consequence of antibiotic use, also seriously threaten human health (Prescott et al., 1999). Due to these drawbacks, the use of antibiotics in feeds has been entirely banned by the European Union due to the concern that it may

cause bacterial resistance and threaten human health by leaving residues in the animal by products (Dafwang et al., 1984; Aydın and Koçak, 1999; Demirel and Gürbüz, 1999; Keser and Bilal, 2008; Adıyaman and Ayhan, 2010; Chattopadhyay, 2014; Ng and Koh, 2017).

With this ban, studies on the development of feed additives to protect the health of the digestive system ecology, control bacterial diseases and strengthen the immune system of animals gained momentum. Studies conducted in this direction show that enzymes can be used as feed additives (Alp and Kahraman, 1996; Buchanan et al., 1997; Cavazzoni et al., 1998; Erdogan, 1999; Nir and Şenköylü, 2000; Çiftçi, 2001; Sullivan and Nord, 2002 ; Karademir and Karademir, 2003; Drew et al., 2005; Kutlu and Serbester, 2014; Yıldırım et al., 2014).

Among them, various enzymes such as protease, glucanase, lichenase, cellulase, pectinase, amylase, phytase, and lipase are frequently used in the feed industry by adding to mixed feeds as single or enzyme complexes. With the use of enzymes, the digestibility of the feeds and the feed efficiency of the animals increase (Karademir and Karademir, 2003). In this context, studies on probiotics, prebiotics, organic acids, antimicrobial and antioxidant effective herbal extracts, enzymes and toxin binders are continuing (Kutlu and Serbester, 2014).

Advantages of Industrial Enzymes Used in Feeds

Enzymes are biomolecules produced by living cells in protein structure or partly protein. In addition, they are biological catalysts that serve in specific biochemical reactions and accelerate metabolic reactions.

Enzymes have been used in processes such as beverage, bread and cheese making without knowing their existence and function for thousands of years. Today, thousands of enzymes are consciously used for different purposes. Those used as feed additives are enzymes that fall under the hydrolase class.

Enzymes used as feed additives are of fungal and bacterial origin. Among them, various enzymes such as protease, glucanase, cellulase, pectinase, amylase, phytase and lipase are used alone or in combination in the feed industry by adding to mixed feeds (Yiğit and Koca, 2011).

Enzymes used in feeds break down starch, protein and fat and facilitate digestion. Thus, the degree of digestibility and feed efficiency of animals is increased (Karademir and Karademir, 2003). After it was understood that the adding of enzymes in the rations gave positive results, many studies were started on this subject (Deguara et al.,1999).

Although the serum immunoglobulin level of animals fed with glucan-containing feeds increases, they become more resistant to infectious diseases. However, the external addition of beta-glucan to fish feeds is costly and increases the cost of feed. Nowadays, with the introduction of modern production techniques, the enzyme industry has made significant progress in the last half-century. Parallel to this, enzyme biotechnology has made significant progress and enabled the production of industrially important enzymes in a more pure, cheap and abundant amount.

Although it is known that the enzymes used in the industrial field are of vegetable, animal and microorganism origin, they are mainly isolated from microorganisms. Because plant and animal enzymes can not meet the industrial needs. Therefore, the interest in this field has gradually led to microbial enzymes. This interest is because microbial enzymes do not form unwanted by products, have high catalytic activities, are more stable and cheaper, and are obtained in large sizes and in high purity (Wiseman, 1987; Horikoshi, 1999).

Today, more than 50% of industrial enzymes are produced from molds and yeasts and about 30% from bacteria. Enzymes of animal origin share 8% and enzymes of plant origin have a share of 4%. As can be seen, more than 90% of industrial enzymes originate from microorganisms, and this shows that microbial sources that are more suitable for genetic manipulation are preferred compared to animals and plants (Sutay Kocabaş, 2021).

Enzymes can be produced using wild-type or genetically modified microorganisms. The most commercially used microorganisms are *Aspergillus (A. niger), Saccharomyces (S. cerevisiae), Mucor, Serratia, Bacillus (B. subtilis, B. amyloliquefaciens,* and *B. licheniformis), Lactobacillus (L. casei, L. acidophilus,* and *L. delbrueckii), Corynebacterium (C. glutamicum) and Rhizopus (R. oryzae)* can be given as examples (Srivastava, 2019).

The use of microorganisms at such a high rate in enzyme production has made the use of modified microorganisms widespread to increase production. For this purpose, recombinant strains have been developed using genetic engineering techniques and research in biotechnology on industrial enzymes has gained more importance.

