Chapter 9

Probiotics, an Alternative Measure to Chemotherapy in Fish Production

Olumuyiwa Ayodeji Akanmu

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.72923

Abstract

The use of chemotherapy in treating and enhancing the growth of fish has been widely criticized due to its negative environmental consequence. Hence, the use of probiotics which are bio-friendly seems to be a promising alternative. Therefore, the importance of probiotics in fish production was critically reviewed in line with their growth rate, disease treatment, and immune boosting. It was, however, realized that probiotics such as *Lactobacillus fermentum* and *Saccharomyces cerevisiae* cultured from maize slurry and palm wine, respectively, could serve as good probiotics, which could enhance faster growth rate and wound-healing rate. Probiotics are, therefore, recommended to the fish farmers so as to increase the profitability of the aquaculture business.

Keywords: *Lactobacillus fermentum*, *Saccharomyces cerevisiae*, probiotics

1. Introduction

Aquaculture is an ancient occupation of man in which its fast growth due to rapid development has given birth to modern equipment and technology leading to its intensification and commercialization. This development has placed disease problems on the threatening side, making it to be a major constraint to the culture of many aquatic species and consequential impediment on economic and social development in many countries [1]. Owing to the artificial conditions posed by intensive rearing, farmed fish is more susceptible to disease agents than fish in natural aquatic environments [2]. Fish diseases constitute the major limiting factor in aquaculture production since the disease causative agents thrive well in water. Various types of bacterial diseases in fish have been encountered in fresh water fishes across the globe [3]. Jakhar et al. [4] reported that bacterial pathogens cause heavy mortality in both cultured and wild fish/shell species over the world.

However, prevention and control of diseases have led to a substantial increase in the use of broad-spectrum chemotherapeutics, which has been reported to cause development of resistant bacteria, reduction in yield, and introduction of potential hazard to public health, the environment, and killing of the microbial flora in the digestive tracts, which is beneficial to the fish [5]. The development of resistant bacterial genes as a result of exposure to antimicrobial agents has not only made the drugs applied useless, but has also made the animals treated with it not safe for human consumption; therefore, it turned the treatment exercise to a wasteful process, which eventually makes this to be a major disadvantage of using synthetic antibiotics in aquaculture [6]. The success of modern aquaculture among others hinges on the use of biological control agents for diseases, and this depends on the fact of microbial antagonism [3] and triggering immune response to disease challenge.

Generally, the immune system of aquatic organisms is affected by periodic and unexpected changes in their environment. Adverse environmental situations may acutely or chronically, stress the fish, altering some of their biochemical parameters and suppressing their innate and adaptive immune responses [7]. This triggers nonspecific defense mechanisms, which plays important role at all stages of infection. Fish, particularly, depends more heavily on these nonspecific defense mechanisms than mammals. Therefore, there has been an increasing interest in boosting the nonspecific immune system of fish for the treatment and prophylactic measure against disease in the last decade using biological and eco-friendly approach [8].

Probiotics are microbial dietary adjuvant that beneficially affect the host physiology by modulating mucosal and systemic immunity, as well as improve nutritional and microbial balance in the intestinal tract [9]. These biological agents have been utilized for disease control, supplements to improve growth, and in some cases as a means of replacing antimicrobial compounds in aquaculture. Probiotics have proven to inhibit the growth of pathogens through production of antagonistic compounds, competition for attachment sites, nutrients, and alterations of enzymatic activity of pathogens, immune-stimulatory functions, and nutritional benefits such as improvement in digestibility and utilization in feed [10]. Hence, the concept of utilizing probiotics in animal feed, particularly, poultry and fish, is fast gaining acceptance [11]. The objective of prevention and control of disease can be achieved by the use of probiotics. Probiotics are characterized by their ability to adhere and colonize the gastro intestinal tract (GIT) of the hosts and able to replicate to high numbers. These organisms must be able to produce antimicrobial substances and withstand the acidic environment of the GIT of the host animals. Probiotics are known to play an important role in developing innate immunity among the fishes; therefore, help them to fight against any pathogenic bacteria as well as against environmental stressors [11].

Probiotics can be introduced into culture environment to control and compete with pathogenic bacteria as well as to promote the growth of the cultured organisms. The use of probiotics will prove a new eco-friendly alternative measure for sustainable aquaculture. A wide range of gram positive bacteria have been evaluated as probiotics. This includes *Aspergillus oryzae, Lactobacillus, Bacillus, Micrococcus, Carnobacterium, Enterococcus, Streptococcus, and Saccharomyces species*. The products of probiotics could be administered through water or incorporated in feed, either singly or in combination [11]. Administration of the probiotics proved harmless to the host as well as human being; it also results in improved resistance to infectious diseases in the hosts. However, the dimensions of the effects of probiotics have to be assessed for different fish species. Probiotics could be prepared in different types which

http://dx.doi.org/10.5772/intechopen.72923

include: nonviable, which are dried probiotics; freeze-dried, which are probiotics that thrive well at freezing point; fermentation probiotics, which are produced through fermentation; and viable probiotics, which are living probiotics with guaranteed shelf life [12]. Probiotics have been demonstrated to have potentials for enhancing fish immunity [13], growth [14], wound healing [15], and are eco-friendly [16]. A successful probiotic is expected to be antagonistic to pathogens, by producing antimicrobial substances, which are harmful to the pathogens. In addition, the probiotics should have the capacity to colonize the fish by adhesion and produce important substances like vitamins, which has beneficial effect on the host, in the form of growth promotion or protecting the fish against bacterial pathogens [17].

Probiotics such as *Lactobacillus, Saccharomyces species* and their combinations have been found useful in aquaculture production [15, 18]. The administration of diets fortified with these probiotics have improved growth in *Oreochromis niloticus* [14], increased immunity in *Cyprinus carpio* [19], improved wound healing in *Clarias gariepinus* [20], and in *Heterobranchus bidorsalis* [15]. It would be of interest to understand the applicability of this bio-technique in advancing the fate of aquaculture and food security. Maintenance of hygiene and especially, chemotherapeutics are widely used as interventions on control of diseases of aquatic animals. However, intensive use of chemicals had contributed to the development of resistant strains of pathogens. Hence, there is the need for natural preventives for improving resistance in fisheries and aquaculture. Meanwhile, a large percentage of culture systems still depend mostly on the use of chemotherapeutic agents in treating and controlling the widespread of these diseases. The abuse of chemotherapeutics in fish farming has led to development of drug-resistant bacteria and multiple antibiotic-resistant in the aquaculture industry [21]. This approach has sometimes resulted in the spread of epizootic diseases and severe economic losses. Moreover, chemotherapy may kill or inhibit the normal micro flora in the digestive tract, which is beneficial to fish [22]. Therefore, there is an urgent need to develop alternative approach to the indiscriminate use of antibiotics in fish production.

