

Study on effects of different plant and animal dietary protein sources as replacements to fish meal in vannamei (*Litopenaeus vannamei*) reared under varying water salinities

BY

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CERTIFICATE

This is to certify that **Ms. P. Pravallika**, I.D.No. MFM/2013-003 has satisfactorily persecuted the course of research and that the thesis entitled “ **Study on effects of different plant and animal protein sources as replacements to fishmeal in vannamei (*Litopenaeus vannamei*) reared under varying water salinities** ” submitted is the result of her original research work and so sufficiency high in standard enabling its presentation to the evaluation. I also certify that the thesis or part thereof has not been previously submitted by her for a degree or diploma of any University.

Date: 31 -08-2015

D.RAVINDRA KUMAR REDDY

(Chairperson of the Advisory Committee)

CERTIFICATE

This is to certify that the thesis entitled “**Study on effects of different plant and animal protein sources as replacements to fishmeal in vannamei (*Litopenaeus vannamei*) reared under varying water salinities**” submitted in partial fulfillment of the requirements for the award of the degree of **MASTER OF FISHERY SCIENCE in AQUACULTURE** in the **Faculty of Fishery Science** of Sri Venkateswara Veterinary University, Tirupati is a record of the bonafide research work carried out by **Ms. P. Pravallika**, I.D.No.MFM/2013-003 under our guidance and supervision. The subject of the thesis has been approved by the Student’s Advisory Committee.

No part of the thesis has been submitted for the award of any degree or diploma. All assistance and help received during the course of investigations have been duly acknowledged by the author of the thesis.

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DECLARATION

I **Ms. P. Pravallika** , hereby declare that the thesis entitled “**Study on effects of different plant and animal protein sources as replacements to fish meal in vannamei (*Litopenaeus vannamei*) reared under varying water salinities**” submitted to Sri Venkateswara Veterinary University, Tirupati for the degree of **MASTER OF FISHERY SCIENCE** majoring in **AQUACULTURE** is the result of original research work done by me. I also declare that the materials contained in this thesis have not been published earlier.

Date: 31-08-2015

(P. Pravallika)

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ABBREVIATIONS

FM	Fish Meal
CSM	Cottonseed Meal
AI	<i>Acetes indicus</i> Meal
SF	Sunflower meal
MM	Mysid meal
T	Treatment
°C	Degree Centigrade
cm	Centimeter
mg/l	Milligram per liter
ANOVA	Analysis of variance
%	Percentage
Fig.	Figure
gm	Grams
ppm	Parts per million
spp.	Species
Temp.	Temperature
FCR	Feed Conversion Ratio
ppt	Parts Per Thousand
PER	Protein Efficiency Ratio
DO	Dissolved Oxygen
HUFA	Highly Unsaturated Fatty Acids
FRP	Fiber Reinforced Plastic
MBM	Meat- Bone Meal

PBM	Poultry By- Product
RCM	Red crab meal
'/..	Parts per thousand (ppt)

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Abstract

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Major advisor : **Dr. D. RAVINDRAKUMAR REDDY**

University : Sri Venkateswara Veterinary University

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The present study “ **Study on effects of different plant and animal protein sources as replacements to fishmeal in vannamei (*Litopenaeus vannamei*) reared under varying water salinities** ” was conducted in the Wet Laboratory of the Department of Aquaculture, College of Fishery Science, Sri Venkateswara Veterinary University, Muthukur.

Cottonseed meal, (CSM), sunflower meal (SF), *Acetes indicus* (AI) meal, Mysid meal (MM) and Fish meal (FM) were used at three different protein levels (25%, 30% and 35%). Shrimp diet was prepared by mixing all the ingredients in required proportions, ground and

sieved to the required size and made in to pellets. Studies were conducted to evaluate the replacement of fish meal with Cottonseed meal, (CSM), sunflower meal (SF), *Acetes indicus* (AI) meal, Mysid meal (MM) 0ppt, 5ppt, 15ppt and 25ppt salinities.

The experiments were conducted for 63 days in aquaria. Triplicates were maintained for each of the treatment. Feed was given three times a day. Weekly sampling was done for water quality parameters viz., pH, Temperature, Dissolved Oxygen (DO), Total alkalinity and Total hardness. The growth, survival and feed conversion ratio were estimated by sampling the shrimp once in a week.

The temperature values recorded during 63 day period of the experiment at 0 ppt, 5 ppt, 15 ppt ranged between 29.5°C -31.6 °C and at 25 ppt 29.6 °C -31.7 °C overall the lowest temperature was recorded at 0,5,15 ppt during the initial day and the 14 day, whereas the highest temperature was recorded at 25 ppt during the 63 day irrespective of the dietary protein content and experimental diets. Table no-3

The pH values recorded during the 63 day of period of experiment at 0 ppt salinity ranged between 8.2 to 8.3, at 5ppt salinity 8.2 to 8.3, at 15 ppt salinity 8.3 to 8.4 and 25 at ppt salinity 8.4 to 8.5. Overall the pH lowest value was recorded at 0 ppt salinity where as the highest at 25 ppt salinity of all the treatments irrespective of the dietary protein content and experimental diets table no.5

The DO was found to be in the range of 7.84ppm and 6.31ppm at 0ppt, 7.77ppm to 6.08 ppm at 5ppt and 7.50ppm to 6.55ppm at 15ppt and 7.30ppm to 5.55ppm at 25ppt salinity. The pH ranged from 8.2 to 8.3 at 0ppt, 8.2 to 8.3 at 5ppt , 8.3 to 8.4 at 15ppt and 8.4 to 8.5 at 25ppt during the period of experiment. The range of total alkalinity varied between 214 mg/l to 155 mg/l at 0ppt salinity, 315mg/l to 229 mg/l at 5ppt salinity , 625mg/l to 535mg/l at 15ppt and 725mg/l to 645 mg/l at 25ppt salinity. Total hardness of water ranged

between 238 mg/l to 191 mg/l at 0ppt salinity, 322mg/l to 240 mg/l at 5ppt salinity , 675mg/l to 601mg/l at 15ppt salinity and 777 mg/ l to 700 mg/l at 25 ppt .

Weight of shrimp in grams and weight increment data observed weekly for different treatments at different salinities were presented in tables 8 to 9 figure 13,14,15 and 16 statistical analysis table number 10,11,12 and 13 a respectively.

Overall after 63 days, the highest average body weight of 14.01 gms (MM 30) and the lowest average body weight of 9.26 gms (CSM 25) at 15 PPT salinity and 0 PPT respectively.

The highest ABW of 12.30 gms, 13.55 gms, 14.01 gms, and 13.95 gms at 0, 5, 15 and 25 PPT salinities respectively (MM 30). The lowest ABW of 9.26, 10.50, 11.11 and 11.70 gms at 0, 5, 15 and 25 PPT salinities respectively (CSM 25)

Specific growth rates for *L.vannamei* treated with different diets at four different salinities were calculated and presented in tables (14) and figure 21 respectively at 0ppt 5ppt 15ppt and 25ppt Salinities. The specific growth rates by end of the experimental period (63 days) were calculated for all treatments reared at 0, 5, 15 and 25 salinities.

The SGR for *L.vannamei* treated with different diets reared under different salinities were regard between 0.05 to 2.11 for the entire experimental period. At 0 ppt the highest value was recorded 1.7 (FM 35) another lowest value of 0.05 (MM 30); at 5 ppt the highest value was 2.11 (FM 35) and the lowest value was 0.01 (AI 35), at 15 ppt the highest value was 2.02 (FM 35) another lowest value was 0.49 (SF 25) and finally at 25 ppt the highest value was 2.07 (FM 30) and the lowest value was 0.49 (SF 25, MM 25) recorded respectively. Presented at table number 14 figures number 21.

The Feed Conversion Ratio in different experiments of *L. vannamei* groups reared at varying salinities were calculated and presented in the table number 15 and figure number

22. The highest and lowest values were recorded 2.50 (CSM 25, CSM 35, SF 25) at 0 salinity and 1.45 (FM 30) at 25 salinity respectively. At 0 salinity the highest value recorded CSM 25, CSM 35, SF 25 (2.50) and the lowest value for 1.85 (FM 30), where as at 5 ppt the highest value for 2.45 (SF 25) and the lowest value for 1.75 (FM 30), for 15 ppt the highest (2.40 – CSM 25) and lowest (1.59 – FM 30) and finally at 25 ppt the highest (2.30 – SF 25) and the lowest (1.45 – FM 30) respectively.

Survival percentages of *L. vennamei* fed on different experimental diets reared under veering salinities and the statistical analysis were presented in table number 16 figures number 17, 18, 19 and 20 respectively.

Overall the survival percentage varied from 55% (CSM 30, 35 – 63rd day) – 100%. (FM 25,30, 35 AI 25, MM 25, 30, 35 – 7th day).

After final sampling, at 0 ppt the highest survival recorded were 85.% (FM 25, 30,35) and lowest 55% (CSM 30,35); at 5 ppt the highest 90% (FM 25,30,35) and lowest 60% (CSM 30, SF 25, 30 ,35); at 15 ppt the highest 95 % (FM 25, 30, 35) and lowest 60 % (CSM 30, 35, SF 35) and finally at 25 ppt the highest 100 % (FM 20, 25, 30) and the lowest 65% (CSM 30,35) respectively

1. Introduction

Shrimp is one of the world's most popular shellfish. It provides high quality rich protein, calcium and various extractable compounds and minerals for human body, while low in calorie and fat (Abdullah *et al.*, 2009). Marine shrimp continued to dominate crustacean aquaculture, with three major species accounting for over 86% of total shrimp aquaculture production in 2000 (the giant tiger prawn, *Penaeus monodon* the fleshy prawn, *Penaeus chinensis*; and the white leg shrimp, (*Litopenaeus vannamei*).

Global consumption of shrimp and fish has been increasing steadily during the past decade as result of economic growth and expanded aquaculture production. As shrimp aquaculture is expected to continue to increase in coming years, shrimp prices are likely continue to fall as production exceeds demand, therefore challenging the profitability of this industry. (Amaya *et al.*, 2007b) One factor considered to reduce shrimp production costs and increase producers profitability, is the use of feeds with low levels of fish meal and high levels of less expensive, high quality plant protein source. Since 1991, Thailand has been the world's leading shrimp exporter, with black tiger shrimp (*Penaeus monodon*) being the most common cultured marine shrimp from the 1990s until 2004 (Limsuwan and Chanratchakool, 2004). However, since the introduction of the Pacific white shrimp (*Litopenaeus vannamei*) into the country, shrimp farming in Thailand has been undergoing a dramatic transformation. The main reason for this change is that *Litopenaeus vannamei* has a faster growth, higher stocking rate and yield, and incurs lower production costs than *Penaeus monodon*.

Replaced that , *Litopenaeus vannamei* species can be considered as a good source of fatty acid as well as protein. *Litopenaeus vannamei* is the primary aquaculture species produced in the Western Hemisphere. It has the reputation of being a tolerant species, able

to adapt to fluctuations in salinity, pH and dissolved oxygen levels (Rosenberry, 1999).

The culture of marine shrimp in low-salinity water in inland areas has become common in many regions of the world including: Australia, China, Ecuador, India, Thailand, United States, and other countries (Boyd and Thunjai, 2003).

(Litopenaeus vannamei) are easier to reproduce than *Penaeus monodon*. The uniform growth rate of this species is also an advantage in marketing. This species has also been subjected to disease problems lately. To overcome these disease problems, one proposed solution to white leg shrimp production problems is the use of water with salinities lower than sea water. Several researchers like (Van Wyk *et al.*, 1999; Mc Graw *et al.*, 2002; Saoud *et al.*, 2003; Sowers and Tomasso, 2006) have studied the growth of *(Litopenaeus vannamei)* in different salinities.

The Government of India (GOI) permitted pilot-scale introduction of the species in 2003 and subsequently permitted the culture of *(Litopenaeus vannamei)* in the country in 2008 based on a risk analysis carried out by Central Institute of Brackish water Aquaculture (CIBA) and National Bureau of Fish Genetics Resources (NBFGR) which recommended the pilot-scale introduction of *(Litopenaeus vannamei)* culture in India *albeit* with strict regulatory guidelines. Accordingly the nodal research and development agencies have jointly evolved detailed guidelines for importing brood stock, seed production and culture of *(Litopenaeus vannamei)* . (Kumaran *et al.*, 2012).

The basic principle of shrimp culture system is the stocking of various fast-growing, compatible species of shrimp with complementary feeding habits to utilize efficiently the natural food present at different ecological niches in the pond for maximizing shrimp production (Albertoni *et al.*, 2003).

L.vannamei offers numerous advantages over *P.monodon* for the Asian shrimp farmer. Other specific advantages include: rapid growth rate, tolerance of high stocking density, tolerance of low salinity and temperature, lower protein requirements (and therefore production costs), certain disease resistance (related to SPR stocks), high survival during larval rearing, and some marketing advantages.

The Pacific White shrimp *Litopenaeus vannamei* has become the main crustacean species produced through aquaculture, with production exceeding that of the black tiger shrimp *Penaeus monodon* (FAO, 2007; Briggs *et al.*, 2004; Liao and Chien, 2011).

L.vannamei is the dominant penaeid species currently cultivated in the Western Hemisphere. Temperature and salinity are two very important environmental factors in the culture of this and other shrimp species. In view of consistencies in published information regarding salinity effects on shrimp survival and growth, the optimum salinity for *L.vannamei* is still not conclusive. The species is found in the waters with a wide salinity range (1 to 40ppt). The high tolerance of *L.vannamei* to low salinity and the year round availability of healthy post-larvae make this species an excellent candidate for inland farming. *L.vannamei* species can be readily reproduced in captivity, has wide tolerance to environmental parameters, better utilizes low-protein containing diets, and grows fast compared to other penaeid shrimp species (Wyban, 2007).

As the Indian shrimp farming industry has exploded from a minor producer of shrimp to one of global importance, several factors have stimulated efforts to find alternatives for animal protein sources in the manufacture of shrimp feeds. Undoubtedly, price is the key reason to look for alternatives. The supply and price of high quality fish meal can vary dramatically from year to year. Fish meal is often scarce and expensive especially good quality brands, hence the cost of fish production and nutrition is often very high.

Fish meal is a preferred ingredient in balanced rations for shrimp because of its high protein content, essential amino acid composition (particularly lysine and methionine), omega-3 fatty acids, mineral content and acceptable palatability and digestibility (Zaldivar, 2002). Fish meals, as well as other marine animal meals such as krill, shrimp, squid, and scallop waste are often included in aquatic diets as they are considered an excellent source of high-quality proteins, highly unsaturated fatty acids, minerals, and attractants (Tacon and Akiyama, 1997). However, fish meals and marine by-products are commodities for which supplies are limited and demand is expected to continue to increase. Hence, maintenance of the economical viability of commercial aquaculture will require the replacement of expensive marine proteins, with lower cost ingredients for which production is not limited. Protein sources that can be utilized to replace marine protein sources, either partially or completely, include both terrestrial plant and animal sources that are either locally available or traded on the world market. Replacement of marine proteins in shrimp feeds has met with different degrees of success.

(Amit 2012). Reported that Even though fish meal and other marine meals is excellent sources of protein and other essential nutrients for aquaculture diets, their demand is subject to competition from other sectors of the agriculture industry.

Fish meal high prices can create the perverse incentive to increase fishing effort when anchoveta are scarce. This possibility as caused some concern among those who fear that higher fish meal and oil demand will lead to greater fishing pressure on stocks of reduction fish (Naylor et al., 2000). Animal origin ingredients such as fish meal, poultry by-product meal and meat and bone meal are considered the most suitable protein sources for shrimp feeds. Also, the supply, quality, and price often fluctuate from year to year due to both market constraints and natural phenomena. Marine fish meal is often utilized in aqua

feeds because it offers a balanced source of indispensable amino acids, essential fatty acids, vitamins, minerals, and generally enhances palatability. (Hernandez *et al.*, 2008).

Fish meal is one of the most expensive ingredients of aquaculture diets. It is estimated that more than 50% of the variable costs of fish production are feed costs (Woods 1999), so profitability of production is significantly influenced by feed. Fish meal is a protein source traditionally used in aquaculture diets, yet it is a limited resource and is expensive (FAO, 2006). Fish meal has an almost ideal available amino acid profile for most aquatic species and is highly palatable, making the finished feed very attractive and promoting maximum feed intake.

The production of fish meal from major producers with Chile and Peru combined providing about 40% of production. This drop in production due to El Niño in 2006 and tight food regulations from CEP nations combined with continuing strong demand, particularly on China, resulted in a strong rise in price over last few years. This in turn has reinforced the concern of some that fishmeal and oil are going to become limiting factors to aquaculture production. Fish meal is preferred among other protein sources because it is an excellent source of essential nutrients such as protein and indispensable amino acids, essential fatty acids, cholesterol, vitamins, minerals, attractants and unidentified growth factors (Swick *et al.*, 1995; Samocha *et al.*, 2004). However, limited availability and high demand make fish meal a costly ingredient.

Hence, fish meal replacement strategy and dietary crude protein of aquatic feeds have gradually become primary concern. Any substitute for fishmeal, shall provide adequate nutrition and shall be economically feasible. In the formulated practical diets, protein is the most expensive energy component, and its quality represents a very important nutritional aspect in aquaculture. In Andhra Pradesh, a number of factors have limited expansion of Tiger shrimp (*Penaeus monodon*) including the high cost

of coastal real estate and the constant appearance of viral diseases such as white spot which has brought the industry near to collapse and 90% of *Penaeus monodon* farms have been converted to *L. vannamei* by the farmers. The Union Agriculture Ministry Government of India has finally granted permission to culture *Litopenaeus vannamei* (white leg shrimp) in India during the year 2009, a long-pending demand of aquaculture and seafood export industry of the country. According to the Government of India, permission for importing brood stock of *L.vannamei* would be granted by the Coastal Aquaculture Authority (CAA) Chennai, keeping the annual requirements in mind. Notification to this affect was issued Under Livestock Importation Act, 1898 (Amended in 2001) for The Operation of Aquatic Quarantine for the Import of *L. vannamei*.

In this context, research efforts have been directed to identify novel, alternative and economically viable plant protein sources for partially or totally replacing fish meal in the shrimp feed. One of the possible alternative plant protein sources is cottonseed meal, and sunflower meal. The replacement of fish meal with cottonseed meal in shrimp diets has met with varying degrees of success. There is a growing demand to substitute fish meal with less expensive protein-rich plant sources (Rumsey 1993) and in this respect, legume seeds and oilseed meals have economic potentials (De la Pena *et al.*, 1987; Lim and Dominy 1991).

Due to the fishmeal relatively high cost, cost variability, and growing environmental concerns about harvesting wild fish to produce fish meal, it is desirable to replace fish meal, with less expensive protein sources. The ultimate aim of artificial feeding in aquaculture is to achieve maximum protein deposition with minimum inputs of feeds at a minimum cost. The development of commercial aqua feeds has been traditionally based on fish meal (FM) as the main protein source due to its high protein content and balanced essential amino acids (EAA), digestible energy sources, minerals and vitamins. Because of their low price and

consistent quality, plant proteins are often an economically and nutritionally viable source of protein.

Replacement of fish meal with cheaper ingredients of either vegetable or animal origin in fish feed is necessary because of rising cost and uncertain availability of fish meal. It is desirable that diets be prepared with locally available ingredient to make formulation easier and to lower cost of production.

The Total replacement of fish meal with commercially available plant and animals meals is more problematic than the replacement of fish meal. However an attempt has made for total replacement of fish meal with plant and animal proteins. *L.vannamei* has high growth rate, adoption to various culture systems, high market value, excellent response to compounded feed and high resistance against diseases, *L.vannamei* has become the primary culture shrimp through domestication.

Among the alternative animal protein sources *Acetes indicus* , and *Mysid meal* may be considered to be a suitable ingredients for replacing fish meal in fish and crustacean diets because of its high nutritional value (Anh *et al.*, 2009). In Andhra Pradesh *Acetes* spp. formed 48.74% of the non-penaeid prawn catch. Non-penaeid prawns constitute one of the important fishery resources in India, contributing 4.47% to the total marine fish production of the country. The non-penaeid prawn resource is multispecies, mainly constituted by the tiny species of prawns, viz. *Acetes indicus* Milne Edwards. Among these prawns the sergested prawn *Acetes indicus* is the most abundant and occurs almost all along the coastline of India (Deshmukh, 1993).

Mysid shrimp are commonly used by aquarium hobbyists, and sometimes commercial aquaculturists, to feed to seahorses (Garrick-Maidment 1997; Hilomen-Garcia 1999; Forteach 2000) because they appear to promote good growth and survival.

The shrimp farming industry is currently experiencing major crop losses due to disease outbreaks sometimes associated with the deterioration of water quality, which makes the establishment of salt water ponds economically risky. Instead, the culture of penaeid shrimp in low-salinity waters, which not only provides a cost-effective solution to prevalent disease problems but also improves the use of saline-alkaline land waters, is now popular in many regions of the world (Bray *et al.*, 1994; Dong 2003). The Pacific white shrimp *Litopenaeus vannamei* possesses the ability to tolerate low salinities. Recently, there has been increasing interest in the culture of this species in low salinity waters or fresh water (Saoud *et al.*, 2003), for which information is required on the effects of salinity performance, principally growth and survival. Little is known about the feasibility of using *Sunflower meal*, *cottonseed meal*, *Mysid meal* and *Acetes indicus* as a source of dietary protein in feed formulations for *L.vannamei*. The objective of the present study was to demonstrate the effects of the fish meal replacement by cottonseed meal, sunflower meal plant protein and *Acetes indicus*, mysid meal animal protein on the growth performance and feed efficiency in *L.vannamei* reared under different salinities.



Plate.1: *Litopenaeus vannamei* (White leg shrimp)

Shrimp farming has increased over the years, culture methods are becoming more intensive. Intensification of the culture system means higher dependence on the use of manufactured dry feed that may represent an important part, or be the single greatest expense, of the variable cost of the operation. Protein sources that can be utilized to replace marine protein sources, either partially or completely, include both terrestrial plant and animal sources. One factor considered to reduce shrimp production costs and increase producers profitability, is the use of feeds with low levels of fish meal and high levels of less expensive, high quality plant and animal protein sources.

