

**INFLUENCE OF AQUALASE AND GROBIOTIC®- A
ON GROWTH, DIGESTIVE ENZYME ACTIVITIES,
SURVIVAL AND *STREPTOCOCCUS FAECIUM*
RESISTANCE IN RAINBOW TROUT
(*ONCORHYNCHUS MYKISS*) JUVENILES**

by

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LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
AOAC	Association of Official Analytic Chemists
AQ1	Aqualase 0.1%
AQ2	Aqualase 0.2%
ADG	Average Daily Growth
BA	Blood agar
BKD	Bacterial Kidney Disease
BHI	Brain heart infusion broth
BSA	Bovine Serum Albumin
C	Non-probiotic, prebiotic diet (control)
CF	Condition factor
CFU	Colony forming units
CL	Chemiluminescent
DE	Digestible energy
DP	Degrees of polymerization
DGGE	Denaturing gradient gel electrophoresis
EC	Electrical conductivity
FAO	Food and Agricultural Organization
FCR	Feed conversion ratio
FDA	Food and Drug Administration
FE	Food efficiency
FFT	Fingerling feed rainbow trout
FI	Total feed intake per fish
FOS	Fructooligosaccharides
G-A1	GroBiotic [®] -A 0.5%
G-A2	GroBiotic [®] -A 1%
G-A3	GroBiotic [®] -A 1.5%
G-A4	GroBiotic [®] -A 2%
G-A5	GroBiotic [®] -A 2.5%
G-A6	GroBiotic [®] -A 3%
GE	Gross energy
GFT1	Grow out feed rainbow trout
GOS	Galactooligosaccharide
Hb	Haemoglobin
HIS	Hepatosomatic index
IFRO	Iranian Fisheries Organization
IHN	Infectious hematopoietic necrosis
I	Intestine

IP	Intraperitoneal injection
IPN	Infectious pancreatic necrosis
ISA	Infectious salmon anemia
LAB	Lactic acid bacteria
LC	Leukocyte count
LD ₅₀	Lethal Dosage 50%
Lf	Lactoferrin
MCH	Mean corpuscular hemoglobin
MCHC	Mean corpuscular hemoglobin concentration
MCV	Mean corpuscular volume
ME	Metabolizable energy
MOS	Mannan oligosaccharide
MS-222	Tricane methane sulfonate
MR	Muscle rate
NRC	National Research Council
NFE	Nitrogen free extract
OD	Opaque density
PBS	Phosphate buffered saline
PI	Protein intake
PD	Pancreas disease
PER	Protein efficiency ratio
PCV	Packed red cells
RBC	Red blood cell count
RGR	Relation growth rate
SGR	Specific growth rate
SCFAs	Chain Fatty Acids
SR	Survival rate
S & Pc	Stomach and Pyloric caeca
US\$	US Dollar
VHS	Viral haemorrhagic septicaemia
VSI	Visceral somatic index
WBC	White blood cell

**PENGARUH AQUALASE DAN GROBIOTIC® KE ATAS PERTUMBUHAN
DAN AKTIVITI ENZIM PENCERNAAN, KEMANDIRIAN DAN KETAHANAN
KEPADA *STREPTOCOCCUS FAECIUM* DALAM JUVENIL RAINBOW TROUT
(*ONCORHYNCHUS MYKISS*)**

ABSTRAK

Kajian telah dijalankan untuk menentukan kesan penambahan supplemen probiotik Aqualase (THEPAX) dan prebiotik GroBiotic-A terhadap pertumbuhan dan prestasi kesihatan rainbow trout, *Oncorhynchus mykiss*. Dua siri eksperimen telah dijalankan untuk menentukan kegunaan kedua-dua supplemen dalam makanan komersial trout (Chineh Co., Iran) terhadap bakteria patogenik ikan, *Streptococcus faecium* dan kesannya terhadap prestasi pertumbuhan, parameter haematologi dan imunologi serta aktiviti spesifik enzim rainbow trout, *O. mykiss*. Dalam kedua-dua eksperimen, darah disampel untuk parameter haematologi dan imunologi selepas 4, 8 dan 12 minggu pemberian makanan. Pada akhir eksperimen dijalankan, gastrointestinal ikan dikeluarkan untuk menentukan aktiviti enzim penghadaman dan pengiraan bilangan bakteria dalam usus. Dalam eksperimen pertama, lima diet telah disediakan yang terdiri daripada diet kawalan (tiada penambahan supplemen); penambahan G-A pada kadar 1 dan 2% (diet prebiotik) dan Aqualase pada kadar 0.1 dan 0.2% (diet probiotik) masing-masing. Kesemua diet secara triplikat diberikan kepada 300 individu *O. mykiss* bersaiz jejari (11.30 ± 0.21 g), dua kali sehari dalam tangki konkrit selama 12 minggu. Prestasi pertumbuhan dan kadar survival adalah lebih tinggi bagi ikan yang diberi makan kedua-dua diet supplemen G-A dan AQ berbanding diet kawalan. Walau bagaimanapun, berat akhir dan survival ikan yang diberi makan diet G-A2% adalah tertinggi antara kesemua diet. Parameter haematologi tidak menunjukkan perbezaan signifikan antara diet tetapi parameter imunologi yang terdiri daripada Igm (56.10 ± 3.44 mg/ml) dan lysozyme