The principal objectives of the food security through aquaculture can only be achieved in the face of increase in growth and survival, feed efficiency, and disease resistance of culturable fish species, which reflects positively on production costs. The use of probiotics, which control pathogens through a variety of mechanisms that targets these attributes are viewed as an alternative to the use of antimicrobial agents [23] but the potentials of this technique have to be tested in many indigenous culturable fish species. With increasing demand for eco-friendly aquaculture, the use of probiotics in aquaculture is now widely accepted [24]. Positive effects of applying certain beneficial bacteria in aquaculture have also been well documented [25].

2. Standards considered in selecting microorganisms as probiotics

For a microorganism to be considered as a good probiotics candidate, it should be able to exhibit these properties: antagonistic properties through the production of antimicrobial materials such as hydrogen peroxide [26] or siderophores [27]. They should be able to colonize other microorganisms in the fish organ through adhesion [17]. The microorganisms are expected to be viable for long period of time under storage [28]. Adhesion is one of the most important criteria for probiotic bacteria because it is considered a pre-requisite for colonization [29]. Probiotic microorganisms will of course have to be nonpathogenic and nontoxic in order to avoid undesirable side effects when administered to fish. Tests of antagonisms, which include studies of adhesion and in-vitro challenged tests, challenged experiments in which fish treated with friendly bacteria are subjected to pathogens in order to evaluate the efficacy of the probiotics by using survival rate as an indicator are important considerable factors in selecting probiotics [30]. The interest of the probiotic use is centered on terrestrial organisms and the term probiotic inevitably is referred to gram positive bacteria associated with the genus *Lactobacillus species.* Panigrahi et al. [28] submission, however, requires some considerations to humans and terrestrial animals. It could be assumed in aquaculture that the intestinal microbiota does not exist as an entity by itself but there is a constant interaction with the environment and the host functions [31]. The bacteria in the aquatic medium could either be ingested with the feed or when the host drinks water. Terrestrial animals (mammals) inherit an important part of the initially colonizing bacteria through contact with the mother, while aquatic species usually spawn eggs in water, without further contact with their parents. This allows the ambient bacteria to colonize intestinal tract, gills, or skin of newly born animals/larvae, which have not fully developed.

3. Test for pathogenicity of the selected strains

Microorganisms considered as probiotic candidates should be scrutinized for pathogenicity on the host animals by challenging the target animals with the probiotic microorganisms. The challenged organisms could be administered to the target species through injection, immersion, or addition into the feed. The test of pathogenicity could either be carried out in-vitro or in-vivo.

a. **In-vitro antagonism tests:** Common way to screen the candidate probiotics is to perform in-vitro antagonism tests in which the pathogens are exposed to the candidate probiotics or their extracellular products in liquids [32] or solid medium. Depending on the extract arrangement of the tests, candidate probiotics can be selected based on the competition for nutrients [32]. The pre-selection of probiotics candidate based on these in-vitro antagonism tests has often led to the finding of effective probiotics [26].

b. **In-vivo antagonism tests:** Pathogenicity effects of microorganisms considered as probiotics could also be tested in-vivo to determine the safety level of the tested probiotic candidate.

4. The probiotics characteristics of Lactic acid bacteria and Yeast

Lactic acid bacteria are potential probiotic candidates in aquaculture and are also known to be a normal inhabitant in the intestine of healthy fish [14]. Most lactic acid bacteria are harmless, while some strains have been reported to have beneficial effects on fish health and are antagonistic to pathogens [32]. Strains of lactic acid bacteria are the most common microbes employed as probiotics. Most probiotic strains belong to the genus *Lactobacillus*. *Lactobacillus species* have

http://dx.doi.org/10.5772/intechopen.72923

the ability to degrade organic materials, reduce ammonia, and inhibit the growth of pathogens by outcompeting them [33]. Lactic acid bacteria are a heterogeneous group of bacteria that are generally considered safe for use in food and food products. Lactic acid bacteria have been used for lactic acid fermentation of sorghum-or maize-based cereals used as infant weaning foods, for example, Pap (*ogi*) prepared from maize slurry [34]. Lactic acid bacteria are spherical, cocci, coccobacilli, or rods and divide in one plane only with the exception of *Pediococcus species* [35]. Lactic acid bacteria have no strict taxonomic significance although they have been shown by serological techniques and 16S ribosomal RNA cataloging to be phylogenetically related. They share a number of common features as earlier stated. Most of these organisms are aero-tolerant anaerobes, which lack cytochromes and porphyrins. The lack of these two components in their systems explains why they are negative to catalase and oxidase tests [36]. The antibacterial effect of lactic acid bacteria (LAB) is therefore ascribed to its tendency to produce antibiotics-like substances (bacteriocins) such as *Acidophilin, Lactolin,* and *Lactocidin*.

Saccharomyces cerevisiae is budding yeast species commonly used in baking and brewing, owing to its fermenting property or ability. The cells are ovoid in shape, 5–10 μm in diameter. It reproduces by a division process known as budding. All strains of *S. cerevisiae* can grow aerobically on glucose, maltose, and trehalose but cannot grow on lactose and cellobiose. It is a single-celled organism, which can easily be cultured with short generation time of about 1.5–2 hours doubling time at a temperature of 30°C. It contains various immune-stimulating compounds such as β-glucans, nucleic acid, and mannan oligosaccharides, which have been reported to enhance immune response and growth of various fish species [37]. Mesalhy et al. [38] recorded higher growth rate in the study carried out using probiotic-supplemented diets on *Oreochromis niloticus* than those kept on basal diet. It was concluded that addition of *Bacillus subtilis* and *S. cerevisiae* enhanced the growth performance, feed utilization, and mitigated the effects of population density, which is the main growth-inhibiting factor in intensive aquaculture systems. The best food conversion rate (FCR) values were recorded in probiotic-supplemented diets, and it was concluded that the probiotic used improved feed utilization, which practically showed that the probiotic used can reduce the amount of feed necessary for animal growth and thus, reduce the cost of production [37].