Marine protein sources are often utilized in aquatic feeds because they are an excellent source of indispensable amino acids, essential fatty acids, vitamins, minerals, and generally enhance palatability. Marine by-products such as scallop waste, lobster waste, squid viscera and shrimp head meal have been evaluated as alternative marine protein sources (Carver *et al.*, 1989; Sudaryono *et al.*, 1995). Much attention has been devoted to the evaluation of plant proteins such as: soybean meal (Lim and Dominy, 1990; Piedad- Pascual *et al.*, 1990; Tidwell *et al.*, 1993; Sudaryono *et al.*, 1995)., solvent-extracted cottonseed meal (Lim, 1996) lupin meals (Sudaryono *et al.*, 1999) various legumes, cowpea, green mungbean, rice bean. and leaf meals (Eusebio, 1991; Eusebio and Coloso, 1998) and papaya or camote leaf meal (Penaflores, 1995). Replacement of marine proteins in shrimp feeds has met with different degrees of success. Studies on the protein requirement of the prawns and shrimps have been reviewed in detail by New (1976, 1979, 1980), Wickens (1976), Gopal (1986) and Ali (1988). Many of these authors also had discussed the problems associated with conducting the basic nutrient requirement studies in crustacean.

2.1 PROTEIN:

Guillaume (1997), defined protein requirement as the minimum or the maximum amount of protein needed per animal per day. Protein requirements changes with respect to biotic factors (e.g., species, physiological state, size) and dietary characteristics (e.g., protein quality, energy: protein ratio). Abiotic factors such as temperature and salinity may also effect the protein requirement (Guillaume 1997). The protein requirement of a given species is often based on the response (e.g., growth, feed efficiency, protein conversation ratio) of the animal to varying levels of dietary protein under a given set of circumstances. Hence, the requirement is generally described as the optimal protein content of the diet. It has also been shown with shrimp that size (weight) affects growth response relative to the protein content of the diet.

(Millward 1989). Reported that the protein requirement of any organism need, not only to satisfy the organism's need for substrates, for the maintenance and growth, but also, to exert a regulatory influence on the organism which activates the various processes associated with the growth. The protein requirement for shrimp is often described as the optimal protein content of the diet (i.e., 20, 30, 40% crude protein) and its determination is usually based on the response of experimental animals (i.e., growth performance) to varying levels of dietary protein under some specific conditions (dose-response method) (Colvin and Brand, 1977; Kanazawa, 1990; Cousin *et al.*, 1991; Shiau *et al.*, 1991; Aranyakananda and Lawrence, (1993); However, by this method different investigators have reported different levels of protein as the best level, resulting in controversy with regards to optimal dietary levels. The quantity and quality of dietary protein are primary factors influencing shrimp growth, nitrogen loading of the culture system and feed costs.

Azad (1996) reported that protein requirements of fish and shellfish are high, both as a source of amino acids for protein synthesis and also for gluconeogenesis. The dietary protein requirements are met as a function of nutrient density (eg., protein level) of the diet and feed intake. Dietary protein

levels ranging from 30 to 60% are recommended for various species and sizes of marine shrimp (Akiyama *et al.*, 1992). The optimal level of dietary protein was determined to be within the range of 52-57% for *P.japonicus* (Deshimaru and Yone, 1978) and later revised not to exceed 42% (Koshio *et al.*, 1993), 40-44% for *P.monodon* (Alava and Lim, 1983; Shiau *et al.*, 1991). The protein requirement has been defined more adequately as the amount of protein required per animal biomass per day (Kureshy and Davis 2002), and it is often based on the response to varying levels of dietary protein under a particular set of conditions that would produce the most favorable production response, i.e. weight gain, feed efficiency or protein conversion efficiency.

2.2 PROTEIN REQUIREMENT IN *Litopenaeus vannami*:

A reasonable amount of work done on the protein sources and amino acids required for *L. vannamei* was first documented by Akiyama *et al.* (1991). Cousin *et al.* (1993) showed that *L. vannamei* responded to the feeds containing fish meal. It is generally believed that post larval shrimp required a higher dietary protein than older shrimp (Colvin and Brand, 1977; Chen *et al.*, 1985; Goddard, 1996), and *L.vannamei* postlarvae are often fed with protein levels of 35% when reared in nursery ponds with natural feed available (Villalon, 1991) whereas in intensive nursery system they are fed diets with protein levels of 40 – 55% (Sturmer *et al.*, 1992; Samocha *et al.*, 1993; Treece and Fox, 1993). Velasco (1998) did not find significant differences between growth at 25 and 33% dietary protein levels with postlarvae.

L. vannamei juveniles were found to have higher survival and growth rates (138mg/d) when fed a diet containing 11% dietary lipid level. *L. vannamei* juveniles performed best with a diet containing 40% protein. Colvin and Brand (1977) reported below 30% to be the protein requirement while Kureshy and Davis (2002) found a maximum protein requirement at 32% for juveniles and sub adults of *L. vannamei*. The first report of protein requirement for PL, raised in tanks during first molt period done by Colvin and Brand (1997) indicated 30-35 % protein levels for better results. Velasco *et al.* (2001)

examined the effect of dietary protein on the post larvae to verify the growth in a range of 10-33% of crude protein. Green *et al.* (1977) studied the effect of diet protein on food conversion during semi-intensive production of *L. vannamei* during dry season and rainy season on Honduras. (Teichert–coddington and Rodriguez (1995) studied different diets containing different levels of crude protein in the semi-intensive commercial grow out of *L. vannamei* in Honduras.

Rosas *et al.*, (2001a) worked on the effect of dietary protein and energy levels on growth, oxygen consumption, haemolymph and digestive gland carbohydrates, nitrogen excretion and osmotic pressure of *L.vannamei* and *L.setiferus* juveniles. Smith *et al.*, (1985). Described growth and digestability of three sizes of *L.vannamei* and effects of dietary of protein level and protein source. A study on the optimal growth or FCR for *L. vannamei* and for absolute requirement of protein for shrimp was carried out by Arayakananda and Lawrence (1993). Lawrence *et al.*, (1995) studied dietary requirement of juveniles and optimal PER using 25%, 35% and 45% of crude protein in levels for *L. vannamei*.

2.3 FISH MEAL:

Fish meal is the most preferred protein source due to its high protein content, good amino acid balance, essential fatty acids lack of anti nutrients, high palatability, and unidentified growth factors (Surik et al 1995) Fish Meal, as well as other marine animal meals such as krill, shrimp, squid, and scallop waste are often included in aquatic diets as they are considered an excellent source of high quality proteins, highly unsaturated fatty acids, minerals, and attractants (Tacon and Akimaya, 1997). Owing to these properties, fish meal has become one of the primary components of commercial diet formulations.

Chamberlain,(1993), Fish meal, is a commercial product made from both whole fish and the bones and offal from processed fish. It is a brown powder or cake obtained by rendering pressing the whole fish or fish trimmings to remove the fish oil. The supply, quality and price often fluctuate from year to year due to both market and environmental constraints. These are also environmental concerns, in terms of pollution, and over-fishing as well as ethical considerations for the use of fish products and could be used directly to feed humans. Due to these constraints replacement of fish meal and other marine proteins with alternative sources of proteins from terrestrial animals and plants has been encouraged (Tacon and Akiyama, 1997).

Fishmeal is a rich source of digestible protein that has an exceptionally good profile of essential amino acids particularly methionine; a rich source of digestible energy; a moderate source of lipids; and an excellent source of minerals and vitamins. Tacon *et al.*, (2006) reported that marine shrimp feed uses 22.8% of the fish meal used in aquaculture feeds. Commercially shrimp production historically contain between 25% to 50% fish meal, representing the primary and most expensive protein ingredient (Derjant-Li, 2002; Tacon and Borg, 1998). Based on numerous research with *L.vannamei* reared under a variety of culture conditions and densities there is clearly no need for fish meal in production diets (Amaya *et al.*, 2007a, b; Browdy *et al.*, 2006; Patnaik *et al.*, 2006, Samocha *et al.*, 2004).

Davis *et al.*, (2004b) studied on Practical diets for *Litopenaeus vannamei* Working and/or all plant production diets. These studies proved that both fish meal and marine oil sources can be removed from shrimp feeds if suitable alternative sources of protein and lipids are proved to meet essential amino acids and fatty acid requirement of the shrimp. Good quality fish meal contain crude protein levels above 66%, fat content around 8-11% and ash generally below 12%. Fishmeal has a number of favourable nutritional properties for the growth and survival of farmed crustaceans, Fishmeal is a dense source of high quality animal protein with a well balanced essential amino acid profile. It is also a good source of digestible energy, mineral, trace elements and

vitamins. Importantly, fishmeal provides omega-3 fatty acids that monogastric animals such as poultry and pigs cannot synthesize. Lysine, methionine and cysteine levels are all higher in fishmeal than vegetable protein meals (Lim, et.al., 1998).

A major portion (>60%) of fish meal produced globally is used for aquaculture (farming of finfish and shrimp). The intensification of aquaculture in Asia, and particularly in China, is increasing the demand for fish meal even though the supply cannot grow accordingly. Natural phenomenon such as the El Nino-Southern Oscillation affects the fisheries in Central America Pacific Coasts, leading to seasonal scarcities and increased prices. In times of high fishmeal prices, many aqua culturists have little attitude in their feed composition choices. Production of both crustaceans and of high-value finfish from aquaculture nearly doubles by 2020, contributing to higher demand for feed inputs. Strikingly, under faster aquaculture expansion scenario, real fishmeal and fish oil increase by about 50% by 2020. Most of the world's fishmeal comes from specialized fisheries (New and Wijkstrom 2002), However, it is probable that a long term increase in relative price of fishmeal to low-value food fish could, at some point, because low-value food fish to be processed into feed.



Plate 2 : Fish Meal

2.4 REPLACEMENT OF FISH MEAL :

Fish meal, the conventional protein source in aquaculture feeds, supports good fish growth because of its protein quality and palatability. Fish meal is often scarce and expensive especially good quality brands, hence the cost of fish production and nutrition is often very high. Fish meal is used in marine shrimp feeds because it is high in protein, digestible energy and is an effective feed attractant. Reasons for current interest in its replacement include irregular availability, variable quality, and perceived contribution to deterioration of fisheries, potential for adulteration, contamination with hydrocarbons and biological pathogens, and increasing cost.

There is a growing demand to substitute fish meal with less expensive protein-rich plant sources (Rumsey 1993) and in this respect, legume seeds and oil seed meals have economic potentials (De la Pena *et al.*, 1987; Lim and Dominy, 1991). The obvious questions arises as to why replace fish meal in commercial production feed formulation for fish. The following reasons are therefore offered:

- 1). Availability is sometimes irregular due to climate change (e.g., El Nino)
- 2). Protein level, quality and thus, digestibility, among types of fish meal are highly variable.
- 3). A perception of exploitation of fisheries
- 4). Because it is commonly adulterated with meat meals (among other things), it has potential to harbor bovine spongiform encephalopathy.
- 5). It can be contaminated with PCBs, Salmonella s. and
- 6) It cost can fluctuate tremendously (e.g., \$600-1,400/MT). In terms of marine and freshwater penaeid shrimp, several past and recent studies have evaluated replacement of fish meal with a variety of plant protein meals.

The major challenge for the aqua-feed industry is to find alternative feed resources that are sustainable and have all the necessary nutrients and qualities of fish meal and fish oil while minimising undesirable side effects such as slower growth, decreased animal health and changes to the nutritional content of the end product. It is imperative that the impact of potential fish meal and fish oil replacements on the nutritional, sensory, processing, and safety characteristics of aquaculture products is also carefully considered.

Fish meal can now be replaced with protein derived from a range of non-fish sources such as by-products from land animal processing, micro-algae, plants, zooplankton or even insects and bacteria. Micro-algae in particular, are seen as very high protein content and may also be rich in omega-3 fatty acids. Most studies show that the partial replacement of fishmeal or oil by vegetable or plant based raw materials does not affect the health or the growth of fish and even though feeds may be based on ingredient which contain lower levels of omega-3 fatty acids. According to the leading fish feed producers in Norway, vegetable based alternatives are now widely in use, and will be increasingly used in the future. Fish farmers have welcomed the use of plant ingredients in fish feed hoping it can help stabilise feed costs. (Schipp *et al.*, 2008).

Replacement of the fish meal currently used in feeds for other livestock is nutritionally easier. Partial or complete substitution already occurs to a limited extent and depends considerably on the relative prices of fish meal and other animal or vegetable proteins. Fish meal production has been static during the past decades and is likely to remain so. Therefore the price of fish meal will increase drastically and aquaculture must gradually reduce its reliance on fish meal as a fish / shrimp feed ingredient during its further production expansion. Increase in use of fish meal , not only for penaeid shrimp culture but also for other types of culture (livestock, fish etc.) has directed attention to assessing alternative ingredients as replacements for fish meal protein (Bharadwaj *et al.*, 2002; El-saidy and Gaber , 2002).

To replace fish meal with plant ingredients, the nutrient requirement of the species to be fed, as well as the nutrient composition and biological availability of the feed stuffs, should be known (NRC, 1993). Amaya *et al.*, (2007), reported the successful replacement of animal protein sources with plant proteins in shrimp feeds containing fish oil. Ideally, these alternative ingredients should have good availability and satisfactory nutritional quality for the

species to feed, and also to be economically practical (D Abramo and Lovell, 1991) many plant, animal by-products and microbial protein sources have been evaluated as replacements for fish meal (Davis and Arnold, 2000; Olvera-Novoa and Olvera- Castillo, 2000; Cruz-Suarez *et al.*, 2004; Yu, 2004).

Listed a large number of possible fish meal replacers, including invertebrate animal by-products (e.g, silkworm pupae, earthworms, and zooplankton), single-cell proteins (mainly from fungal and bacterial sources), oilseeds (e.g. soybean, rapeseed, sunflower, cottonseed), legumes (e.g. beans, peas, lupins) and miscellaneous plant protein products (e.g. corn gluten meal and concentrates made from potatoes and leaves). According to New and Wijkstorm (2002), generally poorer digestibility, lower availability of some essential amino acids, palatability problems and in some cases, the presence of anti-nutritional factors, have limited the replacement of fishmeal by plant proteins.

Recent statistics indicate future continued growth in aquaculture production although it is likely to be constrained by fish meal and fish oil supply (FAO, 2008). In response to this demand for protein sourced from fisheries, the potential replacement of fish meal in aquaculture diets with higher levels of plant derived proteins represents important advantages in terms of both, production economics and sustainable management of marine resources (Amaya *et al.*, 2007 b). Although terrestrial plant proteins tend to be less suitable for marine omnivores and carnivores than the high quality proteins found in marine feedstuffs, they are being used at increasingly high levels of substitution for fish meal. Several different types of nutrient sources are required to feed farmed shrimp and fish in order to maintain the expected rapid growth of this sector. Protein is a key growth related nutrient and constitutes a major proportion of shrimp and fish diets, and the search for new protein ingredients that are both nutritious and cost effective is a major focus for aqua feed producers.

The advantage of incorporating vegetable protein source in the diet of harvesting shrimp may eventually contribute towards a reduction a reduction of fish meal costs and waste products as well as to achieve sustainable shrimp farming as stated by Luis *et al.*, (2006) in their study on energy. To replace marine protein sources and oils in commercial feeds, one must have a complete strategy that allows for the replacement of required nutrients. It is clear that fish meal and most of the marine meals can be replaced either singularly with animal by-product meal or in combination with plant protein sources without affecting the physical and nutritional quality of the feed (Viola *et al.*, 1982, 1988; Tidwell *et al.*, 1993; Sudaryano *et al.*, 1995; Webster *et al.*, 1995; Wu *et al.*, 1995; Davis & Arnold 2000; Samocha *et al.*, 2004).

Many plant and animal by-products and microbial protein sources have been evaluated as replacements for fish meal (Davis and Arnold,2000; Olvera-Novoa and Olivera-Castillo,2000; Cruz-Suarez *et al.*,2004),and some of them are currently used in the feed manufacture industry. Alvarez *et al.* (2007) discussed the importance of substituting fish meal with Soybean meal in practical diets for juvenile white shrimp (*L. schmitti*). The relative complexity of replacing marine protein (Lim and Dominy 1990) has been described previously (Fox *et al.*, 1995, Lim *et al.*, 1997) and there were many interactions at different levels and total replacement of fish meal by plant proteins. Lim and Dominy (1990) described the evaluation of Soybean meal as a replacement for marine animal protein in diets for shrimp *L. vannamei*.

One factor considered to reduce shrimp production costs and increase producers profitability, is the use of feeds with low levels of fish meal and high levels of less expensive, high quality plant protein sources. (Dersjant-Li, 2002; Tacon and Barg, 1998; Gonzalez-Rodriguez and Abdo de la Parra, 2004). Feed cost contribute above 60% of the production cost of intensively reared shrimp (Tan and Dominy 1997). Hence, research needed to identify and utilize

less expensive and more sustainable ingredients in shrimp feeds. Many studies also that poly unsaturated fatty acids were essential in the diet for maximum growth and feed efficiency of *L.vannamei*(Lim *etal.*,1997;Gonzalez *etal.*,2002).

Proteins of plant origin are readily available and inexpensive but have a less than amino acid profile. Limiting essential amino acids could be economically supplemented using ingredients that are rich in those nutrients. Fish meal has been completely substituted by terrestrial protein sources in production diets of various fishes such as cat fish and tilapia (Webster and Lim,2002) and crustaceans such as *M.rosenbergii* (Tide well *et al.*,1993).several past and recent studies have evaluated replacement of fish meal with a variety of plant protein meals. Amaya *et al.*, (2007).studied on alternative diets for the pacific white shrimp *L.vannamei*.

Given the growing demand by animal production industries for fish meal and its limited supply, prices are likely to continue to increase, therefore restraining future use as the main protein source in shrimp feeds. Replace or minimize the inclusion of animal protein sources in commercial shrimp formulation using plant protein(Colvin and Brand,1977; Lim and Dominy,1990; Piedad-pascual *et al.*,1990; Lim *et al.*,1998; Hardy,1999; Divakaran *et al.*,2000; Davis and Arnold,2000; Tacon,2000; Mendoza *et al.*,2001; Dersjant-li,2002; Conklin,2003; Fox *et al.*,2004; Gonzalez-Rodriguez and Abdodelaparra,2004; Samocha *et al.*,2004). According to Davis *et al.*, (2004), the use of all plant protein feed can be limited due to a variety of factors including deficiency or imbalance of essential amino acids, reduced levels of minerals, limited levels of highly unsaturated fatty acids (HUFA), presence of antinutritional factors or toxins and decreased palatability.

Davis *et al.*,(2004), reported the successful replacement of animal protein sources with plant proteins in shrimp feeds containing fish oil was examined both in the laboratory setting and during a full-scale commercial trial of the feasibility of totally replacing the fish meal component of marine

shrimp (*L.vannamei*) diets. Feed represents the largest single operational variable during shrimp production (Molina-Poveda and Morales, 2004) and during semi intensive shrimp culture.

The high cost of shrimp feed mainly responds to the cost of fish meal and therefore finding a relatively lower cost alternative ingredient has been an ongoing research goal (Forster *et al.*, 2003; FAO,2006). In comparison to fish meal, however these products are of only limited utility because they vary in protein quality (amino acid profile) and digestibility (Bureau *et al.*, 2000). Small pelagic fish species from the bulk of capture fisheries landings destined for reduction,with anchovies (Family: Engraulidae) forming 46%; and herrings, pilchards, sprats, sadines and menhadens (Family:Clupidae) forming 40% of estimated landings for reduction in 1999(FAO 2000a).

Under aquaculture conditions shrimp are fed manufactured balanced diets which typically contain approximately 25-30% of fish meal (Tacon & Barg, 1998) The production of fishmeal from the major producers with Chile and Peru combined providing about 40% of production. This drop in production combined with continuing strong demand, particularly in China resulted in a strong rise in the price over the last few years. Allen Davis *et al.*,(1999).Studied on replacement of fish meal in practical diets for the pacific white shrimp, *L.vannamei*. The crustacean aquaculture sector is currently heavily dependent upon capture fisheries for sourcing key nutrients and feed ingredients for compound aqua feeds.

2.5 PLANT PROTEINS:

Plant proteins are produced in larger quantities than fish meals, their production is more stable, they are often expensive, and their expanded use does not threaten over exploitation of a limited resource as can occur with fisheries products. Other sources of plant proteins, such as sunflower meal,

cottonseed meal are commonly utilized. Usually the use of plant proteins shows some limitation due to a variety of factors including a deviancy or imbalance of essential amino acids, presence of anti nutritional factors or toxins, and decreased palatability. The inclusion of plant protein sources in aqua feeds is expanding due to the limited amount of fish meal available for the production of animal feeds. (Glencross *et al.*, 2005; Gatlin *et al.*, 2007). Many of these limitations can be overcome through the use of proper combinations of different types plant proteins to balance essential nutrient profiles (e.g., amino acids and fatty acids); through developing specific processing procedures to inactivate, reduce or eliminate anti nutritional factors.; and or through limiting their inclusion in the diet to a level that does not influence animal performance (Li *et al.*, 2000).

Protein sources that can be utilized to replace marine protein sources, either partially or completely, include both terrestrial plant and animal sources that either locally available or traded on the market. Replacement of marine proteins in shrimp feeds has not met with different degrees of success. Amaya *et al.*, (2007a) reported the use of an all-plant protein feed can be limited due to a variety of factors including deficiency or imbalance of essential amino acids, reduced levels of minerals, limited levels of highly unsaturated fatty acids (HUFA), presence of anti-nutritional factors or toxins and decreased palatability. According to Carver *et al.*, (1989), extrusion has the advantage of inactivating and/or destroying some of the heat-sensitive anti-nutritional factors found in plant protein sources, such as soybean meal, while also gelatinizing starch granules.