Chemical Structure of Glucan

Glucans are glucose polymers obtained from the cell walls of yeast, bacteria and fungi, and grain feeds such as oat, barley and rye. Differences in the way these glucose polymers bond with each other give each glucan its structural changes. Differences in molecular weight, degree of branching, compatibility and intermolecular combinations are the factors that can affect the biological activity of glucan (Keser and Bilal, 2008). There are four types of β-glucans categories based on the type of the glycoside bond they cleave, namely, (1) β-(1,4)-glucanase (EC 3.2.1.4, carboxymethylcellulase), (2) β-(1,3)-glucanase (EC 3.2.1.39, laminarinase), (3) β-1,3(4)-glucanase (EC 3.2.1.6), (4) β-(1,3-1,4)-glucanase (EC 3.2.1.6, lichenase) (Goldenkova-Pavlova et al., 2018) (Figure 1). All of these can reduce β-(1,3-1,4)-glucanases, but there are differences in substrate specificity.

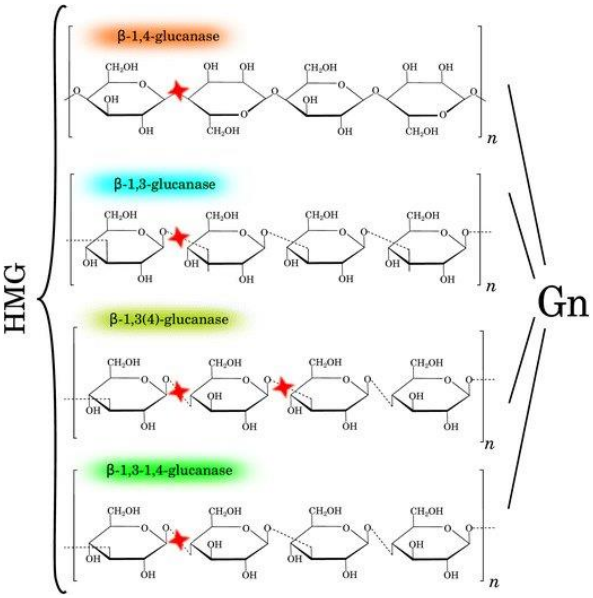

Figure 1. Structures of β-glucans with different type of the glycoside bond. The red tetragons are the cleavage sites of enzymes. *HMG*; high molecular weight β-Glucans, *Gn*; oligosaccharides with different degrees of polymerization (Goldenkova-Pavlova et al., 2018).

The glucans in the cell wall of yeast and fungi are composed of a few 1,6-linked branches and 1,3-linked glycopyranosyl residues. In contrast, oat and barley cell walls contain unbranched glucans containing 1,3- and 1,4-linked glycopyranosyl residues, while glucans of bacterial origin are composed of unbranched 1,3-linked glycopyranosyl residues (Keser and Bilal, 2008).

β-(1,3)-Glucanase (Laminarinase) Enzyme

β-(1,3)-Glucans are essential components of yeast, fungal cell wall and brown marine microalgae (*Laminaria* spp.). Bacterial β-(1,3)-glucanase belongs to the glycosyl hydrolase family 16 (GH 16). β-(1,3)-glucanase isolated from ATCC 21606 is classified in the glycosyl hydrolase 64 family (GH-64). β-(1,3)-glucanase isolated from DSM 10297 strain is classified in glycosyl hydrolase 16 family (GH-16). This situation significantly shows structural diversity expression (Saeki et al., 1994; Coutinho and Henrissat, 1999).

β-(1,3)-Glucans are potential and functional food ingredients because they are small soluble prebiotic substances. However, it is also produced as insoluble by some exopolysaccharide bacteria such as Alcaligenes, Faecalis, and Myxogenes.

Bacterial β-(1,3)-glucanases can be classified as exo- or endo-acting glucanases depending on where they cleave the substrate. Endo-β-(1,3)-glucanases play a major role in yeast cell lysis (Ferrer, 2006). The complex structure of the yeast cell wall consists of β-(1,3)-glucans, β-(1,6)-glucans, mannoproteins and chitins. These complex polymers are hydrolyzed as substrates of related enzymes.