Saccharomyces, Clostridium, Bacillus, Enterococcus, Lactobacillus, Shewanella, Leuconostoc, Lactococcus, Carnobacterium, and Aeromonas species are the commonly used probiotics in fish culture practices [11]. These probiotics have been reported to produce beneficial results to the host organisms. *Bacillus* species increased survival and production of channel catfish (*Ictalurus punctatus*), improved growth and immunity of Nile tilapia (*Oreochromis niloticus*) was achieved through feeding of diet containing *Bacillus subtilis* and *Rhodopseudomonas*, and rainbow trout (*Oncorhynchus mykiss*) was protected against *Vibrio anguillarum* by *Pseudomonas fluorescens* [9]. Generally, probiotics have demonstrated the ability to increase fish growth by enhancing the feed conversion efficiency, as well as confer protection against harmful bacteria by competitive exclusion, production of organic acids (formic acid, acetic acid, and lactic acid), hydrogen peroxide, and several other compounds [37]. They can also effectively trigger the fish immune system [37].

Abdul El-Halim et al. [39] discovered that the addition of living yeast in diet improved the performance of *Oreochromis niloticus*. Scholz et al. [40] also reported improved growth and survival of sea bass fry with *S. cerevisiae* and attributed this to adherence ability of *S. cerevisiae* cells to the gut and secretion of amylase enzymes, which increased digestibility of the diet. The probiotics used by Marzouk et al. [41] enhanced the growth performance of *Oreochromis niloticus* and suppressed the activity of the pathogenic bacteria in the intestine of the tested fish. The disease outbreak was reportedly prevented in fish with the use of *S. cerevisiae* and *Bacillus subtilis* as probiotics. This could have been possible with the ability of these microorganisms to attach and colonize the intestinal walls of the host animals, which eventually prevent other bacterial from getting access to the intestinal walls [41]. Li et al. [42] described positively influenced growth performance of brewer's yeast (*S. cerevisiae*) and feed efficiency of hybrid striped bass (*Morone chrysops xm saxatilis)* and resistance to *Streptococcus iniae* infection. In addition, results of immune response assays illustrated that brewer's yeast can be administered for relatively long periods without causing immune-suppression.

Lactobacillus fermentum and S. cerevisiae have also been reported to improve the growth performance and health status of fish species, *Oreochromis niloticus* [37] and *Mystus montanus* [3]. Various studies have been carried out using some bacteria strains as probiotics on fish species such as *Clarias gariepinus* and *Tilapia species,* but there is little or no information on the use of bacteria strains and yeast species as probiotics on indigenous species such as *H. bidorsalis.* Furthermore, information on the immune response of these probiotics on this fish species is not available [43]. *Lactobacillus fermentum* is a common bacteria strain, which has been used on different fish species. The wide occurrence and high antagonistic effects to the pathogens of the *L. fermentum and the S. cerevisiae* made them a good potential in testing for their probiotic ability and immune response. A beneficial effect by application of certain beneficial bacteria in human, pig, cattle, and poultry nutrition has been well documented by Jong [44]. However, the use of such probiotics in aquaculture is a relatively new concept [45]. Zhou et al. [17] reported the use of beneficial bacteria (probiotics) to displace pathogens by competitive processes being used in animal industry as a better remedy than administering antibiotics. This phenomenon is now gaining acceptance for the control of pathogens in aquaculture.

5. Importance of probiotics in aquaculture

Probiotics have been found beneficial in various ways such as:

- Providing additional nutrients thereby reducing feed costs.
- Maintaining desired conditions within the culture environment.
- Eliminating the stressors like NH_3, NO_2, and NO_3.
- Stabilizing and controlling the microbial populations.
- Maintaining stable water quality parameters.
- Preventing bacterial and viral infections.
- Improving feed and make it to be more attractive.
- Supporting growth through production of vitamins, minerals, nucleic acids, and by stimulation of beneficial gut flora.

- Improving feed conversion rate and survival rate of aquatic species.
- Reducing the use of chemotherapy.

The benefits listed above substantiated [46] who anticipated that bacteria would be found useful both as food and as biological control agents of fish diseases and activators of the rate of nutrient regeneration in aquaculture. Zong-fu et al. [12] stated that potential probiotic microorganisms must be able to colonize the fish intestinal mucosa and produce materials, which are eco-friendly to the host but antagonistic to pathogens. Furthermore, optimal diet utilization by the host animal has been ensured with the use of probiotics, which stimulate the multiplication of gut micro flora in the host fish. It should be noted that an application of probiotics into the water and ponds may also have a positive effect on fish health by improving the water quality, since they modify the bacteria composition of the water and sediments.

5.1. Application of probiotics as biological control agents in aquaculture

Probiotics have been applied in various aspects of aquaculture with promising results, especially in shrimp production [47]. *Luminous vibrio* has also been reported to be completely eliminated from the water column and from the sediment of ponds when probiotic strains selected for their inhibitory effect were used [48]. Hence, disease problems could be overcome by applying probiotic biotechnology, which is an application of microbial ecology [47]. Probiotics are expected to have a direct involvement in nutrients or vitamins [26]. They also enhance the growth of fish [49]. Lack of data on the efficacy of probiotics in commercial aquaculture is still affecting the sustained use of probiotics [50]. Most studies on the effects of probiotics on cultured aquaculture animals have emphasized a reduction in mortality or improved resistance against putative pathogens [51]. Probiotic can be added to the host or its ambient environment in several ways such as:

- Addition to artificial diet
- Addition to culture water
- Bathing
- Addition via live food

Probiotics could be provided to animals in different ways depending on the aim and objective of the study. However, the best method of administration is continuous feeding. This would ensure that the probiotics is present in the gut in a large number and able to metabolize and produce its probiotics effects.

5.2. Probiotic use in fish eggs, larvae, juvenile, and adult fish

The need to control the micro biota in hatching incubators through the alternative means in reducing the use of antibiotics needs to be adequately emphasized. Fish larvae may ingest substantial amount of bacteria by grazing on suspended particles and egg debris [52]. Ringo and Gatesoupe [26] added lactic acid bacteria (LAB) to larvae of some fish species and a significant reduction of larval mortality was recorded when the larvae were challenged with pathogenic microorganisms (*Vibrio*). Ref. [32] fed lactic acid bacteria to *Atlantic Cod* fry to look at the effect of lactic acid bacteria on the growth and survival rate of Atlantic Cod fry. The experimental fish were given short term bathing in a bacteria suspension of probiotic [27]. Long term exposure in the rearing water led to the reduction in mortality of fish [9]. Ref. [23] selected several strains with a positive effect on the survival and growth of artemia juvenile.