Plant proteins low and consistent quality, plant proteins are often an economically and nutritionally viable source of protein. However, due to potential problems with deficient levels of indispensable amino acids (eg.lysine and methionine), anti-nutrients and poor palatability, their use is often limited. Considerable attention has been devoted to the evaluation of plant proteins such as soybean meal Lim and Dominy, 1990; Solvent-extracted cottonseed meal Lim, 1996., lupin meals Sudaryono *et al.*, 1999., And various legumes cowpea, green mung bean, rice bean and leaf meals Eusebio, 1991.

Plant proteins are produced in larger quantities than fish meals, their production year to year is more stable, they are often less costly, and their expanded use does not threaten over exploitation of a limited resources as can occur with fisheries products (Davis *et al.*, 2004b). However, the use of plant protein can be limited due to a variety of factors including, deficiency or imbalance of essential amino acids, reduced levels of mineral, limited levels of HUFA, presence of anti-nutritional factors or toxins and decreased palatability. From a nutritional end point, fish meal, and most of the marine meals, can be replaced totally or almost completely by a single or a combination of plant protein sources (Viola *et al.*, 1982, 1988; Tidwell, *et al.* 1993; Sudaryono *et al.*, 1995; Webster *et al.*, 1995; Wu *et al.*, 1995; Davis and Arnold, 2000; Davis *et al.*, 2004b; Samocha *et al.*, 2004).

Commercial shrimp feed formulations generally include 25% fish meal and it is the primary and expensive protein ingredient. One important factor considered in reducing shrimp production costs and to increase producers' profitability is the use of feeds with low levels of fish meal and high levels of less expensive plant protein sources by optimizing the feed formulations (Davis *et al.*, 2008). Gatlin *et al.* (2007) reviewed the use of sustainable plant products. Optimization of this ingredient in commercial shrimp feed formulations (Akiyama *et al.*, 1989, Lim and Dominy, 1990; Piedad- Pascual *et al.*, 1990; Akiyama, 1991; Lemos *et al.*, 2000; Amaya *et al.*, 2007 b). Efforts were made to develop similar database on nutrient utilization for other plant protein sources in practical shrimp feed formulations like groundnut cake, coconut cake, and gingely cake in Indian white shrimp, *Fenneropenaeus indicus* (Ali, 1992). In the case of Pacific white shrimp, *Litopenaeus vannamei*, earlier work has demonstrated the suitability of plant proteins as an ingredient in feeds for this species.(Divakaran *et al.*, 2000) Four plant protein source such as soybean meal, peanut meal, cottonseed meal and rape seed meal were measured on *L. vannamei*.

2.51.SUNFLOWER MEAL:

In recent studies, some attempts were carried out to replace the high cost animal protein source by sunflower meal (low costs plant protein). Fagbenro and Davies (2000) showed that, replacement of 67% of fish meal by sunflower meal in tilapia diets did not significantly altered the final weight. In this respect, Olvera-Novoa *et al.*, (2002) found the possibility to replace animal protein source in tilapia fry diets with sunflower seed meal up to 20% without significant effect in BW of Nile tilapia fry while the highest replacing levels significantly decreased the BW. In another study, El-Saidy and Gaber (2002) reported that up to 50% dehulled sunflower meal protein could be used to replace fish meal as a protein source in the diet of Nile tilapia, *Oreochromis niloticus* without significant effect on the BW. Abbas *et al.*, (2005) reported that the gradual rise in replacement level of fish meal by sunflower meal negatively affected growth performance of major carps and the minimum decrease in fish production was recorded at 25% replacement level while the maximum decrease was recorded at 75% replacing level of fish meal by sunflower meal. Stickney *et al.*,(1996) found that sunflower protein concentrate can replace 25% fishmeal protein in rain bow trout diets. Dayal, J .S *et al.*,(2011) concluded that maximum inclusion level of sunflower cake could be 5% in tiger shrimp diets at 20% replacement of fish meal and the feed cost can be reduced by more than one rupee/kg.

Sunflower (*Helianthus annuus*) is a widely cultivated oil seed and its cake/meal is the residue of oil extracted from seeds. It is a widely used protein source in live stock, poultry and pig rations. It is a good source of methionine and arginine. Sunflower seedmeal is the fourth most abundant plant protein

source with annual worldwide production between 9 and 15 mmt (Hertrampf and PiedadPascual,2000; FAO, 2005b). However, despite the ready market availability of most oilseeds and pulses and their lower cost compared with fish meal, their use within compound aquafeeds is usually restricted by the presence of one or more endogenous antinutritional factors or antinutrients (NRC, 1993).

Plant proteins appear to be the most suitable alternatives for fishmeal in fish diets. The efficiency of the various alternative protein sources as a partial or complete replacement for fish meal has been individually evaluated in fish diets, e.g. sunflower meal, soybean meal, linseed meal, canola and cotton seed meal. Individually these plant by-product meals are fairly rich in protein and favorable essential amino acid profiles, but they are deficient in one or more essential amino acids and contained various quantities of anti-nutritional factors.

Sunflower meal has been reported to contain a lot of endogenous anti-nutritional factors, such as a protease inhibitor, an arginase inhibitor and the polyphenolic tannin chlorogenic acid (Tacon *et al.*, 1984). It has relatively high crude fiber content, which can reduce the pelleting quality and protein digestibility of the feed included at high levels (Kamarudin *et al.*, 1989). Sunflower meal also contains low levels of lysine. Despite these drawbacks, sunflower meal has been reported to be a good protein (Jackson *et al.*, 1982). Sunflower meal can be a cost-effective alternative to fish meal in tilapia, as shown by several experiments. Sunflower meal could replace 10 to 25% fish meal in the diets of Nile tilapia (*Oreochromis niloticus*) (El-Saidy *et al.*, 2002) and red breast tilapia (*Tilapia rendalli*) fingerlings (Olvera-Novoa *et al.*, 2002).

Feeding tilapia on sunflower meal increased the content in linoleic acid (18:2 ω 6) in the fish but resulted in low levels of desirable eicosapentaenoic (20:5 ω 3) and docosahexaenoic (22:6 ω 3) acids (Maina *et al.*, 2003). Juveniles of gilt-head bream could be fed up to 36% decorticated sunflower meal. However, animal performance and feed efficiency were higher at 10-12% sunflower meal inclusion and the best economical return was obtained at 14%

dietary level (Sanchez Lozano *et al.*, 2007). The use of sunflower in fish feeding; Stickney *et al.* (1996) found that sunflower protein concentrate can replace 25% fishmeal protein in rainbow trout diets. Sunflower meal is economically viable to feed tilapia diets containing a maximum of 20% protein from sunflower seed meal.

El-Saidy and Gaber (2002) found that up to 50% dehulled sunflower meal protein could be used to replace fish meal as a protein source in the diet of Nile tilapia, *Oreochromis niloticus* without significant effect on the fish body weight. Abbas *et al.*, (2005) found that the gradual rise in replacement level of fish meal by sunflower meal negatively affected growth performance of major carps and the minimum decrease in fish production was recorded at 25% replacement level while the maximum decrease was recorded at 75% replacing level of fish meal by sunflower meal.

The sunflower cake inclusion as a replacement of fish meal in shrimp diets y conducting growth and digestibility trail with the juveniles of tiger shrimp, *Penaeus monodon* Faricius (Crustacea: Decapoda: Penaeidae) both in tanks and in net cages. Based on this study ,maximum inclusion level of sunflower cake could be 5% in tiger shrimp diets at 20% replacement of fish meal. Sunflower seed meal has been evaluated in fish diets (Tacon *et al.*, 1984), but reports on its use in crustacean diets are limited.



Plate:3 Sunflower seed(*Helianthus annuus*)



Plate:4 Sunflower meal

2.52 COTTON SEED MEAL:

The increased production of organic cotton over the last decade provides pesticide-free quality oilcake, imperative for the production of human food products (OTA, 2009). Cottonseed by-products rank third in the world among produced vegetable protein concentrates (Lim and Chhorn 1996) and are

available at much lower costs than animal proteins. Cottonseed meal has contains toxic compounds gossypol and this toxicity has severely limited the use of cottonseed meal in diets for Pacific white shrimp, *Litopenaeus vannamei*. In 1996, Chhorn Lim reported that *L. vannamei* that consumed diets containing more than 26.5% cottonseed meal had reduced survival, weight gain and feed consumption, while shrimp fed diets with more than 39.8% cottonseed meal experienced high mortality.

Due to their high protein content of up to 40% (Ramachandran *et al.*, 2007), cottonseed products - oilcake and meal - could be used as an inexpensive protein source in fish feeds. However, gossypol, a secondary metabolite stored in the pigment glands of cottonseed, is well known for numerous anti nutritional effects that limit its use as an alternative protein source. (Rincharad *et al.*, 2003; Yue and Zhou, 2008). Cottonseed meal contains the toxic pigment gossypol and is low in available lysin; it has been used to partially replace soybean meal in catfish feeds. (Dorsa *et al.*, 1982; Robinson and Brent1989) At the end of the study, no significant difference was detected among treatments in mean final weight (20.2 g), feed-conversion ratio (1.23), growth (1.9 g/ week) or survival (97.8%).

Lim (1996) demonstrated that solvent-extracted cottonseed meal can be used to replace 40% of a marine protein mix (53% menhaden fish meal, 34% shrimp waste meal and 13% squid meal) in a 32% crude protein practical diet, containing 45% of the marine protein mix. Gossypol is a naturally occurring polyphenolic compound



Plate 5 : Cotton Seed

present in the pigment glands of cottonseed (*Gossypium spp*); the average gossypol content varying from 0.4-2.4% within glanded cottonseeds to less than 0.01% free gossypol within some low-gossypol cottonseed meals (Castaldo, 1995; Liener, 1980; NRC, 1993; Robinson & Brent, 1989).

Although cottonseed meal is the third most abundant plant protein source in the market and its price is quite competitive, inclusion levels are limited mainly due to presence of free gossypol, low lysine availability, and high levels of crude fiber (Hertrampf and Piedad – Pascual, 2000) In the absence of information on the precise mechanism of gossypol toxicity in fish, and the possibility of gossypol accumulation within the edible tissues of fish fed high-gossypol containing cottonseed meals (Dorsa *et al.*, 1982; Robinson & Brent, 1989; Robinson, 1991), it is therefore recommendable that levels of free-gossypol be kept to below 0.01% within the finished diet.

Cottonseed meal in diets of *L.vannamei* at levels of 0, 13.3, 26.5, 39.8, 53, and 66.3 % of the diet as substitutes of an animal protein mix. It was reported that the three lowest levels (0, 13.3, and 26.5 %) yielded similar weight gain, diet consumption and survival. However, growth performance was affected at levels of cottonseed meal higher than 26.5 % of the diet and free gossypol levels higher than 26.5 % of the diet and free gossypol levels higher than 1,100 ppm. The two highest levels of cottonseed meal diets (53 and 66.3 %) caused reduced weight gain at week 4 and high mortality between weeks 6 and 8 (Lim 1996). The shrimp body tended to show a yellow-green discoloration, probably due to accumulation of free gossypol.

Fernandez and Lawrence (1988) found that *L.vannamei* and *P.stylirostris* weight gain and survival was not affected in a 30 % protein diet that contained 20% cottonseed meal; however, growth depression was observed in *Penaeus setiferus* fed the same diet. When the level of protein was reduced to 20 %, the inclusion of 10 % of cottonseed meal in the diet did not affect growth of *L.vannamei* and *P.stylirostris*, but levels of dietary cottonseed meal higher than 5 % affected growth of *P.setiferus*. Wang 2010 Studied on cottonseed meal as an alternative fishmeal *Litopenaeus vannamei* growth, feed utilization, the 8-week growth experiment. The experimental results show that the feed cottonseed meal *Litopenaeus vannamei* survival and than no significant impact, as cottonseed meal usage increases, the growth of juvenile shrimp decreased after increasing trend.

Based on the previous findings, a maximum level of inclusion of 25 % of cottonseed meal in crustacean diets should be considered. (Rose *et al.*, 1998) the studies were conducted the experiment to evaluate the effects of replacement of fish meal with cottonseed meal on growth performance, feed utilization, body composition and non-specific immune response for the Pacific white shrimp. The poor growth and survival at higher levels suggest gossypol toxicity and the possibility of residual pesticide contamination of CSM.

2.6 ANIMAL PROTEIN:

Feed is one of the important and essential inputs in shrimp farming. Feeding provides proper nutrition for the prawn such that with optimum environmental conditions, the prawn will attain marketable size in the shortest time and at the least cost in order to obtain the largest margin of profit. During the marine prawns growth, the food requirement will increase substantially and if they do not get sufficient food, poor survival and production (Bautista and Subosa, 1997).

The demand for animal protein for human consumption is increasing. It is met largely by the terrestrially farmed animals. There has been a considerable increase in the marine shrimp culture due to its high protein content, less fat, delicious taste and market demand in both national and international market. The feed is the largest operating cost of prawn farming and every effort should be made to ensure efficient utilization of feeds (Narasimhan *et al.*, 2013). To obtain maximum growth, an optimum level of dietary protein is needed.

2.61 *Acetes indicus*:

The species belonging to the genus *Acetes* are called paste shrimp in English, but locally they are called Jawala in fresh and Kolim in dried form, in both Marathi and Gujarati. The *Acetes* spp. due to their small size and delicate nature get rapidly decomposed, therefore they are sundried and generally consumed by the poor people. (Deshmukh 1993). Crustacean tissue suspensions were prepared from the sergestid shrimp, *Acetes* sp., known locally in the Philippines as 'Alamang'. This species was chosen because of its local market availability in Iloilo at the time of the Mission and because this shrimp family constituted on of the original feed sources used by Hameed Ali and co-workers for the preparation of a crustacean wet tissue suspension Jawala paste shrimp - *Acetes indicus*; Hameed Ali, *et al.*, (1982).



Plate 6 :Acetes indicus

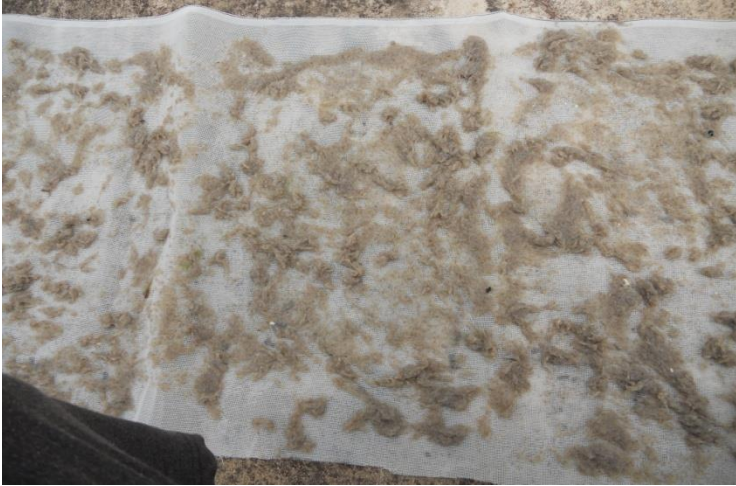


Plate 7 : Acetes indicus –wet position



Plate 8 : *Acetes indicus* dry position

Acetes indicus is a pelagic coastal water species and during certain time of the year, they form conspicuous aggregations near the shore, and are fished mainly with bag net and push net setting near the shore against the flow of the tide. The fishing is generally done during the day. In Asian countries, only a small proportion of the catch is marketed as fresh shrimps; the greater proportion is sun dried, salted or fermented with salt in various ways for food. Shrimp paste and sauce are manufactured extensively throughout Southeast Asia for their esteemed taste and nourishment (Xiao and Greenwood, 1993).

Acetes indicus is a typical neritic shrimp and is common in estuaries and brackish where fresh water from the land greatly influences the situation (Omori, 1975). Fishing grounds of *Acetes* are usually located in calm, muddy or sandy-mud and intertidal zones, where waters are shallower than 5 m. Like many others marine zoo-planktons (e.g. Antarctic krill; mysids), *Acetes* exhibits strong sociality, swarming/schooling on various spatio-temporal scales (Nair,

1977; Bal and Rao, 1984; Xiao and Greenwood, 1993). The population density in the huge swarm may exceed 4500 individuals/m³ (Omori, 1974). Large *Acetes* swarms are usually irregular in shape and elongated parallel to the coastline, while micro swarms are usually oval in shape (Xiao 1990).

Despite great abundance in fishery the biological investigations on the species of *Acetes* are few, probably due to their small size. Deshmukh (1988) carried out detailed investigations on the biology of *A. indicus*. *A. indicus* occurs in surface water (0-5 m) in massive accumulations or swarms which are greatly influenced by the tidal currents, precipitation and winds. The size range of the species, in the *dol* nets, is 6-38 mm but grows up to 40 mm. The males are smaller in size reach in 27 mm in total length while the females grow up to 38 mm. by using scatter diagram technique, showed that the males and females exhibit growth rates of 6.15 mm and 5.96 mm per month and the fishable life span of the species is about 6 months. It breeds throughout the year with peak spawning activity during October-January. The size at maturity is 14-15 mm for males and 24.4 for females. The minimum size of maturity for females is 17 mm and an individual female spawns twice during its short span of life. The fecundity is 4, 333- 10, 300 eggs for the females of 19-33 mm in total length. *A.indicus* forms a feeding basket with the help of setose oral appendages and subsists mainly on detritus and planktonic organisms by filter feeding mechanism. (Deshmukh 1993)

2.62 MYSID MEAL:

Mysid is a protein rich natural food but its large particle size and chitin are the limiting factors resulting in less consumption. Palma *et al.* (2008) conducted experiments to evaluate the efficacy of natural frozen shrimp, mysid and *Artemia* on growth and survival of long snout seahorse (*Hippocampus guttatus*). Hostens (1999) reported that the abundant shrimp *Crangon crangon* in westerschelde, Southern Bight of the North sea is most important predator on mysids.

Mysid shrimp are commonly used by aquarium hobbyists, and sometimes commercial aquaculturists, to feed to seahorses (Garrick-Maidment 1997; Hilomen-Garcia 1999; Forteath 2000) because they appear to promote good growth and survival. Domingues *et al.* (2001) used Artemia and mysid as a food source for fish (*Sepia officinalis*). Cuttle fish fed with mysids grow significantly bigger and survival was higher compared to the group fed with Artemia. Mysid is a protein rich natural food but its large particle size and chitin are the limiting factors resulting in less consumption. Palma *et al.* (2008) conducted experiments to evaluate the efficacy of natural frozen shrimp, mysid and Artemia on growth and survival of long snout seahorse (*Hippocampus guttatus*). Significantly higher growth and survival were observed in mysid fed groups. Most of the mysids are surface dwellers, surface collection constituted 90.71% and stratified collection constituted only 9.92% of the total catch. Population density of mysids in the Bay of Bengal was higher (37.59%) than that of the Arabian Sea (29.29%) and Andaman Sea (33.1%). *Pseudanchialina pusilla* was the most abundant species (64.77%) and *Siriella gracillis* was the second dominant species (28.1%).

Domingues *et al.*, (2001) were conducted experiment on hatchlings of cuttle fish (*Sepia officinalis*) fed with mysid shrimp *Paramysis novelli*. Examined the effect of varying feed ration on cultured seahorse (*Hippocampus abdominalis*) growth and survival using frozen mysids (*Amblyops kempii*) at daily feed rations of 5, 10, 15 or 20% wet body weight. Mysids were most important in the diets of *Pomatoschistus minutes*, *P. lozanoi*, *Trisopterus luscus* and *Merlangius merlangus* and were present in appreciable numbers in *Pleuronectes flesus*, *Trigla lucerna*, *Clupea harengus* and *Pleuronectes platessa*. In Europe, the highest hyperbenthic densities have been reported from the brackish reaches of estuaries the mobility of hyperbenthic species can be an advantage for example allowing an immediate response to adverse oxygen conditions in the unpredictable environment of an estuary (Mee *et*

al.,1993b,1995). Hostens (1999) reported that the abundant shrimp *Crangon crangon* in westerschelde, Southern Bight of the North sea is most important predator on mysids.



Plate 9: Mysid



Plate 10 : Mysid Meal

2.7 SALINITIES:

L.vannamei is the most commonly cultured shrimp in the western hemisphere (Rosenberry 2000) and inhabits and has been cultured in coastal waters ranging in salinity from 1 ppt to 40 ppt (Bray *et al.*, 1994). Because of the ready availability of post larvae (PL), and this species tolerance to low salinity, it is currently grown in inland waters ranging in salinity from 28.3ppt (Smith and Lawrence, 1990) to 0.5 ppt (Samocha *et al.*, 2001).The shrimp-farming industry is currently experiencing major crop losses due to disease outbreaks sometimes associated with the deterioration of water quality, which makes the establishment of saltwater ponds economically risky. Instead, the culture of penaeid shrimp in low-salinity waters, which not only provides a

cost-effective solution to prevalent disease problems but also improves the use of saline-alkaline land waters, is now popular in many regions of the world (Bray *et al.*, 1994; Dong 2003).

A large number of studies have been devoted to the proper acclimation of post-larval shrimp to low salinity waters prior to stocking. When shrimps are properly acclimated, farmers and aqua culturists have been quite successful at rearing shrimp using a variety of different low salinity water sources which have varied greatly both in salinity and ionic profile (Cawthorne *et al.*, 1983; Smith and Lawrence, 1990; Samocha *et al.*, 1998; Davis *et al.*, 2002; Samocha *et al.*, 2002; Atwood *et al.*, 2003; McGraw and Scarpa, 2003; Gong *et al.*, 2004; McNevin *et al.*, 2004; Ur-Rahman *et al.*, 2005; Sowers and Tomasso, 2006; Araneda *et al.*, 2008; Green 2008; Cuvin- Aralar *et al.*, 2009; Parmenter *et al.*, 2009; Roy *et al.*, 2009) .