(12.27 ± 0.97 mg/ml) adalah tinggi bagi ikan yang diberi makan supplemen G-A2%. Di samping itu, aktiviti amylase, protease dan lipase dalam usus (2.21 ± 0.23 , 4.26 ± 0.35 , 3.83 ± 0.86 IU/mg protein, masing-masing) dan perut-pilorik caeca ikan (1.42 ± 0.23 , 4.66 ± 1.08 , 3.74 ± 0.25 IU/mg protein, masing-masing) yang diberi makan G-A2% didapati lebih tinggi berbanding diet lain. Bilangan bakteria aerobik, anaerobik dan asid laktik (LAB) bagi kesemua diet supplemen adalah lebih tinggi secara signifikan ($p < 0.05$) berbanding diet kawalan dengan bilangan tertinggi diperolehi bagi diet G-A2% pada akhir eksperimen. Keputusan menunjukkan bahawa IgM (62.77 ± 4.64 mg/ml) dan lysozyme (12.47 ± 0.70 mg/ml) adalah lebih tinggi secara signifikan bagi ikan yang diberi makan diet G-A2%. Dalam eksepeimen kedua, tujuh diet yang mengandungi G-A pada tahap 0.5, 1, 1.5, 2, 2.5 dan 3% diberi makan kepada 25 ekor ikan *O. mykiss* bersaiz jejari (4.44 ± 0.06 g) secara triplikat. Ikan-ikan tersebut dipelihara dalam tangki fiberglass dan diberi makan dua kali sehari selama 84 hari dan dibandingkan dengan diet kawalan. Prestasi pertumbuhan dan kadar survival didapati lebih tinggi secara signifikan bagi ikan yang diberi makan diet G-A2.5%. Aktiviti spesifik amylase, protease dan lipase dalam usus (3.00 ± 0.24 , 3.06 ± 0.10 , 4.61 ± 0.21 IU/mg protein, masing-masing) and perut-pilorik caeca (2.70 ± 0.16 , 4.50 ± 0.13 , 4.61 ± 0.21 IU/mg protein, masing-masing) didapati lebih tinggi secara signifikan bagi ikan yang diberi makan diet G-A2.5%. Pengiraan bilangan bakteria aerobik, anaerobik dan asid laktik (LAB) didapati lebih tinggi dalam ikan yang diberi makan diet G-A2.5% berbanding diet lain. Di samping itu, ikan yang diberi makan diet G-A menunjukkan pengurangan kematian bagi ikan yang dijangkiti dengan *S. faecium* seperti berikut; 20% (G-A2.5%), 23.3% (G-A4 dan G-A6), 26.7% (G-A3) dan 30% (G-A2) dan 53.3% (kawalan dan G-A0.5%), manakala serum IgM dan lysozyme didapati paling tinggi dalam ikan yang diberi makan

G-A2.5% (53.37 ± 2.23 mg/ml dan 12.72 ± 0.94 mg/ml, masing-masing). Berdasarkan keputusan ini, boleh disimpulkan bahawa penambahan supplemen G.A dalam makanan komersial rainbow trout pada kadar 2.5% dapat meningkatkan survival, kecekapan pemakanan, pertumbuhan dan tahap kesihatan rainbow trout terhadap bacteria *Streptococcus faecium*.

**INFLUENCE OF AQUALASE AND GROBIOTIC®- A ON GROWTH,
DIGESTIVE ENZYME ACTIVITIES, SURVIVAL AND *STREPTOCOCCUS*
FAECIUM RESISTANCE IN RAINBOW TROUT (*ONCORHYNCHUS MYKISS*)
JUVENILES**

ABSTRACT

The present study was carried out to investigate the effects of supplementation with a probiotic, Aqualase (THEPAX®) and a prebiotic, GroBiotic®- A, respectively, on rainbow trout *Oncorhynchus mykiss*, growth and health performance. Two series of experiments were conducted to evaluate the use of these supplements in a commercial trout feed (Chine, Iran) against fish pathogenic bacteria, *Streptococcus faecium* and their effects on growth performance, haematological, immunological parameters and specific enzyme activities of rainbow trout, *O. mykiss*. In both experiments, blood was sampled for haematological and immunological parameters after 4, 8 and 12 weeks, respectively of feeding. At the end of the trials, gastrointestinal tract of experimental fish were removed to assay for digestive enzymes activity and determination of the total bacterial count in the intestines. In the first experiment, five diets were prepared each comprising of a control (not supplemented); 1 and 2% G-A respectively and 0.1 and 0.2% Aqualase. The diets were fed twice daily to triplicate groups of 300 *O. mykiss* fingerlings (average 11.30 ± 0.21 g) in concrete tanks for 12 weeks. The growth performance and survival rate were significantly higher in fish maintained on the both G-A and AQ treatments compared with the control. However, final weight and survival fish fed G-A2% was the highest among all the treatments tested. Haematological parameters showed no significant difference between treatments, whereas immunological parameters consisting IgM (56.10 ± 3.44 mg/ml) and lysozyme (12.27 ± 0.97 mg/ml) were found significantly higher in the fish fed with supplemented G-A2% between groups. Moreover, amylase,

protease and lipase activities in the intestine (2.21 ± 0.23 , 4.26 ± 0.35 , 3.83 ± 0.86 IU/mg protein, respectively) and stomach-pyloric caeca in fish fed diet containing G-A2% (1.42 ± 0.23 , 4.66 ± 1.08 , 3.74 ± 0.25 IU/mg protein, respectively) were detected higher than other groups. The aerobic, anaerobic and lactic acid bacteria (LAB) counts of all supplemented feeds were significantly ($p < 0.05$) higher than the control with G-A2% being highest at the end trial. The challenge results showed IgM (62.77 ± 4.64 mg/ml) and lysozyme (12.47 ± 0.70 mg/ml) were significantly higher the group maintained on the G-A2%. In the second experiment, seven treatments containing 25 individuals of *O. mykiss* fingerlings (4.44 ± 0.06 g) were fed to triplicate group of fish with feed containing G-A at levels of 0.5, 1, 1.5, 2, 2.5 and 3%. The fish were raised in fiber glass tanks and fed twice daily for 84 days and compared with control group. Growth performance and survival rate were found significantly highest in fish fed G-A2.5%. Specific amylase, protease and lipase activities in the intestine (3.00 ± 0.24 , 3.06 ± 0.10 , 4.61 ± 0.21 IU/mg protein, respectively) and stomach-pyloric caeca (2.70 ± 0.16 , 4.50 ± 0.13 , 4.61 ± 0.21 IU/mg protein, respectively) observed significantly higher in juvenile fed with G-A2.5%. The highest count of aerobic, anaerobic and lactic acid bacteria (LAB) were detected the fish that fed with G-A2.5% compared to other groups. Furthermore, fish fed with G-A resulted in decrease in mortality in fish infected with *S. faecium* as follows; 20% (G-A2.5%), 23.3% (G-A4 and G-A6), 26.7% (G-A3) and 30% (G-A2) and 53.3% (control and G-A0.5%), while serum IgM and lysozyme were found the highest in fish fed G-A2.5% (53.37 ± 2.23 mg/ml and 12.72 ± 0.94 mg/ml, respectively). From the results, it can be concluded that supplementing a commercial trout feed with G-A at 2.5% G-A can enhance the survival, feed efficiency, growth and health status of rainbow trout against *Streptococcus faecium* bacteria.