5.3. Improving the immune response of the fish larvae

The level of immune response exhibits by the host animals greatly depend on the immune stimulants such animal is able to produce. Immune stimulants are produced to resist or combat any foreign body or objects intended to infect such animal. The immune systems of fish larvae are less developed; therefore, depend on nonspecific immune response to fight against infection [21]. Observations obtained in experiments with warm-blooded animals indicate that probiotic (lactic acid bacteria) administered orally increased resistance to enteric infections [49]. There are many reports that bacterial compounds act as immune stimulant in fish; however, it is not clear whether bacteria administered as probiotic could have a beneficial effect on the immune response of cultured aquatic species [8]. The role of lactic acid bacteria (LAB) within the digestive tract of endothermic animals and humans has been extensively studied [30]. Few authors have tested in-vivo, the protection conferred by probiotics in fish experimentally infected with pathogens. Bernet et al. [53] found that *Lactobacillus* strains isolated from rotifers increased the resistance of Turbot larvae against a pathogenic *Vibrio* species. Gildberg at al. [32] demonstrated that *Carnobacterium* divergens decreased the mortality rate of Atlantic Cod fry challenged with *Vibrio anguillarum.* Douillet and Langdon [54] also reported that *Carnobacterium* administered to fry and fingerlings of Atlantic salmon reduced the mortality caused by *Aeromonas salmonicida, Vibrio ordalli, and Yersinia ruckeri.* The role of lactic acid bacteria as immune-modulators improves non-specific defenses and is well-known for mammals [31]. Villamil [55] stated that this role has to be determined for fish. Most studies with probiotics conducted to date with fish have been undertaken with strains isolated and selected from aquatic environment and cultured animals.

5.4. Improvement of water quality

Water quality has been recorded to be improved at the addition of probiotics especially, *Bacillus species*. The rationale behind this is that gram positive *bacillus species* are generally efficient in converting organic matters back to CO_2 than gram negative bacteria [8]. Probiotics has also found its usage in water purification, especially with the culture of nitrifying bacteria in bio filters. Nitrifiers are responsible for the oxidation of ammonia to nitrite and subsequently to nitrate. The nitrifying cultures could be added to the ponds or tanks when an incidental increase of ammonia or nitrite levels is observed. Besides ammonia, nitrite toxicity is a common problem in fish culture especially in stagnant pond and re-circulatory system [56].

5.5. Improvement in fish growth

Inclusion of probiotics in the diets of fish species such as hybrid striped bass (*Morone chrysops xm saxatilis*), *Oreochromis niloticus*, catfish, and carp could improve the growth performance, body length, weight gain, and feed conversion ratio (FCR) of fish species [57]. Probiotics could also improve the body composition of fish fed with it. The addition of probiotics in the fish diets was reported to reduce the mortality rate. Gatesoupe [30] showed that turbot (*Scophthalmus maximus*) larvae fed with rotifers enriched with lactic acid bacteria had improved resistance against pathogenic vibrio infection, while noninfected fish showed slight increase in mortality when the level of lactic acid bacteria in the feed was too high.

5.6. Improve the hematology of fish

The hematological parameters of fish have been reported to be improved with the addition of probiotic bacterial into the diets of the experimental fish. For instance, the red blood cell counts (RBC) and white blood cell (WBC) of experimental fish were reported improved after being fed with probiotic bacterial [58]. Probiotics actively stimulate the proliferation of lymphocytes (both B and T cells) and further immunoglobulin production in fish [59]. Application of hematological techniques is, therefore, valuable in fish biology for the assessment of fish health and stress response. In the hemoglobin, oxygen is bound and released easily by iron (Fe) action contained in the hemoglobin molecule as blood transverse the pulmonary capillaries. Red blood cells (RBC), mean corpuscular hemoglobin (MCH), and hematocrit (HCT) have been reported by Adeyemo et al. [60] to indicate secondary responses of an organism to irritants. O'Neal and Weirich [61] describe decrease in erythrocytes to be the major and reliable indicators of various sources of stress in fish. Decrease in white blood cells (WBC) indicates vulnerability to stress and infection [58]. Decrease in red blood cells (RBC) indicates reduction in level of oxygen (O_2), which is being carried to the tissue and carbon dioxide (CO_2) that is returned to the lungs. It also indicates malnutrition in animal. Decrease in mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (McHc) indicates anemia [58]. Differential counts of neutrophils and monocytes give the level of protection against bacterial invasion, while lymphocytes determine the level of immunity. High platelet values show that the fish is likely to withstand and get healed quickly from bruises or wounds, which could be acquired from fight or overstocking. Heterophil/Lymphocyte ratio is a reliable indicator of stress associated with injury [62]. Increase in heterophil/lymphocyte (H/L) ratio indicates stress. Probiotics also actively stimulate the proliferation of lymphocytes (both B and T cells) and further immunoglobulin production in fish [59].

Serum biochemistry deals with the level of various enzymes, minerals, and proteins in the blood. Biochemical values are sometimes variably or invariably affected by blood, sex, age, environment, nutritional status, and experimental factors. Serum is the preferred sample for chemistry analysis, although plasma is often used because of the difficulties of obtaining two samples from one animal. The yield of plasma from a sample is usually greater than that of serum. If plasma is used for the analysis, then the auto coagulant should be considered in result interpretation. Choudhury et al. [63] discovered that dietary supplements of ribonucleic acid significantly influenced the total serum, protein, albumin, and globulin of the experimental fish

(*Labeo rohita*). The highest plasma protein concentration was recorded in fish fed 25% yeast-based diet [64]. Kobeisy et al. [65] studied the roles of 0, 5, 10, and 20% dietary live yeast on the serum glucose of *Oreochromis niloticus* for 13 weeks. They recorded a significant increase in the serum glucose concentration, compared to the control group.

5.7. Stress reduction in fish

Stress is referred to as "the non-specific response of the body to any demand made upon it." Stresses are additives and increase the susceptibility of animals to disease while decreasing their growth rate and feed conversion efficiency [66]. The degree to which stress affects any particular fish is determined largely by the severity of the stress, its duration, and the health of the fish. Reduced or negative growth is commonly observed during stressful periods, while growth rates or derived parameters are often considered reliable indicators of stress and welfare [67]. Fish under intensive culture conditions are exposed to a variety of stressors owing to the economic realities of large scale production [68]. To enhance production, farmers often increase rearing densities beyond system capacities. Rearing at high density can cause stress through deterioration in water quality, overcrowding, or adverse social interactions [69]. High rearing density adversely increases fish susceptibility to disease, possibly as a result of chronically elevated cortisol levels, which have immune-suppressive and catabolic actions in fish [70]. The common symptoms of stress include: gasping at the surface for oxygen, lack of appetite for food, abnormal swimming position, and fish disease. Stocking density is one of the key factors determining profitability and economic sustainability of a fish farm. Meanwhile, farmers often increase rearing densities to intensify production [71] and these suboptimal conditions may result in chronic stress in fish culture [72]. Three types of stress indicators can be detected in fishes: release of corticosteroid hormones (for example cortisol) into blood circulation [73], changes in hematological parameters, and the whole animal performance like growth and survival rate [68].