The White leg shrimp (*L. vannamei*), is considered one of the best suited species for inland culture, because of its higher tolerance to low salinities compared to other species such as *L. setiferus*, *L. stylirostris* and *P. chinensis*. Previous studies have shown that it is possible to successfully grow *L. vannamei* in low salinity waters. Reid and Arnold (1992); Van wyk *et al.*, (1999) Samocha *et al.*, (2004) observed growth and survival rates in *L. vannamei* different salinities and different densities, Davis *et al.*, (2004a) carried out experiments on acclimatizing White leg shrimp to inland, low salinity waters. Ogle *et al.*, (1992) studied the effect of salinity on survival and growth of White leg shrimp post larvae. Samocha *et al.*, (1998) worked on the growth and survival of *L. vannamei* in low salinity water in a semi closed recirculating system. Zhu *et al.*, (2010) studied the effect of dietary protein and lipid levels on the growth and energy productive value of White leg shrimp *L. vannamei* in different salinities.

The White shrimp *Litopenaeus vannamei* has widely replaced *Penaeus monodon* as the cultured species. One of the reasons for this change is the fact that *L. vannamei* can be cultured in higher densities and with a lower exchange of water, which increases the bio-security. *L. vannamei* is a euryhaline

species and can be cultured in salinities from 0 to 50ppt, although the best growth is obtained in salinities between 10 and 25ppt. In lower salinity culture, there is an osmotic pressure between the shrimp body and the surrounding water, resulting in automatic water uptake mainly through gills and intestine. With lower salinity the shrimp face more difficulties to uptake macro-minerals from the water. (Muylder 2010)

The effect of low salinity on growth and survival of post larvae and juveniles of *L. vannamei* were studied by Laramore (2001). Ponce-Palafox *et al.*, (1997) studied the effect of salinity and temperature on the growth and survival rates of juvenile white shrimp (*L. vannamei*). Bray *et al.*, (1994) studied on the effect of salinity on growth and survival of *L. vannamei*, with observations on the interaction of IHHN virus and salinity.

Jiang *et al.*, (2000) studied on the Effects of the temperature and salinity on nitrogenous excretion by *Litopenaeus vannamei*. It is known that *L.vannamei* can tolerate a wide salinity range from brackish water of 1–2 ppt to hyper saline water of 50 ppt (Pante, 1990; Stern *et al.*, 1990). Boyd (1989) considered salinity of 15–25 ppt to be ideal for *L. vannamei* culture. Significant effects of temperature and salinity have been reported on survival (Ogle *et al.*, 1992), and growth of *L. vannamei*. (Huang 1983; Wyban *et al.*,1995). In laboratory salinity preference experiments at 26–32 °C, Mair (1980) demonstrated *L. vannamei* has a relatively low salinity preference (1–8 ppt). In nature, the abundance of post larval *L.vannamei* is associated with low salinity and high temperature.

Mc Graw *et al.*, (2002) studied on Acclimation of *Litopenaeus vannamei* post larvae to low salinity: Influence of Age, Salinity Endpoint, and Rate of salinity reduction. Adequate growth rates and acceptable production of *Litopanaeus vannamei*, is possible. (Scarpa and Vaughn, 1998; Samocha *et al.*, 1999; Rosenberry 1999). Samocha *et al.* observed that *L.vannamei* growth did not differ in culture systems with 2, 4, or 8 ppt saline water. Of the commercial species cultured in western hemisphere, *L.vannamei* is probably one of the most adapted a species for culture in waters of low salinity.Mair (1980) stated

that out of four species endemic to the west coast of Mexico, *L.vannamei* is the most tolerant to low salinity. Van Wyk *et al.*, (1999) reported that 48h were necessary to acclimate *L.vannamei* (PL₁₂) down to fresh water (0.5 ppt). Their recommended acclimation rate decreased from 2 ppt/h for the first 8 h down to 0.063 ppt/h for the last 8 h.

Gunalan *et al.*, (2013) studied on an experimental study of the effects of different salinities on the growth and survival of *L.vannamei*. Salinity is important parameters to control growth and survival of shrimps. *L.vannamei* may have the ability to survive in a wide range of salinities but its growth can vary significantly among different salinity conditions. (Laramore *et al.*, 2001) found that there was a significant difference in growth for post larval shrimp that were kept in 2 and 3ppt treatments as compared to 30ppt treatments. Not only growth was significantly lower for the shrimp grown in the lower salinities they also showed decreased survival. The shrimp in the 2ppt treatments had a 20% survival while in 30ppt treatment had 80% survival and there was no survival of shrimp in treatments below 2ppt. (Bray *et al.*, 1994) recorded that higher growth in 5 and 15ppt treatments were compared to treatments of 25, 35, and 49ppt. No significant difference in survival was observed among different treatments. In yet another study, results showed the best growth at salinities above 20ppt and temperatures between 20 and 30°C (Ponce-Palafox *et al.*, 1997). At high salinity the shrimp will grow slowly but they are healthy and resistance to diseases. (Van Wyk *et al.*, 1999) reported that salinities less than 0.5 ppt put this species at its physiological limit and cause a large proportion of its energy to be used in osmoregulation, therefore limiting growth and preventing it from reaching commercial sizes.

Cao Mei (2004) studied on effects of salinity and dietary carbohydrate levels on growth and energy budget of juvenile *Litopenaeus vannamei*. The results showed that no shrimp survived in tap water at the end of the experiment irrespective of dietary carbohydrate. The ability to grow *L.vannamei* in fresh water may also be advantage in the USA market, based on results of a consumer acceptance test run by the UF/IFAS Food Science and Human

Nutrition Department of the University of Florida. This study concluded that USA consumers preferred fresh water prawn *L.vannamei* over those grown in brackish water or salt water or harvested from the sea. This was due to better aroma, appearance, flavour and texture characteristics of freshwater grown shrimp. They stated that there was a strong consumer demand in the USA for a higher quality product than that currently available.

The Pacific white shrimp *Litopenaeus vannamei* possesses the ability to tolerate low salinities. Recently, there has been increasing interest in the culture of this species in low salinity waters or fresh water (Saoud *et al.*, 2003), for which information is required on the effects of salinity on performance, principally growth and survival. A reduction in salinity can significantly affect the survival and growth of penaeid shrimps, although they possess the ability to tolerate low salinities (Huang 1983, Bray *et al.*, 1994). Galli and Stern (1998) reported that the survival of *L. vannamei* was drastically reduced when salinity decreased below 0.18 ppt. Bray *et al.*, (1994) reported that *L. vannamei* juveniles had growth optima between 5 ppt and 15 ppt. Currently, no shrimps survived in tap water (about 0.2 ppt, salinity), the survival increased with increasing salinity from 1 ppt to 2 ppt, and they grew well in salinities [greater than or equal to] 4 ppt, indicating the limited capability for *L. vannamei* juveniles to tolerate very low salinities.

Previous studies had shown that holding penaeid shrimps at salinities near isosmotic and points could result in an increase of food consumption (Staples Heales 1991). Rosas *et al.*, (2001a) reported that *L. vannamei* juveniles maintained at 15 ppt salinities consumed 1.69 times more food than those at 5 ppt. and Food consumption generally increased with increasing salinity within the range of 1-16 ppt with the maximum value at 16‰, which coincided with the salinity at which the highest specific growth rate was observed, indicating that at salinities. The isosmotic point for penaeids ranged from 23 to 30 ppt (Dall 1981; Castille and Lawrence 1981). It is generally believed that shrimps near the isosmotic point expend the least energy on respiration and excretion, and so they exhibit the best growth rate and the most efficient energy conversion (Panikkar 1968).

3. Materials and Methods

The experiment was conducted in the Wet Laboratory of the Department of Aquaculture, College of Fishery Science, Sri Venkateswara Veterinary University, Muthukur, for a period of 63 days. (From 1-3-2015 to 2-5-2015).

3.2 .EXPERIMENTAL ANIMALS AND THEIR ACCLIMATIZATION:

Litopenaeus vannamei (1500 numbers) seed were brought from the CP Hatchery and reared in farmer's pond till it reaches an approximately weight of 2.5g for 25 days at 30 ppt salinity. Then the seed were acclimatized to the required salinity, i.e., 0 ppt, 5 ppt ,15 ppt and 25ppt by slowly adding fresh water at the rate of reduction of 2 salinity per hour in the Wet Laboratory in the Department of Aquaculture.

3.3. EXPERIMENTAL DESIGN:

The aquarium tanks used for experiments were of size 60x30x40 cm. Eighty-one aquariums were stalked on iron racks. Aquariums were located in a secured place where there is no direct sunlight and covered all the sides with black paper to avoid algal growth in the tank. Water in the aquariums was aerated by using air stones connected to the air compressor. Biological filters are prepared and used for filtering the aquarium water. The saline water was taken into a tank and allowed to aerate for 48 hours and was used for filling the aquaria. The water is allowed to filter for 24 hours before introducing the shrimps into the aquaria.

Ten numbers of Shrimps with initial average weights of 2.5 were introduced into each aquarium and triplicates were maintained for each treatment Fish Meal 25 (FM25), Fish Meal 30(FM30) , Fish Meal 35 (FM35),Cotton Seed Meal 25 (CSM25), Cotton Seed Meal 30 (CSM30), Cotton Seed Meal 35(CSM35) *Acetes indicus* 25(AI 25) , *Acetes indicus* 30 (AI 30) , *Acetes indicus* 35 (AI 35), Sunflower meal 25(SF 25),Sunflower meal 30(SF 30),Sunflower meal 35(SF35) , Mysid meal 25(MM 25),Mysid meal 30 (MM 30),Mysid meal 35(MM 35), at four different salinities of 0 ppt ,5ppt, 15ppt and 25 ppt. Regular water exchange of 50% was done every day. Left over feed, excreta and other debris was siphoned off from the bottom of the tank without disturbing the shrimps.

3.4. EXPERIMENTAL FEED PREPARATION AND FEEDING:

In the experiment, formulated feed with the crude protein were used for feeding. Experimental diets were prepared with Fish meal at 25%, 30 %, 35% protein levels, Cottonseed meal at 25%, 30 %, 35% protein level, Mysid meal at 25%, 30 %, 35% protein level, Sunflower meal at 25%, 30 %, 35% protein level and *Acetes indicus* at 25%, 30 %, 35% protein inclusion levels. Other ingredients used in the experimental diets were de oiled rice bran and maize, 2% of vitamin and mineral mixture added to experimental diets. All the ingredients that are Fish meal, Cottonseed meal, *Acetes indicus* meal, Sunflower meal, Mysid meal, de oiled rice bran, maize, vitamin and mineral mixture used in feeds were obtained from local markets. Ingredients used in the feed and all the experimental diets were estimated for proximate composition.

Each ingredient was procured in required quantity and ground into powder and sieved. All the ingredients were then mixed in required proportion and water was added at the rate of 30 ml per every 100g of feed and dough was prepared. Maida (1%) was used as a binding agent in the feed. The dough was cooked for 20 minutes in pressure cooker and then cooled. Vitamin and mineral mixture were then added. The homogenous dough was pressed

through a hand pelletizer with a sieve of 1 mm diameter. The feed was dried in shade and then in hot air oven at 80-90°C to reduce the moisture content to 10% and stored properly in dry and air tight bottles and kept in dark cool place.

Table.1: Feed formulation of the diets used in the experiment:

(Ingredients g/100g)

Note:
FM25

NAME OF THE INGRADIENT	FM			CSM			AI			SF			MM		
	25	30	35	25	30	35	25	30	35	25	30	35	25	30	35
FM	31.5	43	54.5	-	-	-	-	-	-	-	-	-	-	-	-
CSM	-	-	-	30.5	48.2	83.2	-	-	-	-	-	-	-	-	-
AI	-	-	-	-	-	-	28.68	40.0	50.5	-	-	-	-	-	-
SF	-	-	-	-	-	-	-	-	-	54.5	75.0	95.4	-	-	-
MM	-	-	-	-	-	-	-	-	-	-	-	-	25.5	35.0	44.5
DOB	33.25	27.5	21.75	33.75	24.90	7.4	34.66	29.0	23.75	20.5	13.0	1.0	36.5	33.0	30.0
Maize	33.25	27.5	21.75	33.75	24.90	7.4	34.66	29.0	23.75	23.0	10.0	1.6	36.0	30.0	23.5
	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
TOTAL	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100

	:	Fish meal containing 25% crude protein
FM30	:	Fish meal containing 30% crude protein
FM35	:	Fish meal containing 35% crude protein
CSM25	:	Cottonseed meal containing 25 % crude protein
CSM30	:	Cottonseed meal containing 30 % crude protein
CSM35	:	Cottonseed meal containing 35% crude protein
AI25	:	<i>Acetes indicus</i> meal containing 25% crude protein
AI30	:	<i>Acetes indicus</i> meal containing 30% crude protein
AI35	:	<i>Acetes indicus</i> meal containing 35% crude protein
SF25	:	Sunflower meal containing 25% crude protein
SF30	:	Sunflower meal containing 30% crude protein
SF35	:	Sunflower meal containing 35% crude protein
MM25	:	Mysid meal containing 25% crude protein
MM30	:	Mysid meal containing 30% crude protein
MM35	:	Mysid meal containing 35% crude protein

Table.2: Proximate composition of the various ingredients used formulated feeds (% on dry matter basis):

INGREDIENT	FM	CSM	AI	SF	MM	DOB	Maiz
Moisture	7.03	7.69	12.45	11.6	12.4	7.7	9.3
Crude protein	55	40	58.4	36.4	64.2	12.5	11.3
Ether extract	4.02	6.65	6.03	3.5	2.4	4	-
Crude fiber	3.1	6.9	4.1	9.5	5	-	4.4
Total ash	3.47	3.57	11.7	8.9	4.6	3.9	-
Crude fat	-	-	-	-	-	-	4.1

3.4.1. PROXIMATE COMPOSITION:

Proximate analysis of the feed was estimated by the method of AOAC, 1995.

3.4.1.1. MOISTURE:

A known weight of the feed sample was taken and dried in an oven at 105°C to constant weight and the moisture content was calculated by using the following formula:

$$\text{Weight of wet sample} - \text{Weight of dried sample}$$

$$\text{Moisture (\%)} = \frac{\text{Weight loss}}{\text{Weight wet of sample}} \times 100$$

Weight wet of sample

3.4.1.2. CRUDE PROTEIN:

Nitrogen content of the sample was estimated by Kjeldahl method and the crude protein was estimated by multiplying nitrogen percentage by a constant factor 6.25.

$$\text{Crude protein (\%)} = \text{Nitrogen (\%)} \times 6.25$$

3.4.1.3. ETHER EXTRACT:

Ether extract was estimated by soxhlet apparatus using petroleum ether as a solvent.

Weight of initial sample – Weight after extraction

$$\text{Ether extract (\%)} = \frac{\text{Weight of initial sample} - \text{Weight after extraction}}{\text{Weight of initial sample}} \times 100$$

Weight of initial sample

3.4.1.4. ASH:

Ash content was estimated by taking a known weight of sample in silica crucible and placing it in a muffle furnace heated at 600°C for 6 hours.

$$\text{Ash (\%)} = \frac{\text{Weight of ash}}{\text{Weight of sample}} \times 100$$

3.4.1.5. CRUDE FIBER:

Crude fiber was estimated by treating the moisture and fat free sample successively with dilute acid (1.25%) and alkali.

$$\text{Crude fiber (\%)} = \frac{\text{Weight of crude fiber}}{\text{Original weight of sample}} \times 100$$

Shrimps were fed at the rate of 5% body weight. The pelleted feed was made into small granules of 1mm size and fed thrice a day (6.00AM, 5.00PM and 10.00PM). Left over feed and faeces in all the aquarium tanks were removed after one hour of feeding.

3.5 GROWTH PERFORMANCE:

The growth parameters of all the shrimps of each aquarium were individually estimated by taking their total body weight at 7 days interval.

3.5.1. WEIGHT INCREMENT:

Weight increment was obtained by subtracting initial body weight from the final body weight.

Weight increment (gm) = Final body weight (gm) – Initial body weight (gm).

3.5.2. SPECIFIC GROWTH RATE:

Specific growth rate was calculated by the formula

$$\frac{[(L_n \text{ FBW} - L_n \text{ IBW})]}{\text{Day}} \times 100$$

FBW -- Final body weight

IBW -- Initial body weight

Ln -- Logarithm

Day -- duration of experiment (63 days)

3.6. SURVIVAL RATE:

Survival of the animal at each fortnight was noted down and survival rates are calculated as below

$$\text{Survival (\%)} = \frac{\text{Total number of animal survived}}{\text{Total number of animal stocked}} \times 100$$

3.7. FOOD CONVERSION RATIO (FCR):

Feed Conversion Ratio was calculated by dividing feed given (dry weight) by body weight gain (wet weight)

$$\text{Feed Conversion Ratio} = \frac{\text{Feed given (dry weight)}}{\text{Body weight gain (wet weight)}}$$

Body weight gain (wet weight)

3.8. PHYSICO -CHEMICAL PARAMETERS OF WATER:

The water quality parameters were observed weekly during the experimental period. Water quality parameters observed during the study are:

S.No.	Parameter	Method
1.	pH	Digital pH and temperature meter
2.	Temperature	Digital pH and temperature meter
3.	Dissolved oxygen	Titrimetric Winklers method (APHA, 1985)
4.	Total alkalinity	Titrimetric method (APHA, 1985)
5.	Total hardness	Titrimetric method (APHA, 1985)

3.9. STATISTICAL ANALYSIS:

The data obtained on Growth, Survival and Food Conversion Ratio was statistically analyzed by applying Randomized Block Design (RBD) of two-way classification.



Plate No. 11 : Partial setup of experimental animals reared at 0, 5, 15 and 25

PPT salinity



Plate No. 12 : Partial setup of experimental animals reared at 0, 5, 15 and 25

PPT salinity



Plate No. 13 : Formulated feeds prepared for experiment



Plate No. 14 : Length measurement of *L.venamnei*



Plate No. 15 : Weight measurement of *L.vennamei*

4. Results

4.1. WATER QUALITY PARAMETERS:

In the present study important water quality parameters such as Dissolved oxygen, Temperature, pH, Total alkalinity , Total Hardness were observed weekly at every sampling in all aquaria at four different salinities , 0ppt, 5ppt Salinity, 15ppt Salinity and 25ppt Salinity, and presented in tables no 3-16. And in figures from 1-22

4.1.1.TEMPERATURE:

The temperature values recorded during 63 day period of the experiment at 0 ppt, 5 ppt,15 ppt ranged between 29.5°C -31.6 °C and at 25 ppt 29.6 °C -31.7 °C overall the lowest temperature was recorded at 0,5,15 ppt during the initial day and the 14 day, whereas the highest temperature was recorded at 25 ppt during the 63 day irrespective of the dietary protein content and experimental diets. Table no-3

4.1.2. pH:

The pH values recorded during the 63 day of period of experiment at 0 ppt salinity ranged between 8.2 to 8.3, at 5ppt salinity 8.2 to 8.3, at 15 ppt salinity 8.3 to 8.4 and 25 at ppt salinity 8.4 to 8.5. Overall the pH lowest value was recorded at 0 ppt salinity where as the highest at 25 ppt salinity of all the treatments irrespective of the dietary protein content and experimental diets table no.5

4.1.3. DISSOLVED OXYGEN (PPM):

At 0 ppt salinity the dissolved oxygen values were observed during the initial sampling were between 7.81 to 7.69 mg/lit a highest and lowest dissolved oxygen were observed in MM-30,MM-35 and FM-25,CSM-30 respectively. During the seventh day sampling dissolved oxygen value range between 7.84 to 7.14 mg/lit the highest and lowest dissolved oxygen values were observed in the treatments of FM-25, and MM-25 Results of the 14th day sampling recorded highest value for 7.7 mg/lit (MM-25) and while the lowest being for 6.99 (FM-25) 21st day observation range between 7.15 to 6.69 mg/lit highest in AI-30 and lowest in FM-25. On 28th days highest and lowest values recorded were for 7.21 (MM-30) and 6.89 mg/lit CSM-35 respectively. The 35th day observation ranged between 7.2(FM-25) to 6.15(AI-25) The sampling results of the treatments of the 42nd day were 7.11 mg/lit to the highest CSM-35 while lowest in 6.31 mg/lit (AI-25) Among these values observed for 49th day were highest values was for 7.11 mg/lit (CSM-35) and lowest being for 6.66 mg/lit MM-35) The 56th day observation ranged between 7.01 mg/lit (SF-30) to 6.66 mg/lit (FM-25,CSM-25,AI-25) The final day (63rd day) sampling results were 6.88 mg/lit (AI-35) in (highest) and 6.66 mg/lit (FM-35,AI-35) (lowest) During this sampling among all 0 ppt salinities, highest dissolved oxygen were recorded the observation given table no 5 (fig no. 1)

At 5 ppt salinity the dissolved oxygen values were observed during the initial sampling were between 7.77 to 7.50 mg/lit a highest and lowest alkalinity values were observed in MM-30 and FM-25 respectively. During the seventh day sampling dissolved oxygen range between 7.77 to 7.0 mg/lit the

highest and lowest dissolved oxygen values were observed in the treatments of MM-30, and FM-25 Results of the 14th day sampling recorded highest value for 7.70 mg/lit (AI-25) and while the lowest being for 7.0 (FM-30) 21st day observation range between 7.17 to 7.0 mg/lit highest in AI-30 and lowest in SF-35. On 28th days highest and lowest values recorded were for 7.2 (MM-35) and 7.0 mg/lit CSM-30 respectively. The 35th day observation ranged between 7.01 (FM-35) to 6.75 (MM-35) The sampling results of the treatments of the 42nd day were 7.05 mg/lit to the highest CSM-35 while lowest in 6.08 mg/lit (MM-35, MM-30) Among these values observed for 49th day were highest values was for 7.10 mg/lit (AI-35) and lowest being for 6.80 mg/lit MM-25) The 56th day observation ranged between 7.07 mg/lit (MM-35) to 6.85 mg/lit (FM-25) The final day (63rd day) sampling results were 6.85 mg/lit (SF-35, MM-25) in (highest) and 6.66 mg/lit (MM-30) (lowest) During this sampling among all 5 ppt salinities, highest dissolved oxygen were recorded the observation given table no 5 (fig no. 2)

At 15 ppt salinity the dissolved oxygen values were observed during the initial sampling were between 7.50 to 7.05 mg/lit a highest and lowest dissolved oxygen were observed in FM-30, FM-35 and CSM-30 respectively. During the seventh day sampling dissolved oxygen range between 7.20 to 7.0 mg/lit the highest and lowest dissolved oxygen were observed in the treatments of MM-30, and FM-30 Results of the 14th day sampling recorded highest value for 7.18 mg/lit (AI-35) and while the lowest being for 6.84 (FM-30) 21st day observation range between 7.02 to 6.89 mg/lit highest in SF-35 and lowest in SF-30. On 28th days highest and lowest values recorded were for 7.02 (MM-35) and 6.76 mg/lit CSM-25, CSM-30 respectively. The 35th day observation ranged between 6.95 (AI-25) to 6.69 (SF-25) The sampling results of the treatments of the 42nd day were 6.91 mg/lit to the highest FM-35 while lowest in 6.66 mg/lit (SF-30) Among these values observed for 49th day were highest values was for 6.91 mg/lit (AI-30) and lowest being for 6.70 mg/lit MM-30) The 56th day observation ranged between 6.88 mg/lit (AI-35, SF-25) to 6.66 mg/lit (MM-25) The final day (63rd day) sampling results were 6.75 mg/lit (AI-30) in

(highest) and 6.55 mg/lit (MM-30) (lowest) During this sampling among all 15 ppt salinities, highest dissolved oxygen were recorded the observation given table no 5 (fig. no-3).