CHAPTER 1: INTRODUCTION

1.1 Background of Aquaculture

Aquaculture accounted for 46 percent of total food fish supply in 2008 (FAO. 2011). The fisheries and aquaculture sector is crucial to food security, poverty alleviation and general well-being. In 2008, the world consumed 115 million tonnes of fish, and demand is expected to rise. Fish and fishery products are a vital and affordable source of food and high-quality protein, in 2008 fish as food reached an all-time high of nearly 17 kg per person, supplying over 3 billion people with at least 15 percent of their average animal protein intake (FAO. 2011).

Trout farming began in Europe and has expanded to more than 60 countries. Most freshwater farmed trout is rainbow trout. The main rainbow trout producing countries, in descending order of production, are Iran, France, Italy, Denmark, the United States and Spain (FAO. 2011). Furthermore, the farming of salmonid fishes, especially Atlantic salmon (*Salmo salar*) and rainbow trout (*O. mykiss*) is a good example of commercially successful intensive aquaculture in the world. Rainbow trout is a large economic fish and recently, the artificial propagation of the species has increased in commercial farms, making it the second most cultured fish species in Iran (FAO, 2011).

The successful commercialization of the salmonid industry was achieved through many processes including investment, innovative studies like disease control, technological advancement and creative marketing strategies. However, negative impacts of infectious diseases still persist in various aquaculture systems, costing losses in revenues from mortality amounting to millions of dollars, thus reducing the means of

combating diseases. Even though the concept of functional feed is a relatively new phenomenon in the aquaculture industry, it however, represents an emerging process of developing diets which could extend beyond satisfying the basic nutritional requirements of the farmed fish, to improve the health and the resistance to stress and cause of the disease (Gatlin, 2002). Prevention of diseases and treatment strategies such as the use of vaccines and drugs, are limited in most aquaculture operations due to the constraints of regulation or administrative protocol inconveniences (Gatlin, 2002).

Lately, with increase in water contamination and the intensive expansion in fish-culture, there are progressively more types of diseases in freshwater fish, resulting into severe financial losses every year in many countries around the world. Agenew and Barnes (2007) reported that among the diseases, the most important agents causing severe losses in the fish farming industry are bacteria. On the other hand, the most prevalent disease-causing bacteria are gram-negative, although there are still several types of gram-positive bacteria pathogens of fish, which cause Streptococcosis (Swenshon *et al.*, 1998). Streptococcal disease in fish was first reported in 1957, affecting reared rainbow trout in Japan (Hoshina, *et al.*, 1958). Since then, many other species of fish were reported vulnerable to being infected (Klesius *et al.*, 2000; Buller, 2004; Vendrell *et al.*, 2006; Austin and Austin, 2007). Yanong and Francis-Floyd, (2002) noted that Streptococcosis in fish could cause high mortalities (more than 50 percent) within a period of 3 to 7 days. Some outbreaks are however, more constant in nature and mortalities could extend beyond several weeks, but with only a few fish dying per day. Bunch and Bejerano (1997) observed that *Streptococcus* spp. is an opportunistic pathogen because they are capable of extensively spreading over fish farm environments and cause stress effects to assert pathogenicity.

In Iran, Streptococcosis was identified during the past 3-5 years as one of the most important infectious bacterial diseases in cold freshwater fish (such as rainbow trout) in commercial farms (Soltani *et al.*, 2005). This was identified as a serious problem as it caused huge economic losses every year in the aquaculture industry in some provinces of Iran, according to Ghiasi, *et al.*, (2000), Akhlaghi and Keshavarzi (2002), Soltani *et al.*, (2005, 2008), Saeedi *et al.*, (2009). During the past 3-5 years, Streptococcosis was detected in rainbow trout (*Oncorhynchus mykiss*, Walbaum) in many farms in Iran, causing significant economic losses to the aquaculture industry, whereby approximately 40% of the specimens tested noted to be infected with *Streptococcus* spp. (Pourgholam *et al.*, 2010). Consequently, five pathogenic species have been identified, which include *S. iniae* in Fars province, *S. faecium* in Mazandaran province, *S. agalactiae* in Gilan and Mazandaran provinces, *S. dysgalactiae* in Lorestan, Kohgiluyeh and Boyer-Ahmad, Gilan and Kermanshah provinces, while *S. uberis* is common to all the provinces (except Mazandaran and Lorestan) (Pourgholam *et al.*, 2010). The most dominant species (based on important species index) are *S. uberis*, *S. dysgalactiae* and *S. agalactiae*, respectively (Pourgholam *et al.*, 2010). Annual losses by streptococcosis/lactococcosis have been estimated at 100 million USD (Nomoto *et al.*, 2004). In fact, the economic impact due to this bacterial disease is estimated to be higher than 100 million USD. For instance, Iran is one of the leading trout producing countries producing more than 60000 tonnes per year and annual estimated losses due to this zoonotic disease is about 15 million USD (Haghighi *et al.*, 2010).

The zoophagous distinction of rainbow trout, which intensifies when the fish is cultured under highly stressful, intensive culture situation, is a major concern. The condition is exacerbated by poor nutritional inputs, a strategy which minimizes

production costs under intensive culture in accordance with the growth rates, further compromising fish health. The use of antibiotics to reduce the growth of potential pathogenic bacteria and, thereby prevent disease prevalence has been demonstrated as not sustainable, due to the risk in the development of antibiotic resistant strains (Capone *et al.*, 1996; Karunasagar *et al.*, 1994). Additionally, increasing concerns in the use of antibiotics have resulted in a ban on sub-therapeutic antibiotic use in Europe, with the potential for a ban in the United States and other countries (Patterson and Burkholder, 2003). The use of complementary diets could have positive impacts on aquaculture and has therefore prompted the interest in developing alternative strategies for disease control. Apart from vaccine development, dietary supplementation including probiotics, prebiotics and immunostimulants are receiving increasing attention. A rapidly expanding body of research has established that many intestinal microbial species could make beneficial influence on the growth of fish (Irianto and Austin, 2002) and that dietary composition influences the intestinal microflora of fish species (Ring *et al.*, 1998; Ring and Olsen, 1999).