Hormonal and blood parameters have frequently been used as indicators of stress in sturgeons [74]. Stocking density has been studied in many bony fishes [72]. Stocking density is one of the most important factors in aquaculture because it directly influences survival, growth, behavior, health, feeding, and production of fish under farmed conditions [75]. The effect of stocking density as a major factor affecting fish growth has been the subject of many studies [76]. Hematological parameters are important indices related to response of fish to different environmental conditions. They are considered as important stress indicators in estimating reactions of fish to various environmental conditions and assessment of its general physiological status [77]. The level of hematological and growth indices in fishes is an important parameter to evaluate the stress responses to various environmental conditions [78]. Many studies have confirmed the significance of the hematological parameters to assess the response of organism to the environment condition and their importance for estimating its general health condition and possible effect of exposure to stressors [79]. Stocking density is considered an environmental stressor in aquaculture [80]. This constitutes an important item in any fish culture operation. The result of the improvement in output with respect to stocking density is essential in an intensive production system [81] with the objective of profit maximization.

http://dx.doi.org/10.5772/intechopen.72923

Production economics revealed that high stocking density of 40 fingerlings/m^3 gave the highest profit index and best cost ratio. At high stocking density (40 fingerlings/m^3), raising of *C. gariepinus* is more profitable [82]. Therefore, to increase fish production, appropriate environmental conditions must be provided [83]. Sohrab et al. [84] reported that growth and nutritional indices were appropriate indicators in assessing the impact of the induced stresses in the Caspian roach larvae owing to stocking density. Many studies have mentioned the importance of the hematological indices to evaluate the stress status of fish as a result of stocking density [84]. Hematological parameters such as hematocrit (HCT), hemoglobin (HB), number of red blood cells (RBC), eosinophil (EOS), and heterophil (HET) in Beluga (*Huso huso*) were not affected by increasing stocking density [85]. Binukumari and Anbarasi [86] recorded decreased trend in neutrophil numbers, RBC, and WBC (white blood cell) count in the stressed fish.

5.8. Accelerates wound healing in fish

Wound occurs when the integrity of any tissue is compromised for instance: skin breaks, muscle tears, burns, or bone fractures. This is very common with scale less fish such as catfish. Fish skin is divided into three layers namely: epidermis, dermis, and hypodermis. The skin is the outer covering of an animal, which forms a barrier against harmful microorganisms and chemicals entering the body. It has the ability to constantly renew itself after injury. It is highly vulnerable to injury owing to its position outside the body of the animal. The process of wound healing depends on how deep the wound is. Healing of wounds is characterized by synthesis of collagen. Wound-healing studies have been carried out on *Heterobranchus bidorsalis* juveniles [15], rainbow trout [87], channel catfish [88], and Nile tilapia [89]. Erazo-Pagador and Din [20] reported *Clarias gariepinus* fed with diets with dietary ascorbic acid had more rapid and complete wound healing. Histological examination by Erazo-Pagador and Din [20] revealed that at 14 days after wounding, fish fed with diets without ascorbic acid had normal epidermis and dermis but muscle tissues were still regenerating, whereas fish fed with diets containing ascorbic acid had normal epidermis, dermis, and muscle tissues. Rapid wound healing is especially important in the intensive culture of African catfish. This is because these species behave aggressively, have no scales, and have strong pectoral spines that can inflict wounds, especially at high stocking densities [20].

6. Conclusion

Having mentioned all the benefits that could be derived from using probiotics in fish production, it will be very imperative to embrace the use of this eco-friendly method in fish culture.

Author details

Olumuyiwa Ayodeji Akanmu

Address all correspondence to: olumuyiwa.akanmu@uniosun.edu.ng

Department of Fisheries and Wildlife Management, Faculty of Agriculture, Osun State University, Osogbo, Osun State, Nigeria

References

[1] Adedeji OB, Okocha RC. Constraint to Aquaculture Development in Nigeria and Way Forward. Nigeria: Veterinary Public Health and Preventive Medicine, University of Ibadan; 2011

[2] Salinas I, Cuesta A, Esteban MA, Meseguer J. Dietary administration of *Lactobacillus delbriieckii* and *Bacillus substilis*, single or combined on *Gilthead seabream* cellular innate immune responses. Fish & Shellfish Immunology. 2006;**19**:67-77

[3] Veni T, Chelladuari G, Mohanraj J, Vijayakumari I, Petchimuthu M. Dietary administration of L. acidophilus as probiotics on health, survival and gut microbial load in catfish (*Mystus montanus*). International Journal of Research in Fisheries and Aquaculture. 2012;**2**(4):48-51

[4] Jakhar V, Gahlawat SK, Sihag RC. Review article on bacterial diseases in freshwater prawn *Macrobrachium rosenbergii*. Ecotech. 2010;**2**:39-45

[5] World Health Organization. Joint FAO/NACA/WHO Study Group on Food Safety Issues Associated with Products from Aquaculture. WHO Technical Report Series. 883; 1999

[6] Sorum H. Antimicrobial drug resistance in fish pathogens. In: Aerestrup FM, editor. Antimicrobial Resistance in Bacteria of Animal Origin. Washington DC: ASM Press; 2006. pp. 213-238

[7] Giron-Perez MI, Santerre A, Gonzalez-Jaime F, Casas-solis J, Hermandez-Coronado M, Peregrina-Sandoval J, Takemura A, Zaitseva G. Immunotoxicity and hepatic function evaluation in Nile Tilapia (*Oreochromis niloticus*) exposed to diazinon. Fish & Shellfish Immunology. 2007;**23**:760-769

[8] Nayak SK. Probiotics and immunity: A fish perspective. Fish & Shellfish Immunology. 2010;**29**:2-14

[9] Geng X, Dong XH, Tan BP, Yang QH, Chi SY, Liu HY, Liu XQ. Effects of dietary probiotic on the growth performance, non-specific immunity and disease resistance of cobia, *Rachycentron canadum*. Aquaculture Nutrition. 2012;**18**(1):46-55