At 25 ppt salinity the dissolved oxygen values were observed during the initial sampling were between 7.30 to 6.66 mg/lit a highest and lowest dissolved oxygen were observed in FM-30, FM-35 and AI-35 respectively. During the seventh day sampling dissolved oxygen range between 7.0 to 6.41 mg/lit the highest and lowest dissolved oxygen were observed in the treatments of FM-25, and SF-35. Results of the 14th day sampling recorded highest value for 6.6 mg/lit (FM-25) and while the lowest being for 6.1 (AI-25, AI-30) 21st day observation range between 6.50 to 5.95 mg/lit highest in CSM-30 and lowest in AI-35. On 28th days highest and lowest values recorded were for 6.33 (FM-35, MM-30) and 5.95 mg/lit AI-30 respectively. The 35th day observation ranged between 6.25 (MM-30) to 5.88 (AI-35) The sampling results of the treatments of the 42nd day were 6.30 mg/lit to the highest MM-30 while lowest in 5.80 mg/lit (AI-25) Among these values observed for 49th day were highest values was for 6.25 mg/lit (FM-35) and lowest being for 5.70 mg/lit MM-25) The 56th day observation ranged between 6.02 mg/lit (CSM-30) to 5.70 mg/lit (FM-25)

The final day (63rd day) sampling results were 6.01 mg/lit (FM-30) in (highest) and 5.55 mg/lit (SF-25) (lowest) During this sampling among all 25 ppt salinities, highest dissolved oxygen were recorded the observation given table no 5 (fig. no-4)

4.1.4. TOTAL ALKALINITY (mg/l):

At 0 ppt salinity the total alkalinity values were observed during the initial sampling were between 155 to 170 mg/lit a highest and lowest alkalinity values were observed in SF-35 and FM-30 respectively. During the seventh day sampling total hardness value range between 166-181 mg/lit the highest and

lowest total alkalinity values were observed in the treatments AI-25, and CSM-35 Results of the 14th day sampling recorded highest value for 182 mg/lit (SF-35) and while the lowest being for 166 (MM-25) 21st day observation range between 192 to 177 mg/lit highest in CSM-35 and lowest in SF-35,FM-30.On 28th days highest and lowest values recorded were for 200 (MM-25,SF-35) and 177 mg/lit CSM-30 respectively. The 35th day observation ranged between 203 (MM-35) to 177(AI-30) The sampling results of the treatments of the 42nd day were 200 mg/lit to the highest MM-35 while lowest in 177 mg/lit (FM-30) Among these values observed for 49th day were highest values was for 208 mg/lit (MM-35) and lowest being for 190 mg/lit FM-25) The 56th day observation ranged between 207 mg/lit (SF-30) to 188 mg/lit (FM-25,FM-30) The final day (63rd day) sampling results were 214 mg/lit (MM-35) in (lowest) and 197 mg/lit (FM-25) (highest) During this sampling among all 0 ppt salinities, highest total hardness were recorded the observation given table no 5 (fig. no-5).

At 5 ppt salinity the total alkalinity values were observed during the initial sampling were between 242 to 229 mg/lit a highest and lowest alkalinity values were observed in MM-30,MM-35 and AI-25,AI-35 respectively. During the seventh day sampling total alkalinity value range between 255-239 mg/lit the highest and lowest total alkalinity values were observed in the treatments FM-30, MM-35 and AI-25 Results of the 14th day sampling recorded highest value for 271 mg/lit (AI-25) and while the lowest being for 255 (MM-30,SF-25,FM-25) 21st day observation range between 269 to 257 mg/lit highest in MM-25 and lowest in AI-30,SF-35. On 28th days highest and lowest values recorded were for 269 (SF-35) and 262 mg/lit CSM-30,CSM-35 respectively. The 35th day observation ranged between 292 (MM-30) to 276(CSM-35) The sampling results of the treatments of the 42nd day were 295 mg/lit to the highest MM-35 while lowest in 279 mg/lit (SF-30, CSM-25)Among these values observed for 49th day were highest values was for 299 mg/lit (MM-30) and lowest being for 280 mg/lit FM-30) The 56th day observation ranged between 307 mg/lit (MM-25) to 288 mg/lit (FM-25) The final day (63rd day) sampling results were

315 mg/lit (MM-25, MM-35) in (highest) and 288 mg/lit (FM-25,FM-30) (lowest) During this sampling among all 5ppt salinities, highest total hardness were recorded the observation given table no 6 (fig. no-6).

At 15 ppt salinity the total alkalinity values were observed during the initial sampling were between 565 to 552 mg/lit a highest and lowest alkalinity values were observed in MM-30,MM-35 and FM-35 respectively. During the seventh day sampling total alkalinity value range between 582-535 mg/lit the highest and lowest total alkalinity values were observed in the treatments MM-35and AI-30 Results of the 14th day sampling recorded highest value for 577 mg/lit (MM-35) and while the lowest being for 541 (FM-30,FM-35) 21st day observation range between 585 to 560 mg/lit highest in MM-35 and lowest in FM-25,FM-35

On 28th days highest and lowest values recorded were for 606 (MM-35) and 574 mg/lit FM-30 respectively. The 35th day observation ranged between 606 (MM-35) to 577(FM-25)The sampling results of the treatments of the 42nd day were 610 mg/lit to the highest SF-35 while lowest in 591 mg/lit (FM-35) Among these values observed for 49th day were highest values was for 610 mg/lit (MM-30,MM-35) and lowest being for 595 mg/lit FM-30,FM-25)The 56th day observation ranged between 625 mg/lit (MM-30) to 599 mg/lit (CSM-30)The final day (63rd day) sampling results were 604 mg/lit (FM-35) in (highest) and 604 mg/lit (MM-30,MM-35) (lowest) During this sampling among all 15ppt salinities, highest total hardness were recorded the observation given table number 6presented in figure number 7.

At 25 ppt salinity the total alkalinity values were observed during the initial sampling were between 675 to 653 mg/lit a highest and lowest alkalinity values were observed in MM-35 and AI-25 respectively. During the seventh day sampling total alkalinity value range between 689-645 mg/lit the highest and lowest total alkalinity values were observed in the treatments MM-35 and FM-25 Results of the 14th day sampling recorded highest value for 705 mg/lit

(MM-35) and while the lowest being for 659 (AI-30) 21st day observation range between 701 to 655 mg/lit highest in MM-35 and lowest in FM-35, CSM-35. On 28th days highest and lowest values recorded were for 700 (MM-35) and 660 mg/lit FM-25 respectively. The 35th day observation ranged between 710 (MM-35) to 670 (FM-35)

The sampling results of the treatments of the 42nd day were 707 mg/lit to the highest MM-35 while lowest in 675 mg/lit (FM-35, FM-30). Among these values observed for 49th day were highest values was for 711 mg/lit (MM-35) and lowest being for 691 mg/lit (FM-25). The 56th day observation ranged between 708 mg/lit (MM-35) to 691 mg/lit (FM-35). The final day (63rd day) sampling results were 725 mg/lit (MM-35) in (highest) and 700 mg/lit (FM-25) (lowest). Out of all four salinities the total alkalinity values ranged from 155 FM-30 at 0 salinity to 725 mm-35 at 25 salinity the observation given the table no 7 (fig. no-8).

4.1.5. TOTAL HARDNESS (mg/l):

At 0 ppt salinity the total hardness values were observed during the initial sampling were between 199 to 191 mg/lit a highest and lowest hardness values were observed in SF-25, MM-35 and FM-25 respectively. During the seventh day sampling total hardness value range between 211-199 mg/lit the highest and lowest total hardness values were observed in the treatments MM-35 and FM-30, CSM-25. Results of the 14th day sampling recorded highest value for 215 mg /lit (MM-35, MM-30) and while the lowest being for 202 (FM-30, CSM-25). 21st day observation range between 220 to 202 mg/lit highest in MM-35 and lowest in FM-25. On 28th days highest and lowest values recorded were for 217 (MM-35) and 208 mg/lit FM-30, FM-35 respectively. The 35th day

observation ranged between 218(AI-35, MM-30) to 213(FM-25,AI-30) respectively. The sampling results of the treatments of the 42nd day were 214 mg/lit to the lowest FM-30, CSM30 while the highest in 222(MM-25, SF-35) Among these values observed for 49th day were highest values was for 229 mg/lit (MM-35 and lowest being for 215 mg/lit FM-30) The 56th day observation ranged between 229 mg/lit (MM-30, MM-35) to 219 mg/lit (FM-30) The final day (63rd day) sampling results were 220 mg/lit (FM-35) in (lowest) and 238 mg/lit (MM-25) (highest)

During this sampling among all 0 ppt salinities, highest total hardness were recorded the observation given table number 7 presented in figure number 9.

At 5 ppt salinity the total hardness values were observed during the initial sampling were between 252 to 240 mg/lit a highest and lowest hardness values were observed in SF-25, MM-35 and FM-30 respectively. During the seventh day sampling total hardness value range between 259-240 mg/lit the highest and lowest total hardness values were observed in the treatments AI-30 and CSM-30. Results of the 14th day sampling recorded highest value for 289 mg /lit (AI-30,AI-35) and while the lowest being for 264 (FM-30) 21st day observation range between 300 to 281 mg/lit highest in MM-35 and lowest in FM-35.

On 28th days highest and lowest values recorded were for 303(MM-35) and 279 mg/lit FM-25 respectively.The 35th day observation ranged between 299(CSM-35 to 281 AI-30) respectively. The sampling results of the treatments of the 42nd day were 281 mg/lit to the lowest CSM-25 AI 35,SF-30 while highest in 300 mg/lit (mm-35). Among these values observed for 49th day were highest values was for 299 mg/lit (MM-35 and lowest being for 263 mg/lit CSM-30) The 56th day observation ranged between 305 mg/lit (MM-35) to 285 mg/lit (CSM-30,SF-30) The final day (63rd day) sampling results were 322 mg/lit (MM-35) in (lowest) and 300 mg/lit (SF-25) (highest) During this sampling among all 5 ppt salinities, highest total hardness were recorded the observation given table number 7 presented in figure number 10.

At 15 ppt salinity the total hardness values were observed during the initial sampling were between 612 to 601 mg/lit a highest and lowest hardness values were observed in AI-25 and FM-25 respectively. During the seventh day sampling total hardness value range between 622-609 mg/lit the highest and lowest total hardness values were observed in the treatments SF-35, MM-30,MM-35 and FM-25 Results of the 14th day sampling recorded highest value for 635 mg /lit (AI-35,MM-35) and while the lowest being for 619 (FM-30,SF-35) 21st day observation range between 639 to 621 mg/lit highest in MM-25 and lowest in FM-25. On 28th days highest and lowest values recorded were for 651 (MM-35,MM-25) and 630 mg/lit FM-30 respectively. The 35th day observation ranged between 649 (MM-25) to 631 (FM-30) The sampling results of the treatments of the 42nd day were 650 mg/lit to the highest MM-30 while lowest in 635 mg/lit (FM-30,FM-35,CSM-35)

Among these values observed for 49th day were highest values was for 656 mg/lit (MM-30 and lowest being for 639 mg/lit FM-30) The 56th day observation ranged between 666 mg/lit (MM-35) to 639 mg/lit (FM-30) The final day (63rd day) sampling results were 675 mg/lit (MM-35) in (lowest) and 639 mg/lit (FM-30) (highest) During this sampling among all 15 ppt salinities, highest total hardness were recorded the observation given table number 7 presented in figure number 11.

At 25 ppt salinity the total hardness values were observed during the initial sampling were between 710 to 700 mg/lit a highest and lowest hardness values were observed in MM-25 ,SF-35 and FM-25,FM-35 respectively. During the seventh day sampling total hardness value range between 720 - 705 mg/lit the highest and lowest total hardness values were observed in the treatments MM-35 and FM-25 Results of the 14th day sampling recorded highest value for 739 mg /lit (MM-35) and while the lowest being for 718 (FM-30)

21st day observation range between 740 to 722 mg/lit highest in MM-25 and lowest in FM-35. On 28th days highest and lowest values recorded were for 750 (MM-35) and 729mg/lit FM-35, CSM-25 respectively. The 35th day observation ranged between 251 mg/lit (MM-25, MM-35) to 739 (FM-30, FM-35) The sampling results of the treatments of the 42nd day were 755 mg/lit to the highest MM-30 while lowest in 733 mg/lit (FM-30) Among these values observed for 49th day were highest values was for 755 mg/lit (MM-30, AI-35) and lowest being for 739 mg/lit (FM-35) The 56th day observation ranged between 763 mg/lit (MM-35) to 745 mg/lit (FM-30, FM-35) The final day (63rd day) sampling results were 777 mg/lit (MM-35) in (highest) and 749 mg/lit (FM-30) (Lowest) During this sampling among all 25 ppt salinities, highest total hardness were recorded the observation given table number 7 presented in figure number 12.

4.2 Growth Parameters

4.2.1. AVERAGE BODY WEIGHT AND GROWTH PERFORMANCE OF

***L vannamei* FED ON DIFFERENT DIETS:**

Weight of shrimp in grams and weight increment data observed weekly for different treatments at different salinities were presented in tables 8 to 9 figure 13, 14, 15 and 16 statistical analysis table number 10, 11, 12 and 13 a respectively.

Overall after 63 days, the highest average body weight of 14.01 gms (MM 30) and the lowest average body weight of 9.26 gms (CSM 25) at 15 PPT salinity and 0 PPT respectively.

The highest ABW of 12.30 gms, 13.55 gms, 14.01 gms, and 13.95 gms at 0, 5, 15 and 25 PPT salinities respectively (MM 30). The lowest ABW of 9.26, 10.50, 11.11 and 11.70 gms at 0, 5, 15 and 25 PPT salinities respectively (CSM 25)

4.2.2. SPECIFIC GROWTH RATES:

Specific growth rates for *L.vannamei* treated with different diets at four different salinities were calculated and presented in tables (14) and figure 21 respectively at 0ppt 5ppt 15ppt and 25ppt Salinities. The specific growth rates by end of the experimental period (63 days) were calculated for all treatments reared at 0, 5, 15 and 25 salinities.

The SGR for *L.vannamei* treated with different diets reared under different salinities were regard between 0.05 to 2.11 for the entire experimental period. At 0 ppt the highest value was recorded 1.7 (FM 35) another lowest value of 0.05 (MM 30); at 5 ppt the highest value was 2.11 (FM 35) and the lowest value was 0.01 (AI 35), at 15 ppt the highest value was 2.02 (FM 35) another lowest value was 0.49 (SF 25) and finally at 25 ppt the highest value was 2.07 (FM 30) and the lowest value was 0.49 (SF 25, MM 25) recorded respectively. Presented at table number 14 figures number 21.

4.2.3. FEED CONVERSION RATIO:

The Feed Conversion Ratio in different experiments of *L. vannamei* groups reared at varying salinities were calculated and presented in the table number 15 and figure number 22. The highest and lowest values were recorded 2.50 (CSM 25, CSM 35, SF 25) at 0 salinity and 1.45 (FM 30) at 25 salinity respectively. At 0 salinity the highest value recorded CSM 25, CSM 35, SF 25 (2.50) and the lowest value for 1.85 (FM 30), where as at 5 ppt the highest

value for 2.45 (SF 25) and the lowest value for 1.75 (FM 30), for 15 ppt the highest (2.40 – CSM 25) and lowest (1.59 – FM 30) and finally at 25 ppt the highest (2.30 – SF 25) and the lowest (1.45 – FM 30) respectively.

4.2.4. SURVIVAL RATE:

Survival percentages of *L. vennamei* fed on different experimental diets reared under veering salinities and the statistical analysis were presented in table number 16 figures number 17, 18, 19 and 20 respectively.

Overall the survival percentage varied from 55% (CSM 30, 35 – 63rd day) – 100%. (FM 25,30, 35 AI 25, MM 25, 30, 35 – 7th day).

After final sampling, at 0 ppt the highest survival recorded were 85.1 (FM 25, 30,35) and lowest 55% (CSM 30,35); at 5 ppt the highest 90% (FM 25,30,35) and lowest 60% (CSM 30, SF 25, 30 ,35); at 15 ppt the highest 95 % (FM 25, 30, 35) and lowest 60 % (CSM 30, 35, SF 35) and finally at 25 ppt the highest 100 % (FM 20, 25, 30) and the lowest 65% (CSM 30,35) respectively.

**Table No. 17 : Statistical Analysis for percentage of Survival of *L.vennamei*
fed with different experimental dies reared at 0 PPT salinity :**

Treatment means	
S.No	Average
Treatment 1	93.333
Treatment 2	92.222
Treatment 3	92.222
Treatment 4	72.222
Treatment 5	68.333
Treatment 6	72.222
Treatment 7	88.888
Treatment 8	86.666
Treatment 9	86.666
Treatment 10	79.444
Treatment 11	75.555
Treatment 12	77.777
Treatment 13	92.777
Treatment 14	91.111
Treatment 15	91.111

Anova Table					
Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F cal	F prob
Replications	8	7294.818	911.858	60.080	5.260
Treatments	14	9830.373	702.163	46.276	6.589
Error	112	1699.626	15.172	-	-
Total	134	-	-	-	-

Coefficient of Variation = 4.635

Treatments found Significant at 1% and 5% level of significance CD(0.01) = 4.735 CD(0.05) = 3.592

Comparison of Treatment Means with Critical Difference (0.05)

Treatment No.	T 1	T 13	T 2	T 3	T 15	T 14	T 7	T 9	T 8	T 10	T 12	T 11	T 4	T 6	T 5
Treatment Average	93.33	92.77	92.22	92.22	91.11	91.11	88.88	86.66	86.66	79.44	77.77	75.55	72.22	72.22	68.33
	3	7	2	2	1	1	8	6	6	4	7	5	2	2	3
Critical Difference (CD) Compared	a	a	ab	ab	ab	ab	bc	c	c	d	de	ef	f	f	g

Table No. 18 : Statistical Analysis for percentage of survival of *L.vennamei*

fed with different experimental dies reared at 5 PPT salinity :

Treatment means	
S.No	Average

Treatment 1	95.555
Treatment 2	95.555
Treatment 3	96.111
Treatment 4	80.555
Treatment 5	77.777
Treatment 6	79.444
Treatment 7	92.777
Treatment 8	92.222
Treatment 9	92.777
Treatment 10	79.444
Treatment 11	76.111
Treatment 12	78.333
Treatment 13	95.000
Treatment 14	92.222
Treatment 15	92.777

Anova Table					
Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F cal	F prob
Replications	8	7820.000	977.500	59.103	1.100
Treatments	14	7861.111	561.509	33.952	8.836
Error	112	1852.222	16.536	-	-
Total	134	-	-	-	-

Coefficient of Variation = 4.639

Treatments found Significant at 1% and 5% level of significance CD(0.01) = 4.932 CD(0.05) = 3.753

Comparison of Treatment Means with Critical Difference (0.05)

Treatment No.	T 3	T 2	T 1	T 13	T 7	T 15	T 9	T 14	T 8	T 4	T 10	T 6	T 12	T 5	T 11
Treatment Average	96.11 1	95.55 5	95.55 5	95.00 0	92.77 7	92.77 7	92.77 7	92.22 2	92.22 2	80.55 5	79.44 4	79.44 4	78.33 3	77.77 7	76.11 1
Critical Difference (CD) Compared	a	ab	ab	ab	ab	ab	ab	b	b	c	cd	cd	cd	cd	d

Table No. 19 : Statistical Analysis for percentage survival *L.vennamei* fed with reared at 15 PPT salinity :

different experimental dies

Treatment means	
S.No	Average
Treatment 1	98.333
Treatment 2	97.777
Treatment 3	97.777
Treatment 4	82.777
Treatment 5	78.333
Treatment 6	78.888
Treatment 7	92.777
Treatment 8	91.666
Treatment 9	91.666
Treatment 10	79.444
Treatment 11	78.888
Treatment 12	78.888

Treatment 13	93.333
Treatment 14	92.777
Treatment 15	93.333

Anova Table					
Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F cal	F prob
Replications	8	5480.000	685.000	40.663	1.013
Treatments	14	7806.666	557.610	33.104	2.703
Error	112	1886.666	16.842	-	-
Total	134	-	-	-	-

Coefficient of Variation = 4.645

Treatments found Significant at 1% and 5% level of significance CD(0.01) = 4.980 CD(0.05) = 3.791