Considering their physical properties the molecular masses of endo-β-(1,3)-glucanases are primarily between 30 and 50 kDa, although they range from 12 to 230 kDa (Usoltseva et al., 2020). Although the smallest molecular masses are between 12-16 kDa, it has a molecular weight of 12 kDa in *Citrus*

aurantiifolia from plants, 16 kDa in *Rhizoctonia solani* from pathogenic fungi, and 16 kDa in *Flavobacterium* sp. from bacteria (Manners and Wilson, 1973; Carrasco et al., 1983; Usui et al., 1985). The largest molecular masses are 230 kDa in fungi *Aspergillus nidulans*, 190-218 kDa in yeast *Saccharomyces cerevisiae*, and 160 kDa in *Schizosaccharomyces pombe* (Reichelt et al., 1981; Hien et al., 1983; Szilagyi et al., 2010).

When the optimum temperature values of β-(1,3)-glucans are examined, the lowest optimum temperature values are 24-40°C in fungi *Aspergillus fumigates*, 30°C in *Alternaria tenuissima*, 30°C in animals *Tenebrio molitor* and *Mizuhopecten yessoensis*. It has been observed to have a temperature of 30-45°C (Jirku et al., 1980; Likhatskaya et al., 2006; Genta et al., 2009; Hartl et al., 2011). The highest optimum temperature values were 95°C in bacteria *T. maritima* and 100-105°C in *Pyrococcus furiosus* from archaea (Gueguen et al., 1997). Therefore, according to these values, the adaptation potential of bacterial enzymes to high temperatures is much greater than the potential of eukaryotic enzymes. The potential of archaeal enzymes is even greater than that of bacteria. At the same time, endo-β-(1,3)-glucanase molecules of animal origin may show greater flexibility for adaptation to cold water conditions.

When the optimum pH values of β-(1,3)-glucans are examined, it has been observed that they show slightly acidic properties such as the PH value of 5.1. Typical optimum pH values range from 5.5-6.0 for bacterial glucanases, 3.8-6.0 for fungi, and 4.5-6.0 for plants. Considering the lowest optimum pH values, it can be given as 4.0 in *Delftia tsuruhatensis* from bacteria, 4.0-5.5 in *Euglena gracilis* from microalgae, 4.0 in *Lentinula edodes* from fungi (Sakamoto et al., 2011; Takeda et al., 2015). Considering the highest optimum pH values, 9.0-10.0 in *Bacillus* sp., 8.5 in *Zobellia galactanivorans* and 6.0-7.0 in *Thermotoga maritima* can be given as examples (Nogi and Horikoshi, 1990; Liu et al., 2012; Labourel et al., 2014). Optimum pH of β-(1,3)-glucanase forms isolated from *Cellulosimicrobium cellulans* varies according to the substrate, but is around 5.5-8.0. It was observed that the optimum working pH of the BglIIA enzyme of *O. xanthineolytica* LL G109 strain was around 4.0 on average (Ferrer et al., 1996).

The variable pH values of bacteria have brought to mind the idea that more mutations can be made, new properties can be given to proteins without decreasing cell viability and new products can be obtained (Usoltseva et al., 2020).

β-(1.3-1,4)-Glucanase (Lichenase) Enzyme

Lichenases are found in the endosperm cell walls of commercially important grains such as oats, sorghum, wheat, barley, rice and rye. Lichenase enzymes are used in the food and beverage industry, animal feed production, medical and pharmaceutical industries, and genetic engineering (Bhat and Bhat, 1997).

The first fungal gene encoding lichenase was cloned from the anaerobic fungus *Orpinomyces* in 1997 (Chen et al., 1997). Lichenases are found in the cell walls of plants of the higher Poaceae family. In

general, they are linear polysaccharides containing both β-(1,3) and β-(1,4) linked D-glucose, formed by the attachment of β-(1,3) side chains to the β-(1,4) main chain.

In addition to plants, many microorganisms such as bacteria and fungi also contain significant levels of β-(1,3-1,4)-glucanase. Plants also commonly contain β-(1,3-1,4)-glucanase. However, plant and microbial origin enzymes differ from each other in terms of some features such as amino acid sequences and three-dimensional structures. For example, plant enzymes belong to the glycosyl hydrolase 17 family, while microbial enzymes belong to the glycosyl hydrolase 16 family (Henrissat, 1991; Henrissat and Bairoch, 1993, Henrissat and Bairoch, 1996).