[10] Khalafalla MM. Nutritive value of diets containing digeston-1 as a feed additive for Nile tilapia (*Oreochromis niloticus*) fingerlings. Journal of Aquaculture Research and Development. 2013;**4**(5):2-5

[11] Swarnendu C, Urmimala C, Rajarshi B. Development and assessment of a p0fish feed to assist in aquaculture nutrition management. Research. 2010;**2**(5):63-75

[12] Ali A. Probiotics in fish farming – Evaluation of a candidate bacterial mixture. PhD Thesis, Sveriges Lantbruks Universitet, Vattenbruksinstitutionen, SLU, 901 83 Umea; 2000

[13] Kaleeswaran B, Ilavenil S, Ravikumar S. Changes in biochemical, histological and specific immune parameters in *Catla catla* (Ham) by *Cynodon dactylon* (L). Journal of King Saud University-Science. 2012;**24**:139-152

[14] Iman MKA, Amany MK, Taghreed BI, Magdy IH, Soliman WS. Enterococcus faecium probiotic as a growth promoter and its impact on the expression of the host innate immune in cultured *Oreochromis niloticus*. Research Journal of Pharmaceutical, Biological and Chemical Sciences. 2014;**5**(2):1747-1761

[15] Akanmu OA, Emikpe BO, Omitoyin BO, Ajani EK, Alao SO. Evaluation of the wound healing potential of the diets fortified with *Lactobacillus fermentum*, *Saccharomyces cerevisiae* and their combination in *Heterobranchus bidorsalis* juveniles. Zoology and Ecology. 2016;**26**(4):323-330. DOI: 10.10.80/21658005.2016.123468

[16] Parthasarathy R, Ravi D. Probiotic bacteria as growth promoter and biological control agent against *Aeromonas hydrophila* in catla catla (Hamilton, 1822). Indian Journal of Fisheries. 2011;**58**(3):87-93

[17] Zhou Q, Li K, Jun X, Bo L. Role and functions of beneficial microorganisms in sustainable aquaculture. Bioresource Technology. 2010;**100**:3780-3786

[18] Akanmu OA, Ajani EK, Omitoyin BO, Ogunbanwo SK. Organ-bacteriological dynamics in *Heterobranchus bidorsalis* juveniles fed diets fortified with *Lactobacillus fermentum*, *Saccharomyces cerevisiae* and their combination. Bulletin of Animal Health and Production in Africa. 2016;**64**:483-491

[19] Soroush G, Sahar G, Farhoudi M, Reza H. Effects of addition of *S. cerevisiae* and *B. subtilisin* diet on selected haematological and biochemical parameters in common carp (*Cyprinus carpio*). World Journal of Fish and Marine Sciences. 2011;**3**(1):96-99

[20] Erazo-Pagador G, Din SM. Rapid wound healing in African catfish, *Clarias gariepinus*, fed diets supplemented with ascorbic acid. Isreali Journal of Aquaculture Bamdgeh. 2001;**53**(2):69-79

[21] Zong-fu S, Tian-Xing WU, Li-Sheng C, Li-Jing Z, Xiao-dong Z. Effect of dietary supplementation with clostridium butyricum on the growth performance and humoral immune response in *Miichthys miiuy*. Journal of Zhejiang University. Science. B. 2006;**7**(7):596-602

[22] Sugita H, Kawasaki J, Deguchi Y. Production of amylase by the intestinal microflora in cultured freshwater fish. Letters in Applied Microbiology. 1997;**24**:105-108

[23] Verschuere L, Rombaut S, Sorgeloos P, Verstraete W. Probiotic bacteria as biological control agents in aquaculture. Microbiology and Molecular Biology Reviews. 2000;**64**:655-671

[24] Wang Y, Li J, Lin J. Probiotics in aquaculture: Challenges and outlook. Aquaculture. 2008;**281**(1-4):1-4

[25] Decamp O, Moriarty DJW, Lavens P. Probiotics for shrimp laviculture: Review of field data from Asia and Latin America. Aquaculture Research. 2008;**39**:334-338

[26] Ringo E, Gatesoupe RE. Lactic acid bacteria in fish: A review. Aquaculture. 1998;**160**:177-203

[27] Gram L, Mel-chiorsen J, Spanggard B, Huber I, Nielsen T. Inhibition of vibrio anguillarium by *Pseudomonas fluorescens* strain AH2, a possible probiotic treatment of fish. Applied and Environmental Microbiology. 1999;**65**:969-973

[28] Fuller R. Probiotics is man and animals. The Journal of Applied Bacteriology. 1989;**66**:365-378

[29] Panigrahi A, Kirona V, Puangkaewa J, Kobayashi T, Satoh S, Sugita H. The viability of probiotic bacteria as a factor influencing the immune response in rainbow trout (*Oncorhynchus mykiss*). Aquaculture. 2005;**243**:241-254

[30] Gatesoupe FJ. Updating the importance of lactic acid bacteria in fish farming: Natural occurrence and probiotic treatment. Journal of Molecular Microbiology and Biotechnology. 2008;**14**:107-114

[31] Cahill MM. Bacteria floral of fishes: A review. Microbial Ecology. 1990;**19**:21-41

[32] Gildberg A, Mikkelsen H, Sandaker E, Ringo E. Probiotic effect of lactic acid bacteria in the feed on growth and survival of fry of Atlantic cod (*Gadus morhua*). Hydrobiologia. 1998;**352**:279-285

[33] Nikoskelainen S, Salminen S, Bylund G, Ouwehand AC. Characterization of the properties of human and dairy–derived probiotics for prevention of infectious diseases in fish. Applied and Environmental Microbiology. 2001;**67**(6):2430-2435

[34] Ogunbanwo ST, Sanni AI, Onilude AA. Characterization of bacteriocin produced by *Lactobacillus plantarum* and *Lactobacillus brevis* from OG1. African Journal of Biotechnology. 2003;**2**(8):87-92

[35] Prescott LM, Harley JP, Klein DA. Microbiology. New York: Mc Graw Hill Companies Incorporated; 1999

[36] Stainer JR. In: Carr JG, Cutting CV, Whitings GS, editors. Recent Development in the Fermentation of Sauerkrant in Lactic Acid Bacteria, Beverages and Foods. New York: Academic Press; 1975