Comparison of Treatment Means with Critical Difference (0.05)

Treatment No.	T 1	T 3	T 2	T 13	T 15	T 14	T 7	T 9	T 8	T 4	T 10	T 6	T 12	T 11	T 5
Treatment Average	98.33 3	97.77 7	97.77 7	93.33 3	93.33 3	92.77 7	92.77 7	91.66 6	91.66 6	82.77 7	79.44 4	78.88 8	78.88 8	78.88 8	78.33 3
Critical Difference (CD) Compared	a	a	a	b	b	b	b	b	b	c	cd	d	d	d	d

Table No. 20 : Statistical Analysis for percentage survival of *L. vennamei* fed with reared at 25 PPT salinity :

different experimental dies

Treatment means	
S.No	Average
Treatment 1	100.000

Treatment 2	100.000
Treatment 3	100.000
Treatment 4	80.555
Treatment 5	78.333
Treatment 6	80.000
Treatment 7	91.666
Treatment 8	94.444
Treatment 9	91.111
Treatment 10	87.777
Treatment 11	83.888
Treatment 12	84.444
Treatment 13	95.555
Treatment 14	95.000
Treatment 15	95.555

Anova Table					
Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F cal	F prob
Replications	8	2680.000	335.000	24.777	1.467
Treatments	14	7238.888	517.064	38.231	4.320
Error	112	1514.444	13.528	-	-
Total	134	-	-	-	-

Coefficient of Variation = 4.067

**Treatments found Significant at 1% and 5% level of significance CD(0.01) = 4.463 CD(0.05) = 3.395
Comparison of Treatment Means with Critical Difference (0.05)**

Treatment No.	T 1	T 3	T 2	T 13	T 15	T 14	T 8	T 7	T 9	T 10	T 12	T 11	T 4	T 6	T 5
Treatment Average	100.00 0	100.00 0	100.00 0	95.55 5	95.55 5	95.00 0	94.44 4	91.66 6	91.11 1	87.77 7	84.44 4	83.88 8	80.55 5	80.00 0	78.33 3
Critical Difference (CD) Compared	a	a	a	b	b	bc	bcd	cd	de	ef	fg	gh	hi	i	i

Table No. 10 : Statistical Analysis for Growth Performance and average body weight of L.vennamei fed with different experimental diets reared in 0 PPT salinity:

weight of L.vennamei fed

Treatment means	
S.No	Average
Treatment 1	0.927
Treatment 2	1.036
Treatment 3	1.034
Treatment 4	0.758
Treatment 5	0.946
Treatment 6	0.944
Treatment 7	0.955
Treatment 8	1.084
Treatment 9	1.080
Treatment 10	0.823
Treatment 11	0.944
Treatment 12	0.938

Treatment 13	0.915
Treatment 14	1.020
Treatment 15	1.082

Anova Table					
Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F cal	F prob
Replications	8	7.131	0.892	21.638	1.421
Treatments	14	1.107	0.077	1.910	0.033
Error	112	4.616	0.042	-	-
Total	134	-	-	-	-

Coefficient of Variation = 21.011

Treatments found Significant at 5% level of Significance CD(0.05)= 0.186

Comparison of Treatment Means with Critical Difference (0.05)

Treatment No.	T 8	T 15	T 9	T 2	T 3	T 14	T 7	T 5	T 11	T 6	T 12	T 1	T 13	T 10	T 4
Treatment Average	1.084	1.082	1.080	1.036	1.034	1.020	0.955	0.946	0.944	0.944	0.938	0.927	0.915	0.823	0.758
Critical Difference (CD) Compared	a	a	a	a	a	a	ab	ab	abc	abc	abc	abc	abc	bc	c

Table No. 11 : Statistical Analysis for Growth Performance and average body weight of L.vennamei fed with different experimental diets reared in 5 PPT salinity:

Treatment means	
S.No	Average
Treatment 1	1.073

Treatment 2	2.158
Treatment 3	1.215
Treatment 4	0.924
Treatment 5	0.954
Treatment 6	1.026
Treatment 7	1.032
Treatment 8	1.217
Treatment 9	1.083
Treatment 10	0.964
Treatment 11	1.051
Treatment 12	1.042
Treatment 13	1.233
Treatment 14	1.230
Treatment 15	1.257

Anova Table					
Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F cal	F prob
Replications	8	15.639	1.957	3.155	0.009
Treatments	14	11.096	0.796	1.277	0.236
Error	112	69.421	0.618	-	-
Total	134	-	-	-	-

Coefficient of Variation = 67.613

Treatments found to be Non Significant

Table No. 12 : Statistical Analysis for Growth Performance and average body weight of L.vennamei fed with different experimental diets reared in 15 PPT salinity:

Treatment means	
S.No	Average
Treatment 1	1.154
Treatment 2	1.235
Treatment 3	1.170
Treatment 4	0.921
Treatment 5	1.056
Treatment 6	1.070
Treatment 7	1.114
Treatment 8	1.255
Treatment 9	1.356
Treatment 10	0.944
Treatment 11	1.037
Treatment 12	1.048
Treatment 13	1.028
Treatment 14	1.285
Treatment 15	1.244

Anova Table					
Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F cal	F prob
Replications	8	3.372	0.421	6.807	2.924
Treatments	14	2.079	0.140	2.381	0.009

Error	112	6.941	0.060	-	-
Total	134	-	-	-	-

Coefficient of Variation = 22.067

Treatments found Significant at 1% and 5% level of significance CD(0.01) = 0.303 CD(0.05) = 0.230

Comparison of Treatment Means with Critical Difference (0.05)

Treatment No.	T 9	T 14	T 8	T 15	T 2	T 3	T 1	T 7	T 6	T 5	T 12	T 11	T 13	T 10	T 4
Treatment Average	1.356	1.285	1.255	1.244	1.235	1.170	1.154	1.114	1.070	1.056	1.048	1.037	1.028	0.944	0.921
Critical Difference (CD) Compared	a	ab	abc	abc	abc	abcd	abcd	bcde	bcde	bcde	cde	cde	cde	de	e

Table No. 13 : Statistical Analysis for Growth Performance and average body weight of L.vennamei fed with different experimental diets reared in 25 PPT salinity:

Treatment means	
S.No	Average
Treatment 1	1.141
Treatment 2	1.330
Treatment 3	1.220
Treatment 4	1.020
Treatment 5	1.050
Treatment 6	1.060
Treatment 7	1.132
Treatment 8	1.351

Treatment 9	1.266
Treatment 10	0.881
Treatment 11	1.072
Treatment 12	1.054
Treatment 13	1.104
Treatment 14	1.398
Treatment 15	1.312

Anova Table					
Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F cal	F prob
Replications	8	3.804	0.471	6.614	4.660
Treatments	14	2.726	0.199	2.714	0.008
Error	112	8.048	0.078	-	-
Total	134	-	-	-	-

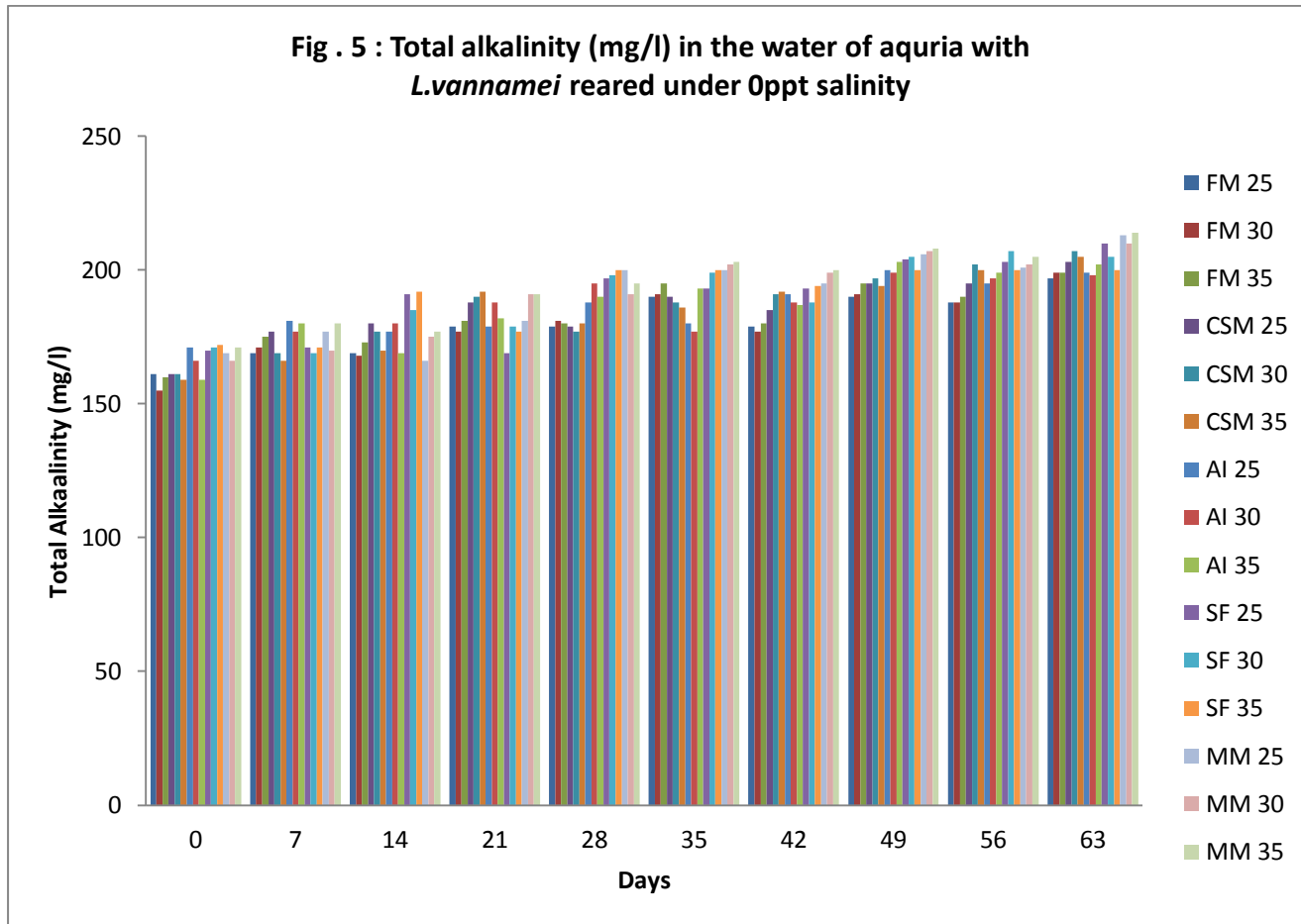
Coefficient of Variation = 23.115

Treatments found Significant at 1% and 5% level of significance CD(0.01) = 0.324 CD(0.05) = 0.246

Comparison of Treatment Means with Critical Difference (0.05)

Treatment No.	T 14	T 8	T 2	T 15	T 9	T 3	T 1	T 7	T 13	T 11	T 6	T 12	T 5	T 4	T 10
Treatment Average	1.398	1.351	1.330	1.312	1.266	1.220	1.141	1.132	1.104	1.072	1.060	1.054	1.050	1.020	0.881
Critical Difference (CD) Compared	a	ab	abc	abcd	abcde	abcdef	bcdef	bcdef	cdefg	defg	efg	efg	efg	fg	g

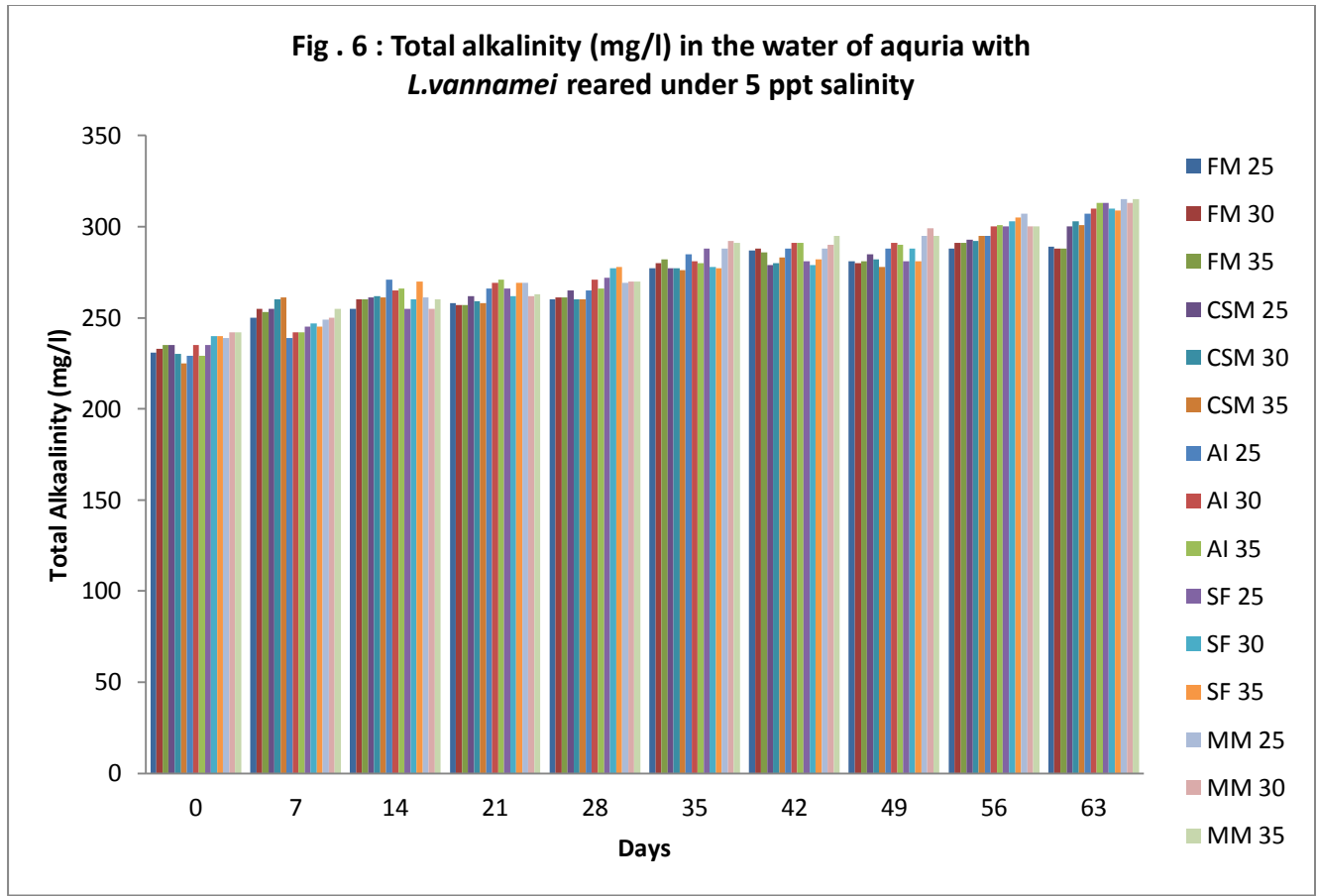
Fig . 5 : Total alkalinity (mg/l) in the water of aquaria with *L.vannamei* reared under 0ppt salinity



Note: FM25 : Fish meal containing 25% crude protein
 FM30 : Fish meal containing 30% crude protein

FM35	:	Fish meal containing 35% crude protein
CSM25	:	Cottonseed meal containing 25 % crude protein
CSM30	:	Cottonseed meal containing 30 % crude protein
CSM35	:	Cottonseed meal containing 35% crude protein
AI25	:	<i>Acetes indicus</i> meal containing 25% crude protein
AI30	:	<i>Acetes indicus</i> meal containing 30% crude protein
AI35	:	<i>Acetes indicus</i> meal containing 35% crude protein
SF25	:	Sunflower meal containing 25% crude protein
SF30	:	Sunflower meal containing 30% crude protein
SF35	:	Sunflower meal containing 35% crude protein
MM25	:	Mysid meal containing 25% crude protein
MM30	:	Mysid meal containing 30% crude protein
MM35	:	Mysid meal containing 35% crude protein

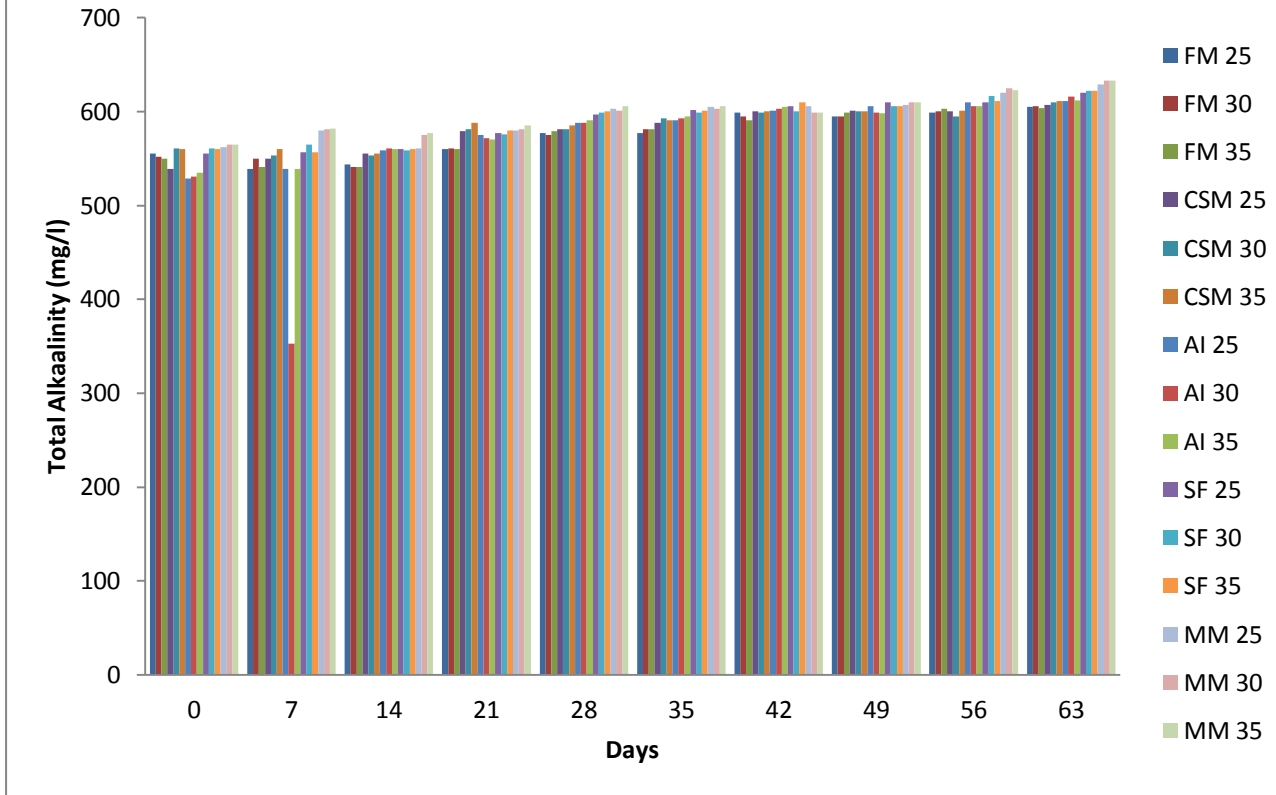
Fig . 6 : Total alkalinity (mg/l) in the water of aquaria with *L.vannamei* reared under 5 ppt salinity



Note: FM25 : Fish meal containing 25% crude protein
 FM30 : Fish meal containing 30% crude protein
 FM35 : Fish meal containing 35% crude protein
 CSM25 : Cottonseed meal containing 25 % crude protein
 CSM30 : Cottonseed meal containing 30 % crude protein

CSM35	:	Cottonseed meal containing 35% crude protein
AI25	:	<i>Acetes indicus</i> meal containing 25% crude protein
AI30	:	<i>Acetes indicus</i> meal containing 30% crude protein
AI35	:	<i>Acetes indicus</i> meal containing 35% crude protein
SF25	:	Sunflower meal containing 25% crude protein
SF30	:	Sunflower meal containing 30% crude protein
SF35	:	Sunflower meal containing 35% crude protein
MM25	:	Mysid meal containing 25% crude protein
MM30	:	Mysid meal containing 30% crude protein
MM35	:	Mysid meal containing 35% crude protein