Considering their physical properties, the molecular weight of *Bacillus* lichenases is generally between 25-30 kDa. However, it was found to be 90 kDa in *R. flavefaciens*, 40.7 kDa in *Talaromyces emersonii*, 38 kDa in *C. thermocellum*, and 37 kDa in *F. succinogenes*. Optimum pH values are 9.0 in *B. brevis*, 6.5 in *S. bovis*, 4.8 in *Talaromyces emersoni*, 6.0 in *Orpinomyces*, 6.6-10.0 in *C. thermocellum* and N137 (80%) in alkalophilic *Bacillus* sp. more than pH 7.0-12.0 is at neutral (pH 6.0-7.5) level, except for enzymes. Optimum temperature values of enzymes also differ between bacteria, 80°C in *C. thermocellum*, 50°C in *F. succinogenes* and *S. bovis*, 55°C in *B. subtilis, B. amyloliquefaciens, B. licheniformis* bacteria, and *B. polymyxa* 45°C, *B. macerans, B. brevis, Bacillus* sp. It is 65°C in N137 origins (Erfle et al., 1988; Schimming et al., 1991; Flint et al., 1993; Chen et al., 1997; Planas, 2000).

Lichenases obtained from recombinant yeasts increase the aroma in wines and hydrolysis of β-(1,3) and β-(1,4)-glucans found in low-quality barley. Lichenases are increasingly coming to the fore in the preparation of animal feeds because they hydrolyze β-glucans at a higher level than other glucanases (Beckmann et al., 2006). Thermostable endo-β-(1,3-1,4)-glucanases are frequently used in the brewery industry to reduce fluidity during the mashing phase, as they are not inactive with temperature during malting and have a high molecular weight (Godfrey, 1983; Stone and Clarke, 1992).

Effect of Glucans on Immunity

Due to their high biological effect, glucans have great importance in areas such as tissue engineering, biomedical, pharmaceutical, apoptosis, anticancer, antitumor, anticoagulant, antioxidant, antibacterial, anti-inflammatory, oxidative stress and immunostimulator (Miao et al., 1995; Kovalchuk et al., 2005; Kim et al., 2006; Kadam et al., 2015; Zargarzadeh et al., 2020; Park et al., 2020).

Antibiotics are often added to the feed of fish and farm animals to control infectious diseases and improve survival and growth. As it is known, excessive use of antibiotics leads to accumulation in the tissues of animals. In recent years, the primary use of glucans to prevent this accumulation, stimulate animals growth, and control pathogens has been on the immune system (Dalmo et al., 1998; Barman et al., 2013; Zhang et al., 2019).

In some studies investigating the effect of β-glucan against deadly pathogen infection in animals, it has been observed that it reduces the death rate by suppressing the infection. For example, it increases the

non-specific immune response and resistance against bacterial infection in Atlantic salmon (Robertsen et al., 1990), and the addition of β-glucan to the diet in sea bass (*Dicentrarchus labrax*) increases plasma complement activity (Bagni et al., 2000), and plentiful yellow croaker. They reported that adding low (0.09%) and high (0.18%) levels of β-(1,3)-glucan to the diet of *Pseudosciaena crocea*) fish increased phagocytic activity in kidney macrophages at low concentration (Ai et al., 2007).

In another study, when the effects of β-glucan addition to the diet of freshwater fish fathead minnow (*Pimephales promelas*) on neutrophil functions in non-stress, acute stress and chronic stress conditions were examined, glucan increased neutrophil degranulation in unstressed fish, while it prevented the decrease in degranulation in fish under acute stress. In fish under chronic stress, the degranulation level reached the level of unstressed fish 3 days after the addition of glucan. As a result of this study, researchers reported that the addition of β-glucan to the diet has the potential to increase the disease resistance and survival rate after transport or exposure to low-quality water by increasing neutrophil functions in fish in acute and chronic stress situations (Palic et al., 2006). In addition, in a study with *Laminaria digitata*, brown algae, they reported that it suppressed human colon cancer cells by 38.8% (Ji et al., 2012).

The increase in growth performance in fish largely depends on the amount of glucan used in the diet, feeding time, ambient temperature and the species studied. It has been reported that feeding strategies should be developed for each fish species concerning the dose and application time of glucan to increase growth performance (Dalmo and Bogwald, 2008).

The Importance of *Bacillus* Bacteria in Gene Cloning

The habitat of bacteria of the genus *Bacillus* is soil or ecosystems indirectly related to the soil. It has also been isolated from plants and animals directly associated with the relevant habitat.

B. subtilis is a motile, rod-shaped bacterium, the majority of which is non-pathogenic and is found almost everywhere in nature. This bacterium is aerobic and gram-positive; its spores are oval and subterminal, it is encapsulated and can grow quickly and easily in the medium without pre-enrichment. *B. subtilis* bacterium divides symmetrically. Since these bacteria are thermophilic, they are not affected by temperature. When exposed to adverse environmental factors such as salt, temperature and acidity, it maintains its vitality by forming spores (Fritze and Pukall, 2001).