[37] Lara-Flores M, Olvera-Novoa MA, Olivera-Castillo L. Effect of inclusion of a bacterial mix (*Streptococcus faecium* and *Lactobacillus acidopilus*) and the yeast (*Saccharomyces cerevisiae*) on growth, feed utilization and intestinal enzymatic activity of Nile tilapia (*Oreochromis niloticus*). International Journal of Fisheries and Aquaculture. 2010;**2**:93-101

[38] Mesalhy ASM, Yousef AGA, Ghareeb AAA, Mohamed MF. Studies on *Bacillus subtilis* and *Lactobacillus acidophilus* as potential probiotics on the immune response and resistance of tilapia (*Oreochromis niloticus*) to challenge infectious. Fish & Shellfish Immunology. 2008;**25**:128-136

[39] Abdul El-Halim A, Nour A, Omar E, Abd ELLatif MG. Effect of different levels of active or inactive yeast on growth performance and feed utilization of Nile Tilapia *Oreochromis niloticus*. 2nd Alex Conf. fd. Sci. Tech.; 1989. pp. 396-405

[40] Scholz U, Garcia Diaz G, Ricque L, Cruiz Suarez E, Vargas Albores E, Latchford J. Enhancement of vibriosis resistance in juvenile *Penaeus vannamei* by supplementation of diets with different yeast products. Aquaculture. 1999;**176**:271-283

[41] Marzouk MS, Moustafa MM, Nermeen MM. The influence of some probiotics on the growth performance and intestinal microbial flora of *Oreochromis niloticus*. 8th International Symposium on Tilapia in Aquaculture (25); 2008. pp. 128-136

[42] Li P, Burr GS, Goff J, Whiteman KW, Davis KB, Vega RR, Neill WH, Gatlin DM III. A preliminary study on the effects of dietary supplementation of brewer's yeast and nucleotides, singularly or in combination, on juvenile red drum (*Sciaenops ocellatus*). Aquaculture Research. 2005;**36**:1120-1127

[43] Folorunso BS. Use of Probiotics for growth enhancement and immuno-modulation of Nile Tilapia and African Catfish, Ph D Thesis. University of Ibadan; 2010. pp. 21-67

[44] Jong SC. Probiotics for humans and animals. ATTC Quarterly Newsletter. 1993;**12**:1-4

[45] Ringo E, Lovmo L, Kristiansen M, Bakken Y, Salinas I, Myklebust R, Olsen RE, Mayhew TM. Lactic acid bacteria vs. pathogens in the gastro-intestine of fish (a review). Aquaculture Research. 2010;**41**:451-467

[46] Yasuda K, Taga N. A mass culture method for *Artemia salina*, using bacteria as food. Mercury. 1980;**18**:53-62

[47] Moriarty DJW. Disease control in shrimp aquaculture with probiotic bacteria. In: Bell CR, Brylinsky M, Johnson-Green P, editors. Microbial Biosystems: New Filters. Proceedings of the 8th International Symposium on Microbial Ecology. Halifax Canada: Atlantic Canada Society for Microbial Ecology; 1999. pp. 237-243

[48] Smith P, Davey S. Evidence for the competitive exclusion of *Aeromonas salmonicida* from fish with stress inducible furunculosis by a florescent pseudomonad. Journal of Fish Diseases. 1993;**16**:521-524

[49] Bogut I, Milakovic Z, Bukvic Z, Birkic S, Zimmer R. Influence of probiotic (*Streptococcus faecium* M 74) on growth and content of intestinal micro flora in carp (*Cyprinus carpio*). Journal of Animal Science. 1998;**43**:231-235

[50] Zizhong Q, Zhang XH, Boon N, Bossier P. Probiotics in aquaculture of China: Current state, problems and prospects. Elsevier Aquaculture Journal. 2009;**290**:15-21

[51] Irianto A, Austin B. Probiotics in aquaculture. Journal of Fish Diseases. 2006;**25**:633-642

[52] Ghazalah AA, Ali HM, Gehad EA, Hammouda YA, Abo-State HA. Effect of probiotics on performance and nutrients digestibility of Nile tilapia, *Oreochromis niloticus* fed low protein diets. Nature and Science. 2010;**8**(5):46-53

[53] Bernet MF, Brassart D, Neeser JR, Servin AL. *Lactobacillus acidophilus* LA 1 binds to cultured human intestinal cells and inhibits cell attachment and cell invasion by enterovirulent bacteria. Gut. 1994;**35**:483-489

[54] Douillet P, Langdon CJ. Effects of marine bacteria on the culture of axenic oyster *Crossostrea gigas* (Thunberg) larvae. The Biological Bulletin. 1993;**184**:36-51

[55] Villamil L, Tafalla C, Figueras A, Novoa B. Evolution of immunomodulatory effects of lactic acid bacteria in turbot (*Scopthalmus maximus*). Journal of Clinical and Diagnostic Laboratory Immunology. 2002;**9**(6):1318-1323

[56] Omitoyin BO. Introduction to Fish Farming in Nigeria. Ibadan, Oyo State: University Press; 2007. p. 90

[57] Noh H, Han KI, Won TH, Choi YJ. Effect of antibiotics, enzymes yeast culture and probiotics on the growth performance of Israeli carp. Korean Journal for Food Science of Animal Resources. 1994;**36**:480-486

[58] Oboh G, Akindahunsi AA. Nutritional and toxicological evaluation of *Saccharomyces cerevisiae* fermented cassava flour. Journal of Food Composition and Analysis. 2005;**18**: 731-738

[59] Al-Dohail MA, Roshada H, Mohamed AP. Effect of probiotic L. acidophilus on the growth performances haematology parameters and immunoglobulin concentration of African catfish (C. gariepinus Burchell, 1822) fingerling. Aquaculture Research. 2009;**40**(14):1642-1652

[60] Adeyemo OK, Ajani F, Adedeji OB, Ajiboye OO. Acute toxicity and blood profile of adult *Clarias gariepinus* exposed to lead nitrate. The Internet Journal of Hematology. 2008;**4**:105-111

[61] O'Neal CC, Weirich CR. Effects of low level salinity on production and haematological parameters of channel catfish (*Ictalurus punctatus*) reared in multi-crop ponds. Book of abstract Aquaculture 2001. International Triennial Conference of World Aquaculture Society; Jan. 21-25, 2001. Disney Coronado Springs Resort Lake Buena Vista, Florida; 2001. 484pp

[62] Moreno J, Merino S, Martinez J, Sanz JJ, Arriero E. Heterophil/lymphocyte ratios and heat-shock protein levels are related to growth in nestling birds. Ecoscience. 2002;**9**:434-439