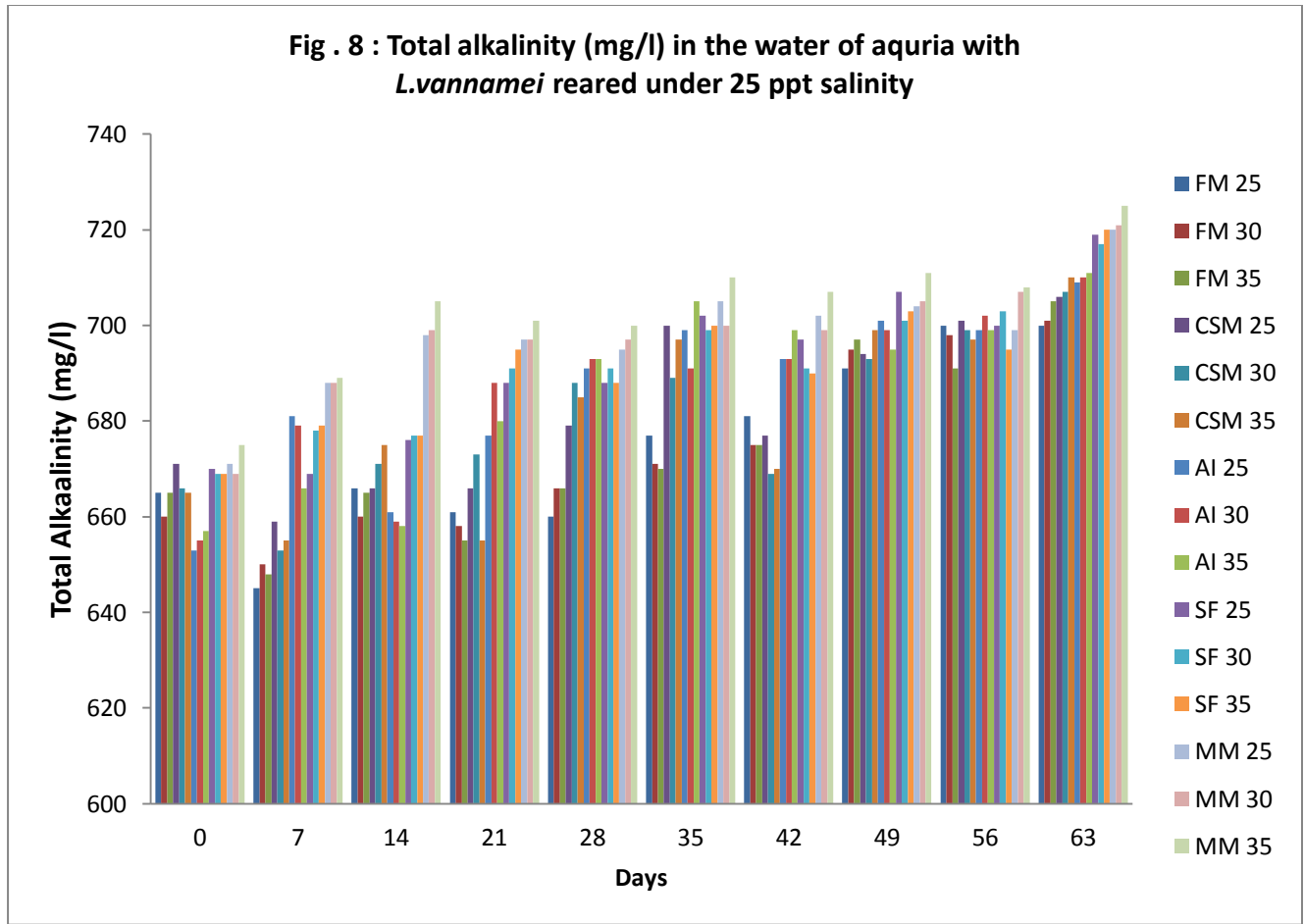
Fig . 7 : Total alkalinity (mg/l) in the water of aquaria with *L.vannamei* reared under 15 ppt salinity



Note: FM25 : Fish meal containing 25% crude protein
 FM30 : Fish meal containing 30% crude protein
 FM35 : Fish meal containing 35% crude protein
 CSM25 : Cottonseed meal containing 25 % crude protein
 CSM30 : Cottonseed meal containing 30 % crude protein
 CSM35 : Cottonseed meal containing 35% crude protein

AI25	:	<i>Acetes indicus</i> meal containing 25% crude protein
AI30	:	<i>Acetes indicus</i> meal containing 30% crude protein
AI35	:	<i>Acetes indicus</i> meal containing 35% crude protein
SF25	:	Sunflower meal containing 25% crude protein
SF30	:	Sunflower meal containing 30% crude protein
SF35	:	Sunflower meal containing 35% crude protein
MM25	:	Mysid meal containing 25% crude protein
MM30	:	Mysid meal containing 30% crude protein
MM35	:	Mysid meal containing 35% crude protein

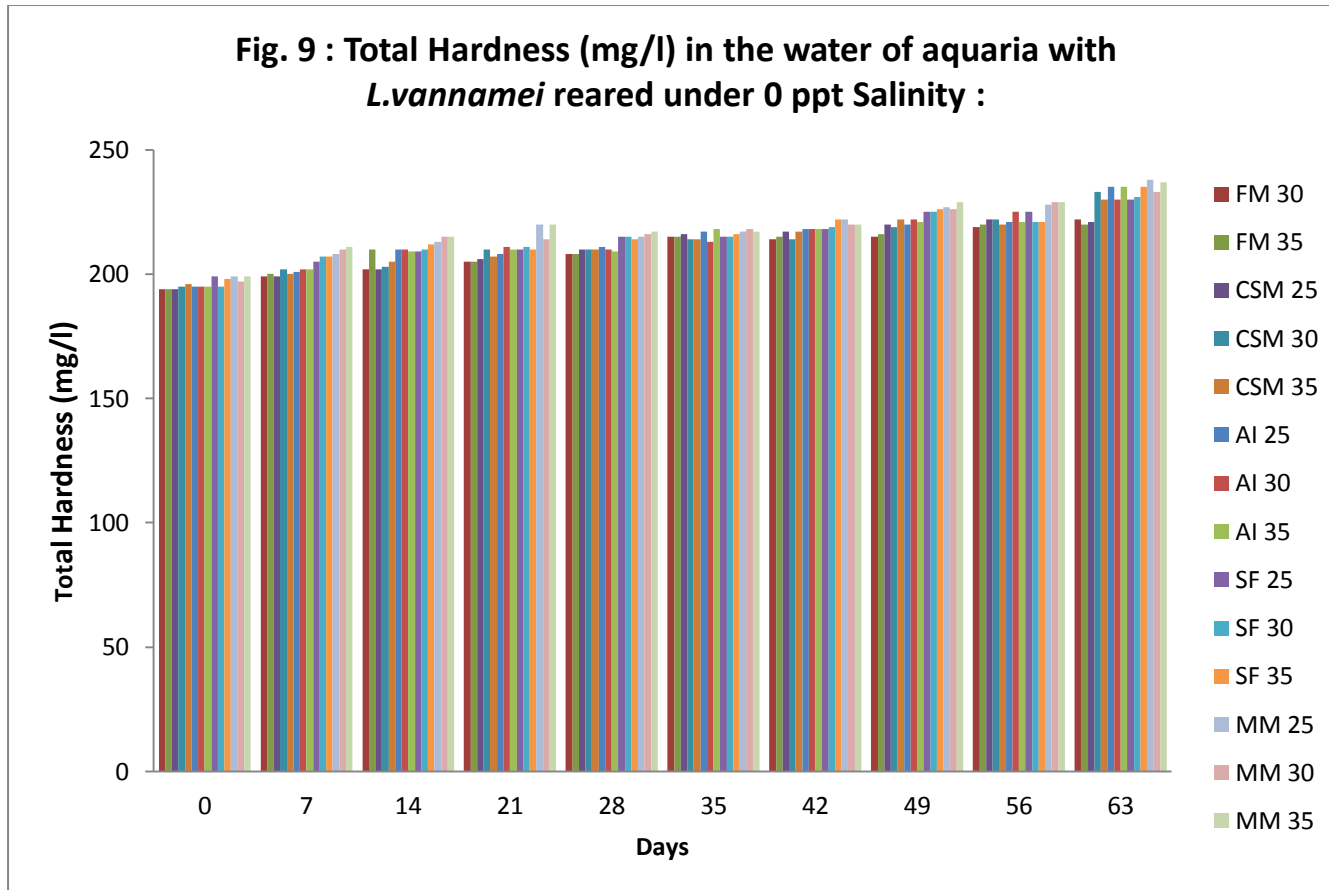
Fig . 8 : Total alkalinity (mg/l) in the water of aquaria with *L.vannamei* reared under 25 ppt salinity



Note: FM25 : Fish meal containing 25% crude protein
 FM30 : Fish meal containing 30% crude protein
 FM35 : Fish meal containing 35% crude protein
 CSM25 : Cottonseed meal containing 25 % crude protein
 CSM30 : Cottonseed meal containing 30 % crude protein

CSM35	:	Cottonseed meal containing 35% crude protein
AI25	:	<i>Acetes indicus</i> meal containing 25% crude protein
AI30	:	<i>Acetes indicus</i> meal containing 30% crude protein
AI35	:	<i>Acetes indicus</i> meal containing 35% crude protein
SF25	:	Sunflower meal containing 25% crude protein
SF30	:	Sunflower meal containing 30% crude protein
SF35	:	Sunflower meal containing 35% crude protein
MM25	:	Mysid meal containing 25% crude protein
MM30	:	Mysid meal containing 30% crude protein
MM35	:	Mysid meal containing 35% crude protein

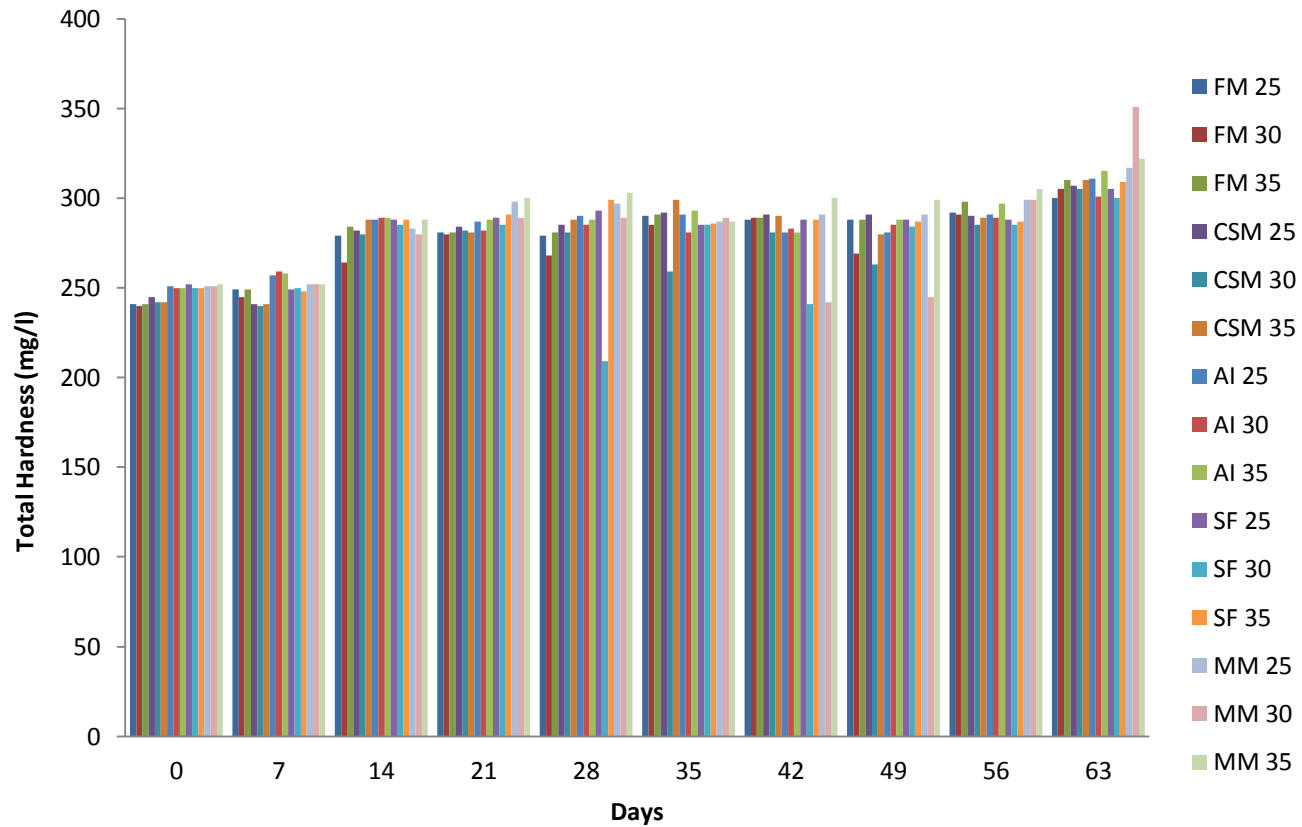
Fig. 9 : Total Hardness (mg/l) in the water of aquaria with *L.vannamei* reared under 0 ppt Salinity :



Note: FM25 : Fish meal containing 25% crude protein
 FM30 : Fish meal containing 30% crude protein
 FM35 : Fish meal containing 35% crude protein
 CSM25 : Cottonseed meal containing 25 % crude protein
 CSM30 : Cottonseed meal containing 30 % crude protein
 CSM35 : Cottonseed meal containing 35% crude protein

AI25	:	<i>Acetes indicus</i> meal containing 25% crude protein
AI30	:	<i>Acetes indicus</i> meal containing 30% crude protein
AI35	:	<i>Acetes indicus</i> meal containing 35% crude protein
SF25	:	Sunflower meal containing 25% crude protein
SF30	:	Sunflower meal containing 30% crude protein
SF35	:	Sunflower meal containing 35% crude protein
MM25	:	Mysid meal containing 25% crude protein
MM30	:	Mysid meal containing 30% crude protein
MM35	:	Mysid meal containing 35% crude protein

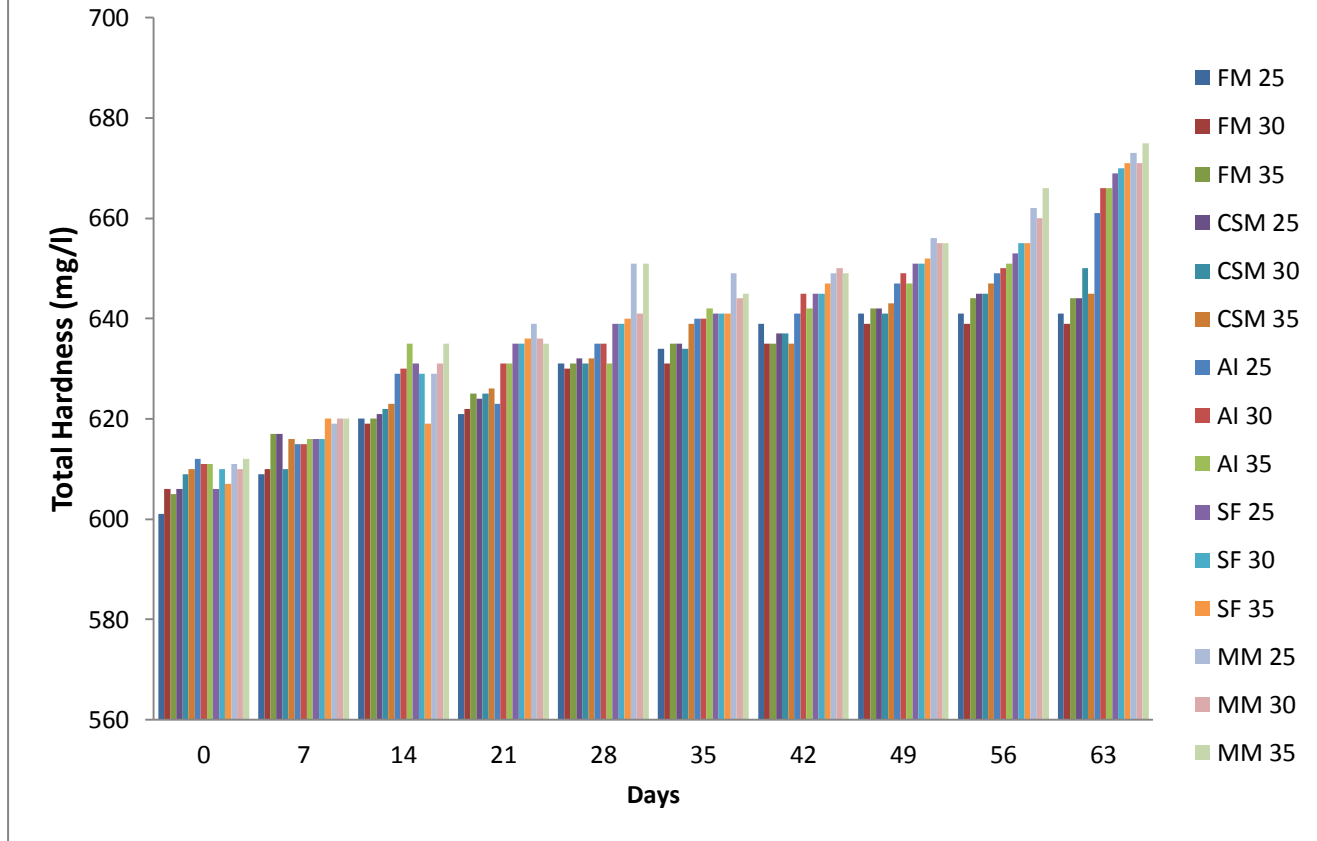
Fig. 10 : Total Hardness (mg/l) in the water of aquaria with *L.vannamei* reared under 5 ppt Salinity :



Note: FM25 : Fish meal containing 25% crude protein
 FM30 : Fish meal containing 30% crude protein

FM35	:	Fish meal containing 35% crude protein
CSM25	:	Cottonseed meal containing 25 % crude protein
CSM30	:	Cottonseed meal containing 30 % crude protein
CSM35	:	Cottonseed meal containing 35% crude protein
AI25	:	<i>Acetes indicus</i> meal containing 25% crude protein
AI30	:	<i>Acetes indicus</i> meal containing 30% crude protein
AI35	:	<i>Acetes indicus</i> meal containing 35% crude protein
SF25	:	Sunflower meal containing 25% crude protein
SF30	:	Sunflower meal containing 30% crude protein
SF35	:	Sunflower meal containing 35% crude protein
MM25	:	Mysid meal containing 25% crude protein
MM30	:	Mysid meal containing 30% crude protein
MM35	:	Mysid meal containing 35% crude protein

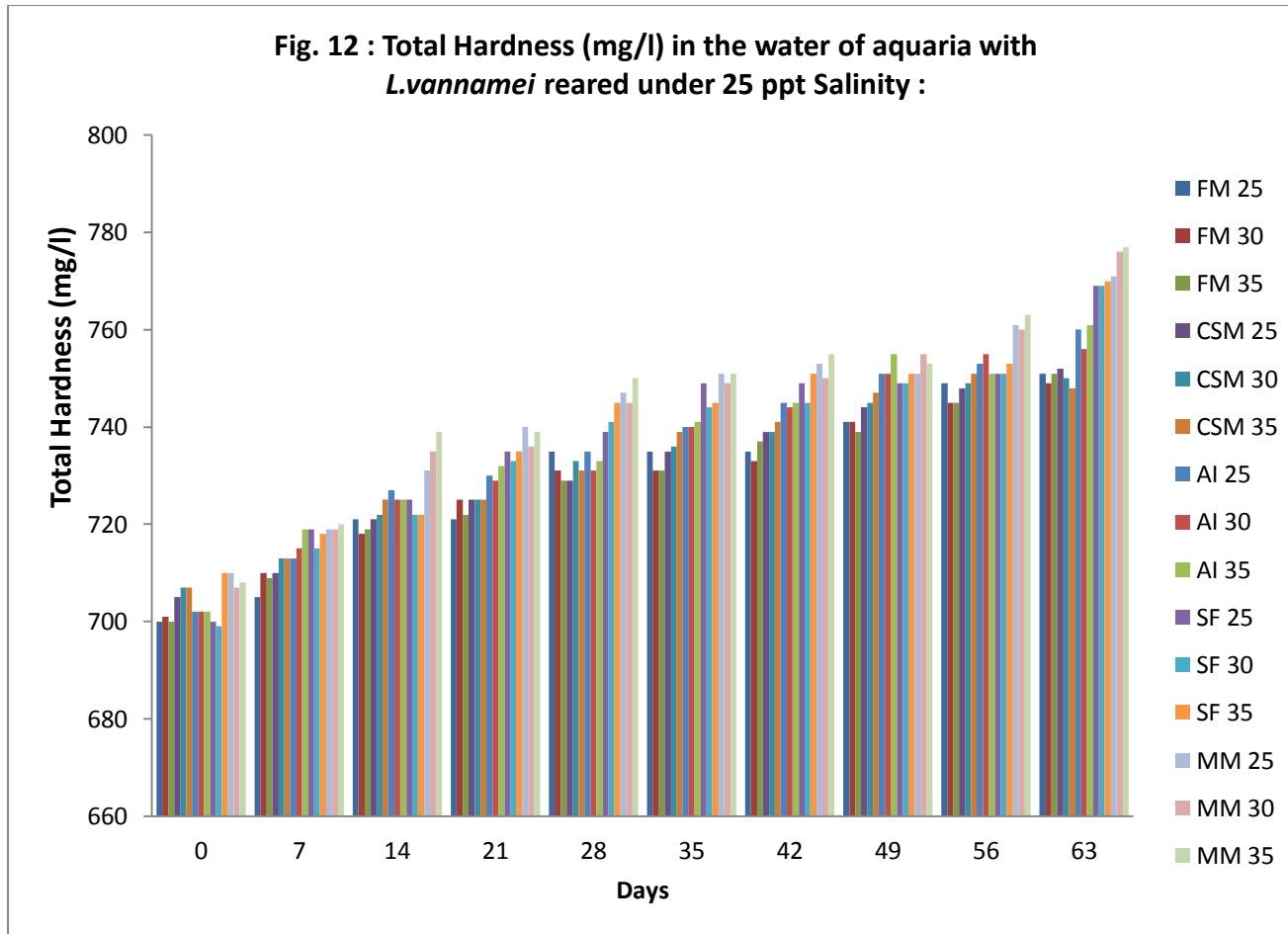
Fig. 11 : Total Hardness (mg/l) in the water of aquaria with *L.vannamei* reared under 15 ppt Salinity :



Note: FM25 : Fish meal containing 25% crude protein
 FM30 : Fish meal containing 30% crude protein
 FM35 : Fish meal containing 35% crude protein

CSM25	:	Cottonseed meal containing 25 % crude protein
CSM30	:	Cottonseed meal containing 30 % crude protein
CSM35	:	Cottonseed meal containing 35% crude protein
AI25	:	<i>Acetes indicus</i> meal containing 25% crude protein
AI30	:	<i>Acetes indicus</i> meal containing 30% crude protein
AI35	:	<i>Acetes indicus</i> meal containing 35% crude protein
SF25	:	Sunflower meal containing 25% crude protein
SF30	:	Sunflower meal containing 30% crude protein
SF35	:	Sunflower meal containing 35% crude protein
MM25	:	Mysid meal containing 25% crude protein
MM30	:	Mysid meal containing 30% crude protein
MM35	:	Mysid meal containing 35% crude protein