Probiotics were identified as live microbial culture from dairy products, which influence intestinal microbial balance (Parker, 1974) and improve the health and nutrition of the host (Salminen, 1996; Schaafsma, 1996). On the other hand, the term prebiotics was introduced by Gibson and Roberfroid (1995) and could be defined as "a non-digestible food component which positively affects the host by selectively enhancing the growth and/or activity of one or a restricted number of bacteria in the colon".

Studies showed that intraperitoneal injection of glucan (a prebiotic) derived from cell walls of the yeast *Saccharomyces cerevisiae* into the gut of Atlantic salmon and

rainbow trout resulted into increased resistance against several bacterial pathogens and reduced fish mortality (Jorgensen *et al.*, 1993; Dalmo and Seljelid, 1995). Recently, evidence of the beneficial effects of probiotics has resulted in the development of the concept of prebiotics. However, the potential use of prebiotics in aquaculture is yet to be pursued extensively (Li and Gatlin, 2005). Previously, the evaluation of a commercial Prebiotic (GroBiotic®-A); a commercial blend of dried fermentation products, partially autolyzed brewer's yeast and dairy ingredients was observed to significantly affect the feed efficiency of juvenile hybrid striped bass (Li and Gatlin, 2004).

Considering these activities, it is important to seek alternative means in aquaculture, to maintain a microbiologically healthy environment for rearing the fish and at the same time, improve production volume to enhance economical profits.

Since last decades, effect of prebiotic and probiotic on fish growth and health have been discussed (Vahabzadeh-Rodsari, 2003; Ziaei-Nejad *et al.*, 2006; farzanfar, 2006). Thus the effects of GroBiotic®-A and Aqualase (THEPAX®) on growth performance, specific digestive activities as well as their effect on the haematological parameters of rainbow trout infected with *Streptococcus faecium* was tested in the current study.

1. 2 Purpose of this study

The overall objective of this study was to compare and evaluate the effectiveness of Aqualase and GroBiotic®-A as a food supplement that can promote growth and health of rainbow trout, *O. mykiss* juvenile.

The specific objectives of the proposed research are:

1. To compare the effects of 2 levels each, of a commercial probiotic (Aqualase) and a prebiotic (GroBiotic[®]-A) respectively, on growth, haematological parameters, digestive enzymes, feed digestibility.
2. To determine the optimal level of GroBiotic[®]-A inclusion for growth, feed utilization in rainbow trout (*O. mykiss*).
3. To determine the optimal level of Aqualase and GroBiotic A that supports growth and fish health and immune response in rainbow trout (*O. mykiss*) challenged with *Streptococcus faecium*.

CHAPTER 2: LITERATURE REVIEW

2.1 Aquaculture challenges

Capture fisheries and aquaculture supplied the world with about 142 million tonnes of fish in 2008. Of this, 115 million tonnes was used as human food, providing an estimated apparent per capita supply of about 17 kg, which is an all-time high (FAO, 2011).

The report world aquaculture 2010 found that global production of fish from aquaculture grew more than 60% between 2000 and 2008, from 32.4 million tons to 52.5 million tons. It also forecasts that by 2012 more than 50% of the world's food fish consumption will come from aquaculture (FAO, 2011). According to FAO (2011), aquaculture is consistently the fastest-growing animal-food-producing sector and has surpassed production from capture fisheries. In order to meet the demands of an and equally rapid growth in global population, the per capita supply from this sector increased from 0.7 kg in 1970 to 7.8 kg in 2008. In terms of production, aquaculture provided less than 1 million tonnes annually in the early 1950s and increased to 52.5 million tonnes, with a value of US\$98.4 billion in 2008. World aquaculture is heavily dominated by the Asia-Pacific region, which accounts for 89 percent of production in terms of quantity and 79 percent in terms of value (FAO, 2011).

For millions of people throughout the world, the fish sector is a source of income and living. Since 2008, employment in fisheries and aquaculture has increased at an average rate of 3.6%. FAO (2011) estimated that in 2008, 44.9 million people were

directly involved in full time or, more frequently part time, in capture fisheries or in aquaculture.

Although these trends are encouraging globally, many restrictions could hamper the growth of aquaculture, with diseases being one of these principal limiting factors (FAO, 2011). Even though global economic losses from diseases in aquaculture have not been recorded, reports of disease from many regions globally have been on the increase, as live aquatic animal trade increases (Subasinghe, 2005). The current approved methods practiced for the prevention and treatment of infectious diseases in the aquatic environment include a regulated number of government-approved antibiotics and chemotherapeutics and limited vaccines, which could be used to support environmental management.

Unfortunately, the use of antibiotics has received heavy criticism, essentially against the development of antibiotic-resistant strains (FAO, 2002). Potential impact on the non-target bacterial communities in the environment (Stickney, 1997) and the risk of antibiotic residues in aquaculture products, which could be harmful to antibiotic-intolerant human consumers (FAO, 2002) are also noted concerns. Furthermore, numerous studies have shown that most antibiotics, including FDA (Food and Drug Administration) proved oxytetracycline are negatively affect natural immunity of fish, increasing the risk of infection from non-bacterial agents like viruses, parasites and fungi (Rijkers *et al.*, 1980, 1981; Siwicki *et al.*, 1989; Taffaella *et al.*, 1999; Luden *et al.*, 1999, 2002). Furthermore, the use of vaccines via injection does not only cause handling related stress which compromise the effectiveness of the vaccines but is also labour intensive (Li, 2005).