Most of the microbial enzymes used in industrial areas are produced by bacteria belonging to the genus *Bacillus*. Since they are the dominant bacteria in microbial fermentation, approximately 75% of the 75 thousand tons of enzymes produced every year in the world are produced by *B. subtilis* bacteria. Most of the enzymes produced by *Bacillus* are used in starch, textile, fruit juice, brewing, detergent and feed industries.

The reasons why *B. subtilis* bacteria are so preferred in industrial areas are that they can easily grow in media without pre-enrichment in antibiotic, toxin and enzyme production studies, their fermentation period is short, they can be easily cultured, their isolation and diagnosis are easy, and there is no

heterogeneity in their structure (Priest, 1977). In addition, they are attractive industrial organisms for many reasons such as being safe (GRAS; Generally Recognized As Safe), having a low degree of pathogenicity in humans, being able to produce various industrial enzymes, not having an outer membrane, and having a high capacity to secrete enzymes produced inside the cell out of the cell.

Bacillus bacteria produce and secrete a large number and variety of hydrolytic enzymes that are active against various complex substrates. Therefore, organisms in the genus *Bacillus* are widely used in the industrial production of enzymes such as α-amylase, β-amylase, protease, β-glucanase, glucose isomerase and endonuclease (Uhlig, 1998).

Microorganisms such as *Bacillus subtilis, Aspergillus niger* and *Aspergillus oryzae* are the most widely used enzyme sources in enzyme production (Glazer and Nikaido, 1995). Many *Bacillus* species produce glucanase enzyme from these enzyme sources. Therefore, the glucanase enzyme genes were cloned and characterized *in B. subtilis, B. lehensis, B. amyloliquefaciens, B. circulans, B. polymyxa, B. licheniformis, B. brevis* bacteria (Cantwell and McConnell, 1983; Hinchliffe, 1984; Hofemeister, 1986; Murphy et al., 1984; Borriss et al., 1988; Bueno et al., 1990a and 1990b; Gosalbes et al., 1991; Lloberas et al., 1991; Louw et al., 1993; Tabernero et al., 1994; Jaafar et al., 2020).

It has been determined that the use of *Bacillus* strains as feed additives in aquaculture causes an increase in feed efficiency, live weight gain, survival rate and growth performance, and positive effects on the immune system. (El-Haroun et al., 2006; Kumar et al., 2006; Bagheri et al., 2008; Merrifield et al., 2010).

Kumar et al. (2006) fed Indian major carp, *Labeo rohita*, with feed containing *B. subtilis* and reported that the proportional weight gain was higher (35.55%) compared to the experimental groups. Bagheri et al. (2008) added different levels (4,8x108 kob/g, 1,2x109 kob/g, 2,01x109 CFU/g, 3,8x109 CFU/g, 6,1x109 CFU/g) of *Bacillus* spp (*B. subtilis* and *B. licheniformis*) to the feed of rainbow trout (*O. mykiss*) fry. They noted that *Bacillus* strains had a positive effect on growth and survival rate at the end of the feeding program.

In another study with rainbow trout (*O. mykiss*), *B. subtilis*, *B. licheniformis* and *E. faecium* bacteria were used as feed additives as single or multiple feed additives and a 10-week feeding schedule was applied. They reported that there was a significant increase in the weight gain of the fish at the end of the experiment (Merrifield et al., 2010).

El-Haroun et al., (2006) investigated the effect of commercial probiotics on growth performance and feed efficiency by adding 4 different rates (0.5%, 1.0%, 1.5% and 2.0%) of probiotic Biogen® (consists of *Bacillus licheniformis* and *Bacillus subtilis*) to the feed of nil tilapia (*O. niloticus*) fingerlings. They stated that feeds with added probiotics provided higher growth performance and feed efficiency than feeds without probiotics at the end of the feeding period.

Conclusion

The development of recombinant *Bacillus* strains protects the health of the digestive system ecology, strengthens the immune system of the animals, and improves the quality of the feed material and facilitates its digestion.

For this purpose, recombinant strains created using biotechnological methods and genetic engineering techniques can be used in molecular studies in the future. It can be used as an alternative probiotic feed additive in the rations of aquatic products, experimental animals and other farm animals. Feeds will be produced more abundantly, economically and sustainably. By using these enzymes, which can be produced commercially as feed additives, animals will be able to benefit from the feed at the maximum rate.

Statement of Conflict of Interest

The authors of the article declare that there is no conflict of interest.

Author's Contributions

The authors declare that they have contributed to the article at a similar rate.

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