[63] Choudhury D, Pala AK, Sahua NP, Kumara S, Dasb SS, Mukherjeeb SC. Dietary yeast RNA supplementation reduces mortality by *Aeromonas hydrophila* in rohu (*Labeo rohita* L.) juveniles. Fish & Shellfish Immunology. 2005;**19**:281-291

[64] Lunger AN, Craig SR, Mc Lean E. Replacement of fish meal in cobia (*Rachycentron canadum*) diets using an organically certified protein. Aquaculture. 2006;**257**:393-399

[65] Kobeisy MA, Hussein SY. Influence of dietary live yeast on growth performance and some blood constituents. In: *Oreochromis niloticus* Proc. 5th Sci. Conf. Animal Nutrition, Ismaila, Egypt, Dec. Vol. 1; 1995. pp. 417-625

[66] Arthi-Manju R, Felicitta J, Sakthivel M, Haniffa MA, Vallimmal S, Chelladural G. Effect of water probiotics on growth performance of *Channa punctatus*. International Journal of Applied Bioresearch. 2011;**1**:25-28

[67] Aluru N, Vijayan MM. Stress transcriptomics in fish: A role for genomic cortisol signaling. General and Comparative Endocrinology. 2009;**164**:142-150

[68] Barton BA, Morgan JD, Vijayan MM. Physiological and condition-related indicators of environmental stress in fish. In: Adams SM, editor. Biological Indicators of Aquatic Ecosystem Health. Vol. 6. Bethesda, M.D.: American Fisheries Society; 2002. pp. 111-148

[69] Procarione LS, Barry TP, Malison JA. Effects of rearing densities and loading rates on the growth and stress responses of juvenile rainbow trout. North American Journal of Aquaculture. 1999;**61**:91-96

[70] Mazur CF, Iwama GK. Effect of handling and stocking density on hematocrit, plasma cortisol, and survival in wild and hatchery-reared chinnok salmon (*Oncorhynchus tshawytscha*). Aquaculture. 1993;**112**:291-299

[71] Iguchi K, Ogawa K, Nagae M, Ito F. The influence of rearing density on stress response and disease susceptibility of ayu (*Plecoglossus altivelis*). Aquaculture. 2003;**220**:515-523

[72] Ramsay JM, Feist GW, Varga ZM, Westerfield M, Kent ML, Schreck CB. Whole-body cortisol is an indicator of crowding stress in adult zebrafish, (*Danio rerio*). Aquaculture. 2006;**258**:565-574

[73] Bolasina S, Tagawa M, Yamashita Y, Tanaka M. Effect of stocking density on growth, digestive enzyme activity and cortisol level in larvae and juveniles of Japanese flounder, *Paralichthys olivaceus*. Aquaculture. 2006;**259**:432-443

[74] Zarejabad AM, Sudagar M, Pouralimotlagh S, Bastami KD. Effects of rearing temperature on hematological and biochemical parameters of great sturgeon (Huso huso Linnaeus, 1758) juvenile. Comparative Clinical Pathology. 2009;**19**:367-371

[75] Salari R, Saad CR, Kamarudin MS, Zokaeifar H. Effect of different stocking densities on Tiger grouper juveniles (*Epinephelus fuscoguttatus*) growth and a comparative study of the flow through and recirculating aquaculture systems. African Journal of Agricultural Research. 2012;**7**(26):3765-3771

[76] Tolussi CE, Hilsdorf AWS, Caneppele D, Moreira RG. The effects of stocking density in physiological parameters and growth of the endangered teleost species *Piabanha bryconinsignis* (Steindachner, 1877). Aquaculture. 2010;**310**(1-2):221-228

[77] Docan A, Cristea V, Grecu I, Dediu L. Haematological response of the European catfish *Silurus glanis* reared at different densities in flow through production system. Achiva Zootechnica. 2010;**13**:63-67

[78] Braun N, Lima RL, Baldisserotto B, Dafre AL, Nuner APO. Growth, biochemical and physiological responses of *Salminus brasiliensis* with different stocking densities and handling. Aquaculture. 2010;**301**:22-30

[79] Tintos A, Miguez JM, Mancera JM, Soengas JL. Development of a microtitre plate indirect ELISA for measuring cortisol in teleosts and evaluation of stress responses in rainbow trout and gilthead sea bream. Journal of Fish Biology. 2006;**68**:251-263

[80] Rafatnezhad S, Falahatkar B, Gilani MHT. Effects of stocking density on haematological parameters, growth and fin erosion of great sturgeon (Huso Huso) juveniles. Aquaculture Research. 2008;**39**:1506-1513

[81] Adikwu IA. Dietary carbohydrate utilization in tilapia: *O. niloticus*. Journal of Agricultural Science and Technology. 2003;**2**(1):33-37

[82] Edward A, Ladu BMB, Elihu A. Growth, survival and production economics of *Clarias gariepinus* fingerlings at different stocking densities in concrete tanks. African Journal of General Agriculture. 2010;**6**(2):59-66

[83] Kamal SM, Omar WA. Effect of different stocking densities on haematological and biochemical parameters of silver carp *Hypophthalmichthys molitrix* fingerlings. Life Science Journal. 2011;**8**(4):580-586

[84] Sohrab A, Soheil E, Imanpour MR. Effect of stocking densities on haematological parameters, growth and survival rate of *Caspian roach* (Rutilus rutilus caspicus) larvae. Journal of Chemical Biological and Physical Sciences. 2013;**3**(2):1320-1326

[85] Asadi R, Imanpour MR. Effects of Stocking Density on some Haematological Parameters and Growth of Great Sturgeon (*Huso huso*) Juveniles; First Conference on Caspian Sea Resources. Gorgan Iran: Gorgan University of Agricultural Sciences and Natural Resources; 2008

[86] Binukumari S, Anbarasi S. Effects of stressors on haematological parameters in the freshwater fish *Cyrinus carpio*. International Journal of Current Research. 2011;**3**(9):42-44

[87] Halver JE, Ashley LM, Smith RR. Ascorbic acid requirements of Coho Salmon and Rainbow trout. Transactions of the American Fisheries Society. 1969;**98**:762-771

[88] Lim C, Lovell RT. Pathology of the vitamin C deficiency syndrome in channel catfish (*Ictalurus punctatus*). The Journal of Nutrition. 1978;**108**:1137-1146

[89] Jauncey J, Soliman AK, Roberts RJ. Ascorbic acid requirements in relation to wound healing in the cultured tilapia *Oreochromis niloticus* (Trewavas). Aquaculture and Fisheries Management. 1985;**16**:139-149