2.2 Biology of rainbow trout (*Oncorhynchus mykiss* Walbaum, 1792)



Plate 2.1: Lateral view of rainbow trout (*Oncorhynchus mykiss*)

Source: Robert MacDowall, Federal government (e.g., U.S. Geological Survey, U.S. Fish and Wildlife Service or their employees, 2001).

Kingdom: Animalia

Phylum: Chordates

Class: Actinopterygii

Order: Salmoniformes

Family: Salmonidae

Genus: *Oncorhynchus*

Species: *Oncorhynchus mykiss*

2.2.1 Description, habitat, distribution and natural feeding habits

Rainbow trout is a streamlined salmonid, with its body shape and coloration varied to reflect habitat, age, sex and degree of maturity. The body size of rainbow trout could range from slender to thick (Greenhalgh, 1989). Rainbow trout usually matures at

the age of between 3 to 5 years, where it grows to between" 15 to 40 cm in length and could live up to 11 years. The cool waters of the Northern Hemisphere are the natural habitats of rainbow trout, although it has been introduced all over the world. The trout is native to the Pacific Coast of Northern America, from Alaska to Mexico, the Pacific Ocean and the eastern coasts of Asia (Roberts, 2001). Two major types of rainbow trout are known; the freshwater river rainbow trout and the part time river, part time sea-going rainbow trout, referred to as steelhead trout. During the last 100 years, all rainbow trout types were introduced into several aquatic environments throughout the world. Both trout types are carnivorous and highly prized by anglers due to their fighting nature when caught on fishing lines (Greenhalgh, 1989). However, trout are predators with a varied diet, eating nearly anything they can grab. When young, insects make up a large portion of their diet, as well as fish eggs, smaller fish (up to 1/3 of their length), with crayfish and other crustaceans making up the remainder.

2.3 Global aquaculture production of rainbow trout

The culture of salmonids particularly rainbow trout (*O. mykiss*), is one important example of successful commercially intensive aquaculture in the world. Rainbow trout (*O. mykiss*) is an important product farmed in both the freshwater and seawater environments in many countries around the world (Figure 2.1).



Fig. 2.1: Map of the world, showing the major countries producing *Oncorhynchus mykiss* (FAO, 2011)

The production of rainbow trout has grown exponentially since the 1950s, especially in Europe and more recently in Chile. This is primarily due to increased inland production in countries such as France, Italy, Denmark, Germany and Spain to supply the domestic markets, and mariculture in cages in Norway and Chile for the export market. Chile is currently the largest producer. Other major producing countries include Norway, France, Italy, Spain, Denmark, USA, Germany, Iran and the UK (FAO, 2011) (Figure 2.2).

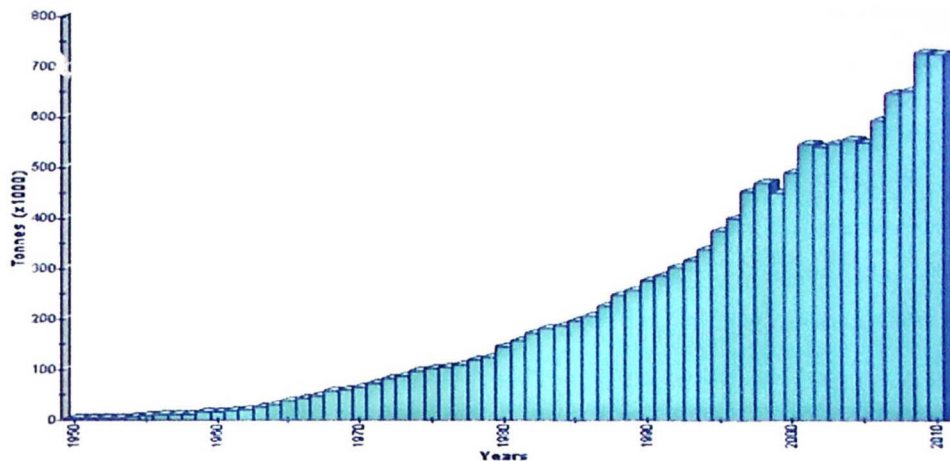


Fig. 2.2: Global aquaculture productions of *Oncorhynchus mykiss* (FAO, 2011)

2.3.1 Aquaculture of rainbow trout in Iran

Across the Islamic republic of Iran, weather conditions vary greatly allowing for a wide range of aquaculture to be practiced. Fish farming in Iran is reported to have begun with the enhancement of fish species selected from the Caspian Sea and continued through the development of semi-intensive aquaculture, using several species of Chinese carp, as well as the rainbow trout (*O. mykiss*). More recently however, shrimp culture (Indian white prawn, *Penaeus indicus*) is fast becoming the principal focus of government investments in the Gulf region, where it is developed in earthen ponds.

Approximately 41458 hectares is reported as the total area for fish farming in Iran. The major cultivated warmwater species are the common carp (*Cyprinus carpio*) and three main Chinese carps namely; grass carp (*Ctenopharyngodon idellus*), silver carp (*Hypophthalmichthys molitrix*) and the bighead carp (*Hypophthalmichthys nobilis*). Chinese carp, rainbow trout, Indian white prawn and aquaculture-based fishery

production represented 48.43%, 35.52%, 2.47% and 13.26 % respectively, of the total Iranian aquaculture productions in 2009 (IFRO, 2011).

A rapid increase of the total aquaculture production from 3219 tonnes in 1978 to over 207353 tonnes in 2009, representing approximately 34.6% increase in production volume was recorded in Iran (IFRO, 2011).

Trout farms are currently distributed across the central, the north-western and western parts of the country mostly in the mountainous areas characterized by cool summers and cold winters. The farming system consists of simple raceways made of concrete troughs with continuous water flow. An increased number of farms, together with improved farming techniques and facilities have enhanced annual production of trout from 280 tonnes in 1978 to over 73 thousand tonnes by 2009 (FAO, 2011) (Table 2.1).

Table 2.1: Aquaculture and aquaculture based fishery production in Islamic Republic of Iran 1997-2009 (FAO, 2011).

Year/Species	Warmwater	Coldwater	Aquaculture based fisheries	Brackish water shrimp	Freshwater shrimp	Total
1997	27183	2514	34780	523	-	65000
1998	27374	4994	38763	869	-	72000
1999	23000	7000	36000	1800	-	67800
2000	27500	9000	25490	4010	-	66000
2001	28060	12170	25785	7624	6	73645
2002	54801	16026	13010	5990	30	89827
2003	61084	23138	18461	7462	30	110175
2004	65400	30000	20230	8903	27	124560
2005	73396	34760	22179	3577	268	134180
2006	77463	46275	24970	5700	270	154678
2007	97262	58761	34888	2508	258	193677
2008	87748	62630	28622	4372	275	183647
2009	100793	73642	27503	5128	287	207353

Total coldwater production in 2009 in the five provinces of Iran accounted for over 21.47% of the total aquaculture production, where rainbow trout alone contributed over 60.5% (Figure 2.3). The major productions of rainbow trout are found in Charmahal va Bakhtiari (16.37%), Lorestan (15.18%), Mazandaran (14.28 %), Fars (20%) and Azarbayjan gharbi 8.07 percent (IFRO, 2011).

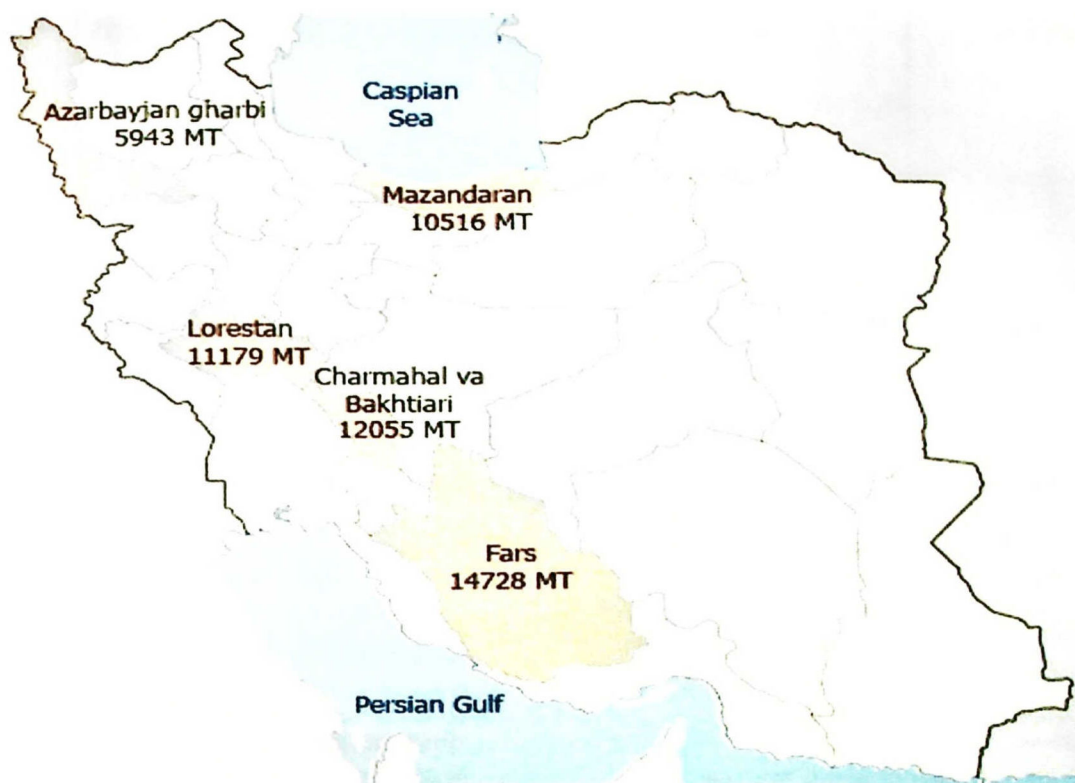


Fig. 2.3: Map of Iran showing the major producing regions of *Oncorhynchus mykiss* (FAO, 2011)

2.4 Fish Disease

In aquaculture, fish disease is an important economic problem (Grisez and Ollevier, 1995). It is thus imperative for an optimum health condition for fish growth to be maintained. The two major goals of aquaculture are rapid growth and high survival of fish to marketable size, which require optimum health conditions. Several factors could trigger the development of diseases, which as well, could be prevented in many different forms. The severity of diseases and duration may vary considerably, depending on the underlying causes. The salmonid culture is affected by diseases and is one of the most important profit-limiting factors in intensive aquaculture. Farmers could avoid the

potential effects of diseases by carefully monitoring the disease and managing the conditions (Halver and Hardy, 2002).

2.4.1 Factors Affecting Fish Health

Both living and nonliving agents could cause various diseases to fish. Examples of living agents are infectious organisms which could be communicable and pathogenic. The living organisms mostly known to cause diseases in fish are bacteria, fungi, viruses and various types of parasites (Noga, 1996). On the other hand, nonliving agents that could adversely affect fish health may originate from inside or outside the fish. Externally, environmental conditions may change the normal physiological processes and cause diseases, while internally; endogenous factors such as fish genetic structure may predispose its defense to less than optimal environmental conditions or infectious agents (Halver and Hardy, 2002). It is well established that various fish species and certain strains within given species may exhibit wide differences in their tolerance to various environmental factors and infectious agents (Plumb, 1994; Wiegertjes *et al.*, 1996).

2.4.1.i Environmental Conditions

Different environmental conditions exist, which if allowed to exceed certain limits, could impair normal physiological processes of fish and as such, compromise their health. An inverse relationship between environmental quality and fish disease has been properly established in aquaculture (Plumb, 1994). The main environmental component which influences fish health is the water which it inhabits. Parameters such

as dissolved oxygen, un-ionized ammonia, nitrite, carbon dioxide and pH are of concern when fish are cultured under intensive conditions and can affect fish health. However, it is important to note that the tolerance levels to different water quality tend to be species specific (Stickney, 1994, Evans, 1993).

Some other important conditions in aquaculture are crowding, antagonistic interactions among fish and handling especially during harvest, sorting or grading which could also impose stress. Improper feeding regimes, inadequate nutrition in the form of nutrient deficiencies or the presence of toxins in the diet could also impose stress on fish. All forms of stress caused by different environmental conditions tend to reduce the health of fish and make them more susceptible to various kinds of pathogenic agents. Physiological changes resulting from these stress conditions are believed to suppress the natural resistance and immunity of fish (Halver and Hardy, 2002).

2.4.1.ii Infectious Agents

Infectious diseases are important causes of economic losses in commercial aquaculture. Different disease-causing organisms are known including; bacterial, fungal, parasitic and viral pathogens which infect fish (Noga, 1996). Some disease-causing organisms are obligate pathogens, requiring a host or an intermediate host to survive. However, facultative pathogens are able to live and multiply in a host or in the environment without the need for a host. Without a diseased condition being produced, the infection of a pathogenic organism cannot occur. Under favorable conditions however, infection by a pathogenic organisms result in diseases. Infectious diseases in aquaculture are very common, where fish could become stressed due to less than optimal

environmental conditions. Therefore, it is important to avoid conditions that induce stress in fish in order to maximize resistance to different disease-causing organisms (Halver and Hardy, 2002).

2.4.2 Diseases Associated with Rainbow Trout

2.4.2.i Bacterial diseases of Salmonids

The most economically important diseases impacting the aquaculture of salmonids are caused by bacteria. The need for the control of bacteria in salmonid farming has been the major reason for the development of good husbandry practices, chemotherapy and vaccination programs in aquaculture. Some bacterial diseases in salmonids appeared only recently in some parts of the world (e.g. *Yersinia ruckeri* spread throughout Europe during the 1980s, as did furunculosis in Norway). Most of the important bacterial diseases of salmonids are widely distributed. In Table 2.2, the bacterial diseases of salmonids, their causative agents and the principal signs of the diseases are shown.

Table 2.2: Principal bacterial diseases in salmonids (Penel and Barton, 1996)

Disease	Causative agents	Signs	Main host and Geographic range
Furunculosis	<i>Aeromonas salmonicida</i>	Internal haemorrhaging, vasodilation, liquefied kidney, furuncles (except Chile)	All salmonids specially <i>salmo</i> spp., FW and SW. Worldwide
Vibriosis	<i>Vibrio anguillarum</i> <i>ordalii</i>	Skin ulceration and haemorrhaging, pale gills, petechial haemorrhages in visceral organs, swollen spleen, liquefied kidney	All salmonids in SW. Worldwide
Coldwater vibriosis	<i>Vibrio salmonicida</i>	Pale gills, petechial haemorrhages in ventral surfaces, swollen inflamed vent. Extensive haemorrhages internally, pale liver	Atlantic salmon, SW. Norway
Enteric redmouth disease (ERM)	<i>Yersinia ruckeri</i>	Reddening and ulceration of mouth region. Internally haemorrhaging and swelling of kidney and spleen	Rainbow trout, FW. Worldwide
Bacterial kidney disease (BKD)	<i>Renibacterium salmoninarum</i>	Darkening, reddening fin bases, exophthalmia. Swollen kidney with whitish patches. Pseudo membrane over visceral organs	All salmonids specially rainbow trout, FW and SW. Worldwide
Columnaris disease	<i>Flexibacter columnaris</i>	Whitish mucoid patches on skin and gills overlying necrotic tissue	Rainbow trout, FW. Worldwide
Fin and tail rot	<i>Flexibacter columnaris</i>	Erosion of fins and tail	Salmonids, FW. Worldwide
Saddleback	<i>Flexibacter columnaris</i>	Grey patch around base of dorsal fin	Salmonids, FW. Worldwide
Salt water columnaris disease	<i>Flexibacter maritimus</i>	Erosion of fins, gill hyperplasia	Salmonids, FW. Tasmania
Bacterial gill disease	<i>Flexibacter branchiophila</i>	Swelling and fusion of gill filaments, excess mucus production by gills	Salmonids, FW. Worldwide
Coldwater disease Peduncle disease Rainbow trout fry anemia	<i>Flexibacter psychrophilus</i>	Severe erosion of tail fin and peduncle, pale gills, anemia	Salmonids, especially coho salmon, rainbow trout fry, FW. Worldwide
Salmonid rickettsial septicemia	<i>Rickettsia</i>	Mottled liver, enlarged kidney, empty gut	Salmonids, SW. Chile, Ireland
Pseudokidney disease	<i>Crnobacterium</i>	Petechial haemorrhage, exophthalmia. Haemorrhage in visceral organs	Salmonids, Europe, N. America

Other pathogens are found in the natural flora of the aquatic environment and are referred to as opportunistic pathogens. The obligate pathogens can often reside in or on the fish producing a "carrier" or "latent" stage of infection where no disease signs are evident. On the other hand, actual outbreaks of bacterial diseases, whether caused by obligate or opportunistic agents, are usually associated with the fish being stressed (Penel and Barton, 1996).

Streptococcus spp.; a genus of gram-positive, facultatively anaerobic cocci occurring in pairs or chains; it is separable into the pyogenic group, the viridians group, the enterococcus group, and the lactic group. It has been reported that *Streptococcal septicemia* (*Streptococcus* spp.) caused diseases in several farmed fish species. However, infection has been reported in rainbow trout in Japan, South Africa, Australia and Italy in water temperatures exceeding 20°C. Infected fish develop acute septicemia, often with high mortalities (Eldar *et al.*, 1994; Penel and Barton, 1996; Kessel *et al.*, 2000). *Streptococcus*-related diseases occur in many cultured marine and freshwater fish. Several streptococcal species are involved, and the relationship between aetiological agents, isolated from the different fish was not fully determined. In Israel, Streptococcosis also affects *Oreochromis* spp., grey mullets (*Mugil cephalus* and *Liza ramada*) and silver carp (Hubbert, 1989).

Aeromonas hydrophila; Motile aeromonad septicemia could be caused by the *Aeromonas hydrophila*, which is common in the aquatic environment especially in warm, organic-rich fresh water. However, such environments are not suitable for the culture of most forms of salmonids. Therefore, this disease is mainly restricted to rainbow trout culture in warm countries. There is some debate as to whether *A. hydrophila* is a primary pathogen of fish, as its infection is usually associated with

severe stress, physical trauma or following infection by another pathogen (Penel and Barton, 1996).

Pseudomonas fluorescens is an opportunistic pathogen which could cause a haemorrhagic bacteraemia or superficial ulcer formation in salmonids. It is associated with elevated temperatures and poor husbandry conditions and is widely distributed in the soil and water (Penel and Barton, 1996).

Pseudomonas anguilliseptica has gross signs of infection which include petechial haemorrhages in the skin, peritoneum and liver. Antibiotic therapy of its infection with oxytetracycline has not been successful (Wiklund and Bylund, 1990). It has been isolated from farmed Atlantic salmon, sea (brown) trout and rainbow trout in Finland (Penel and Barton, 1996).

Mycobacteriosis and Nocardiosis; methods of expression in fish, *Mycobacterium chelonae* has been reported to cause disease in Pacific salmon hatcheries in North America and Australia (Penel and Barton, 1996).

Nocardia asteroides has caused disease in rainbow trout and brook trout hatcheries in North America but these outbreaks appear to be very rare (Penel and Barton, 1996).

Lactobacillus piscicola; for many years, *Lactobacillus piscicola* was linked to the so-called pseudokidney disease in mature rainbow trout. It is a stress-related disease, resulting in low mortality and its main significance is in sharing similarity to *Renibacterium salmoninarum* in gram-stained kidney smears (Penel and Barton, 1996). However, it is readily distinguishable from the latter by serological and biochemical tests (Austin and van Poucke, 1993). On the basis of biochemical, physiological and

chemical criteria, lactobacillus has been classified into a new genus, *Carnobacterium* (Collins *et al.*, 1987).

Epitheliocystis and *Salmonid Rickettsial Septicaemia* is another microorganism that causes diseases in salmonids. *Epitheliocystis* grows in gill epithelial cells, which become swollen and appear as small whitish cysts on the gills. On the other hand, *Piscirickettsia salmonis* causes rickettsial septicaemia and is often accompanied with severe mortalities in Atlantic, Coho and Chinook salmon and rainbow trout from seawater farms in Chile (Cvitanich *et al.*, 1991; Lannan and Fryer, 1993). Recently, outbreak of *piscirickettiosis* occurred in Chile, in rainbow trout reared in fresh water (Bravo, 1994). Rodger and Drinan (1993) also reported a similar rickettsia-like organism in Atlantic salmon post-smolts from the west coast of Ireland (Penel and Barton, 1996).

2.4.2.ii Viral diseases of Salmonids

Viral Haemorrhagic Septicaemia (VHS), Infectious Pancreatic Necrosis (IPN) and Infectious Haematopoietic Necrosis (IHN) are the three major viral diseases identified in salmonid cultivation. All the three diseases are also the main viral problems in rainbow trout (*O. mykiss*) culture. The aetiological agents of these diseases are yet to be identified, although strong evidence exists to indicate viral causes (Penel and Barton, 1996).

2.4.2.iii Fungal diseases of salmonids

Mycosis frequently occurs as a consequence of stress and sexual maturity, as well as directly affecting eggs and fry. In most fungal diseases, there is often one or possibly several predisposing factors which affect the fish or the fungus. These include

pollution, low water levels, overcrowding, mechanical disturbance such as handling and changes in hormonal status and the failure to remove moribund or dead fish or ova (Miyazaki *et al.*, 1977).

Of the four orders of Oomycetes class, *Saprolegniaceae* family has caused the majority of the reported infections in living fish, with the genus *Saprolegnia* being the most significant. *S. parasitica* and *S. diclina* are the principal species involved in disease outbreaks, although this taxonomically difficult group is often referred to as the *Saprolegnia* complex (Neish and Hughes, 1980; Willoughby, 1985). *Achlya*, *Aphanomyces*, *Dictyuchus*, *Leptolegnia*, *Pythiopsis*, *Thraustotheca* and *Pythium* are the other genera involved as salmonid parasites within in Oomycetes (Penel and Barton, 1996).

2.4.2.iv Protozoan parasites of salmonids

The protozoa are a diverse group of unicellular eukaryotic organisms. In most cases, they possess a monogenetic life cycle. Many protozoans are described from salmonids worldwide and only a limited description could be given here. Protozoan parasites that are of significance in salmonids are *Ceratomyxa shasta*, *Ichthyobodo necatrix*, *Myxobolus cerebralis*, *Kudoa thyrsites*, *Hexamita salmonis* and the PKX (Proliferative Kidney Myxosporean) organism (Penel and Barton, 1996).

2.4.2.v Metazoan parasites of salmonids

The metazoan parasites are multicellular organisms. They display both ecto- and endoparasitic life cycles, causing significant diseases and mortalities. Metazoan parasites could be represented by several groups including monogenetic and digenetic trematodes,

cestodes, nematodes, acanthocephalans, leeches, molluscan larvae and crustaceans (Penel and Barton, 1996).

2.4.3 Immunity and Disease Resistance

The fairly well-developed immune system of fish consists of nonspecific and specific components (Iwama and Nakanishi, 1996). The nonspecific component is the natural immunity and consists principally of phagocytic mechanisms associated with macrophages and granular leukocytes such as neutrophils. They are designed to attack microorganisms which invade the skin or mucous membranes of fish. Besides phagocytes, there are other soluble factors such as lysozyme and complements which may assist in destroying invading pathogens. The specific component of the immune system consists of humeral and cell-mediated responses which could provide specific immunological memory, although the immune memory of fish is generally considered to be less developed than that of mammals.

Antibodies are produced against specific pathogens. These bind to the pathogen's membrane so that it could be destroyed via activation of the complement system through its classical pathway (Halver and Hardy, 2002).

2.5 Digestive enzyme activity processes

Enzymes act in the breakdown of nutrients in foods into compounds which could be absorbed across the brush border membrane of the enterocyte. These enzymes are excreted into the lumen, bound to membranes, or contained in supranuclear vacuoles within the enterocyte. This is why in fish, digestion occurs using one or more digestive