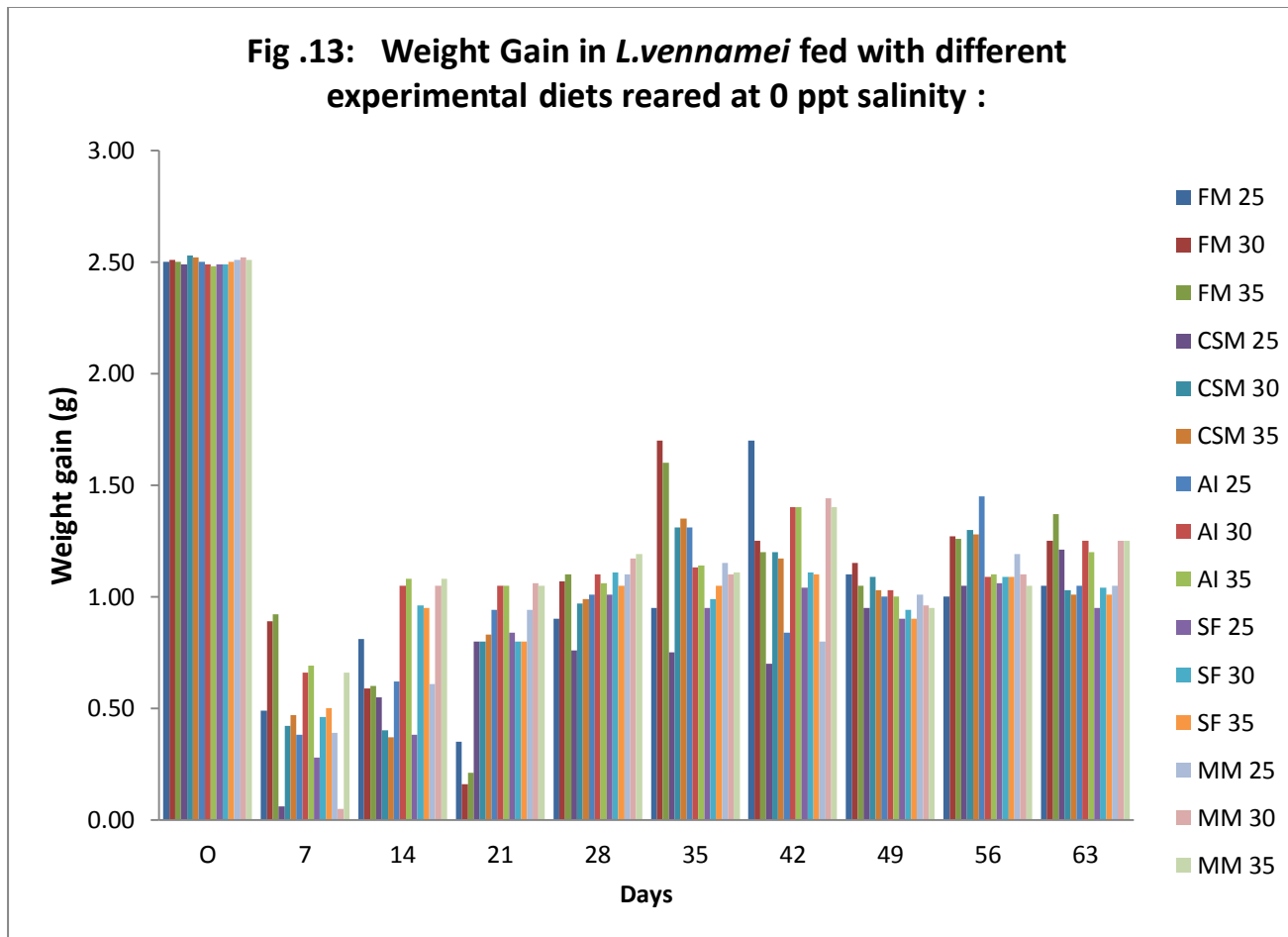
Fig. 12 : Total Hardness (mg/l) in the water of aquaria with *L.vannamei* reared under 25 ppt Salinity :



Note: FM25 : Fish meal containing 25% crude protein
 FM30 : Fish meal containing 30% crude protein
 FM35 : Fish meal containing 35% crude protein
 CSM25 : Cottonseed meal containing 25 % crude protein
 CSM30 : Cottonseed meal containing 30 % crude protein

CSM35 : Cottonseed meal containing 35% crude protein
AI25 : *Acetes indicus* meal containing 25% crude protein
AI30 : *Acetes indicus* meal containing 30% crude protein
AI35 : *Acetes indicus* meal containing 35% crude protein
SF25 : Sunflower meal containing 25% crude protein
SF30 : Sunflower meal containing 30% crude protein
SF35 : Sunflower meal containing 35% crude protein
MM25 : Mysid meal containing 25% crude protein
MM30 : Mysid meal containing 30% crude protein
MM35 : Mysid meal containing 35% crude protein

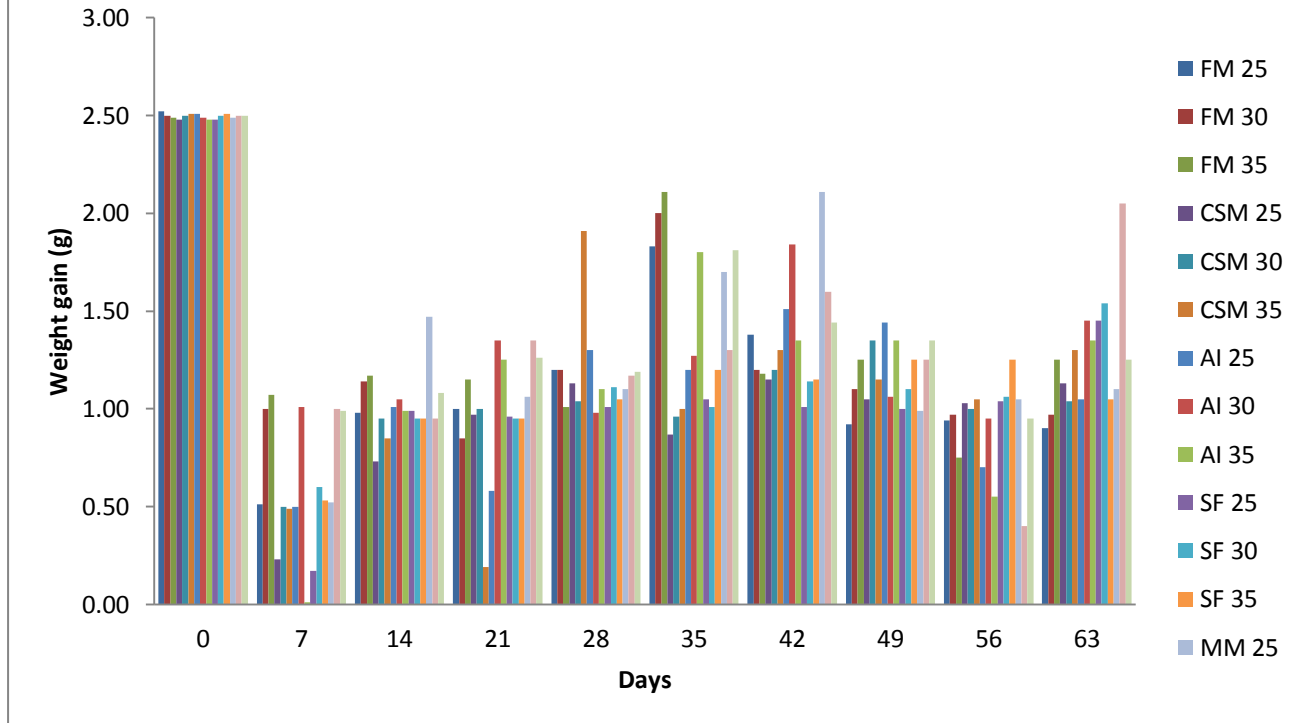
Fig .13: Weight Gain in *L.vennamei* fed with different experimental diets reared at 0 ppt salinity :



Note: FM25 : Fish meal containing 25% crude protein
 FM30 : Fish meal containing 30% crude protein
 FM35 : Fish meal containing 35% crude protein
 CSM25 : Cottonseed meal containing 25 % crude protein

CSM30	:	Cottonseed meal containing 30 % crude protein
CSM35	:	Cottonseed meal containing 35% crude protein
AI25	:	<i>Acetes indicus</i> meal containing 25% crude protein
AI30	:	<i>Acetes indicus</i> meal containing 30% crude protein
AI35	:	<i>Acetes indicus</i> meal containing 35% crude protein
SF25	:	Sunflower meal containing 25% crude protein
SF30	:	Sunflower meal containing 30% crude protein
SF35	:	Sunflower meal containing 35% crude protein
MM25	:	Mysid meal containing 25% crude protein
MM30	:	Mysid meal containing 30% crude protein
MM35	:	Mysid meal containing 35% crude protein

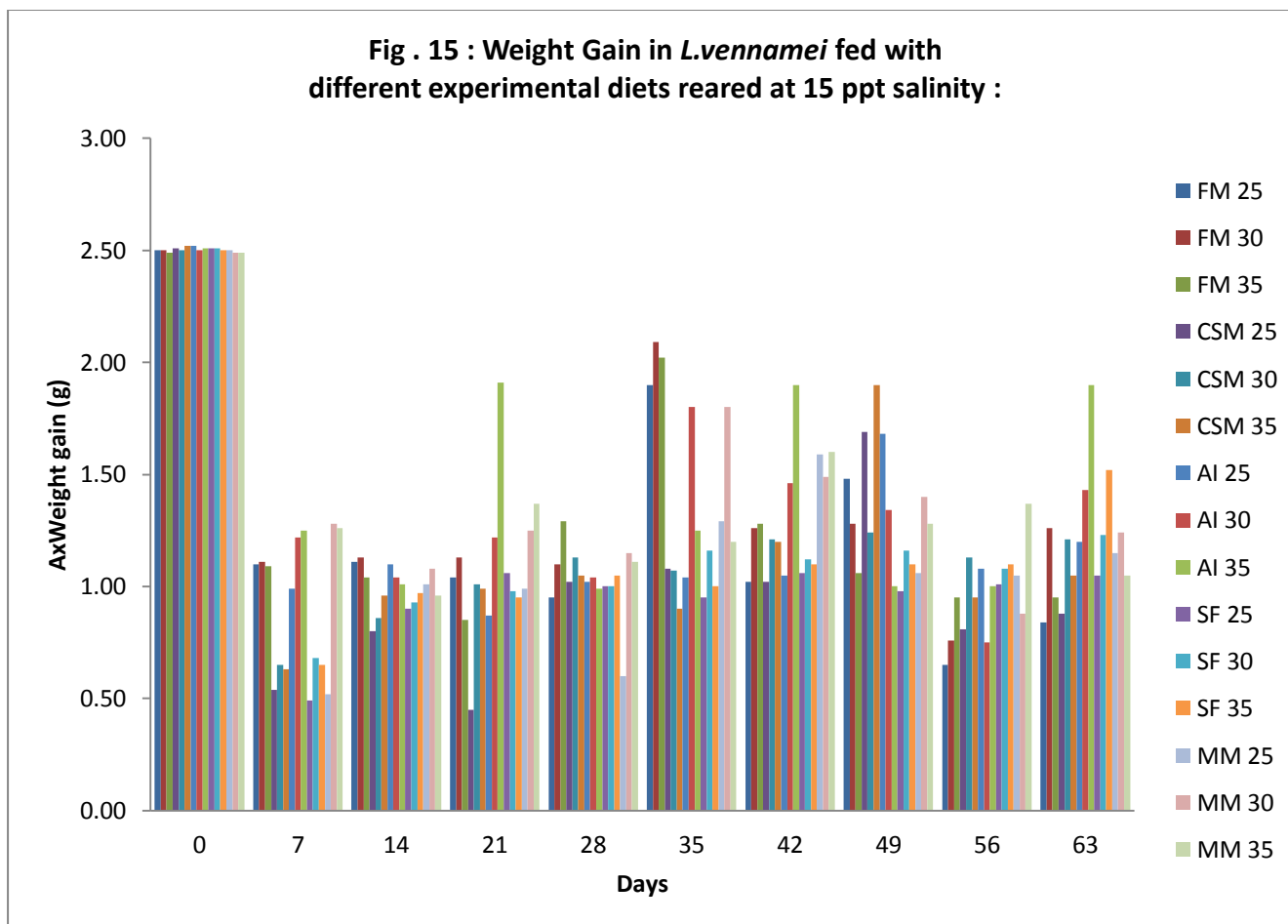
Fig . 14: Weight Gain in *L.vennamei* fed with different experimental diets reared at 5 ppt salinity :



Note: FM25 : Fish meal containing 25% crude protein
 FM30 : Fish meal containing 30% crude protein
 FM35 : Fish meal containing 35% crude protein
 CSM25 : Cottonseed meal containing 25 % crude protein
 CSM30 : Cottonseed meal containing 30 % crude protein
 CSM35 : Cottonseed meal containing 35% crude protein
 AI25 : *Acetes indicus* meal containing 25% crude protein
 AI30 : *Acetes indicus* meal containing 30% crude protein

AI35 : *Acetes indicus* meal containing 35% crude protein
SF25 : Sunflower meal containing 25% crude protein
SF30 : Sunflower meal containing 30% crude protein
SF35 : Sunflower meal containing 35% crude protein
MM25 : Mysid meal containing 25% crude protein
MM30 : Mysid meal containing 30% crude protein
MM35 : Mysid meal containing 35% crude protein

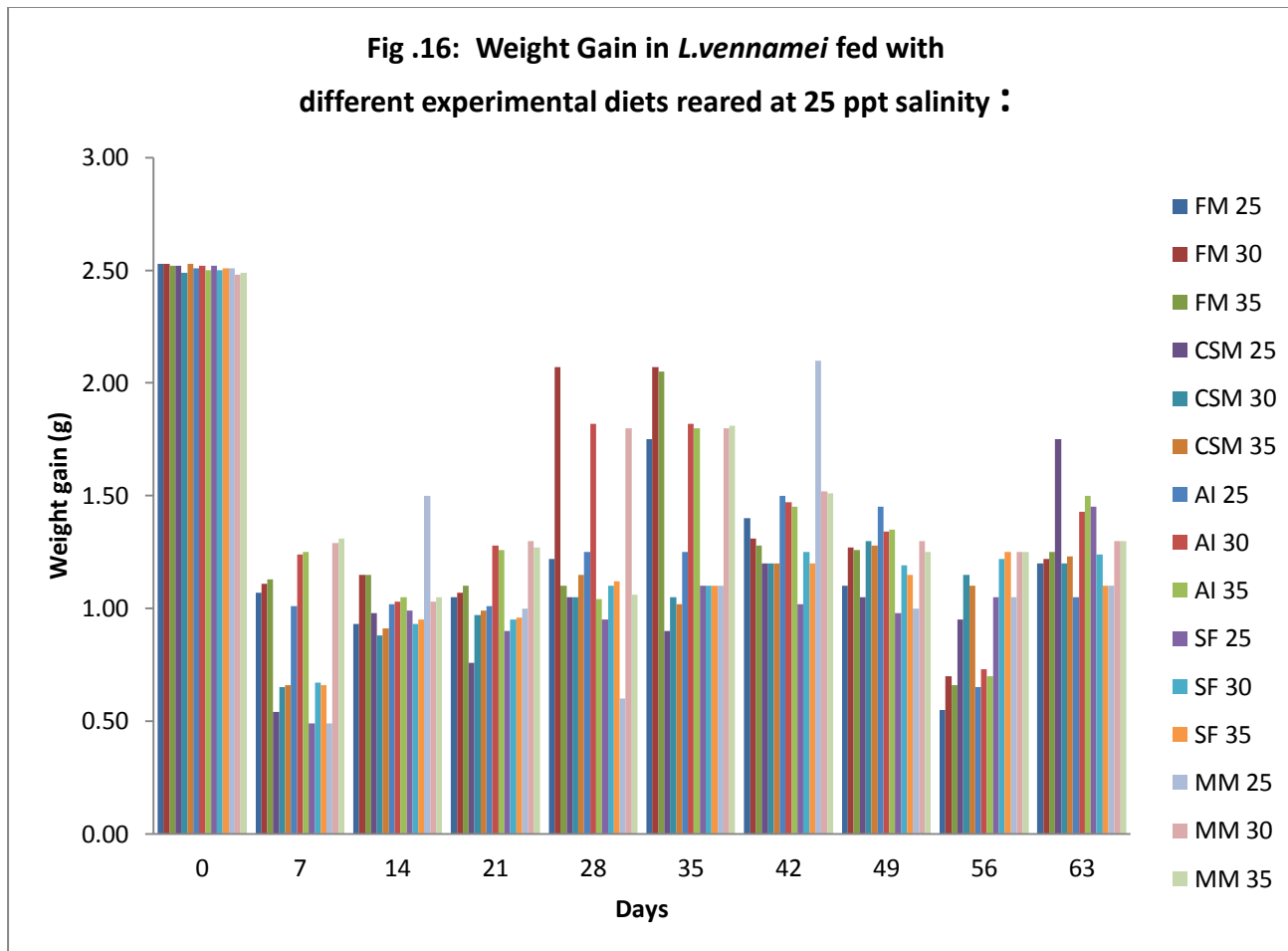
Fig . 15 : Weight Gain in *L.vennamei* fed with different experimental diets reared at 15 ppt salinity :



Note: FM25 : Fish meal containing 25% crude protein
 FM30 : Fish meal containing 30% crude protein
 FM35 : Fish meal containing 35% crude protein
 CSM25 : Cottonseed meal containing 25 % crude protein
 CSM30 : Cottonseed meal containing 30 % crude protein

CSM35	:	Cottonseed meal containing 35% crude protein
AI25	:	<i>Acetes indicus</i> meal containing 25% crude protein
AI30	:	<i>Acetes indicus</i> meal containing 30% crude protein
AI35	:	<i>Acetes indicus</i> meal containing 35% crude protein
SF25	:	Sunflower meal containing 25% crude protein
SF30	:	Sunflower meal containing 30% crude protein
SF35	:	Sunflower meal containing 35% crude protein
MM25	:	Mysid meal containing 25% crude protein
MM30	:	Mysid meal containing 30% crude protein
MM35	:	Mysid meal containing 35% crude protein

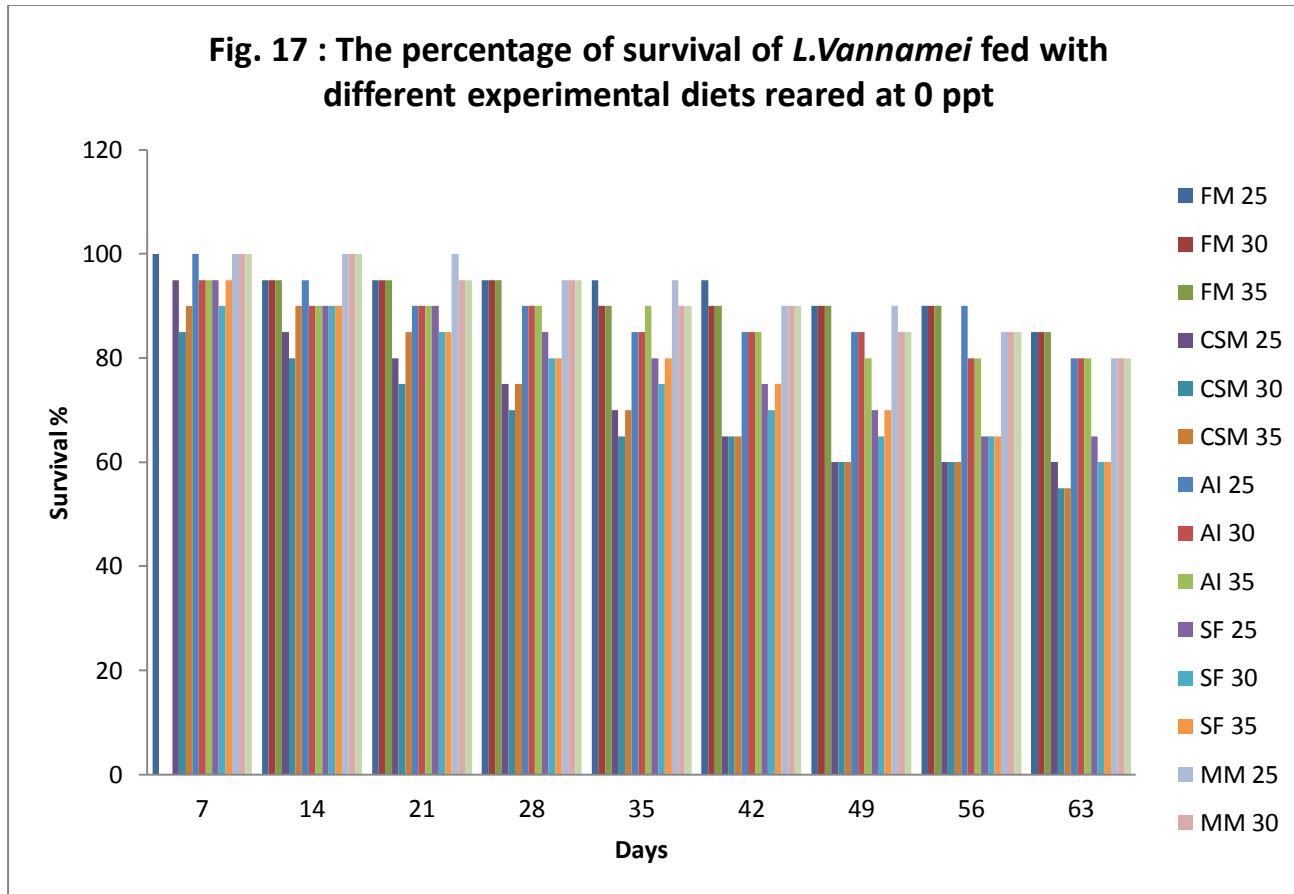
Fig .16: Weight Gain in *L.vennamei* fed with different experimental diets reared at 25 ppt salinity :



Note: FM25 : Fish meal containing 25% crude protein
 FM30 : Fish meal containing 30% crude protein
 FM35 : Fish meal containing 35% crude protein
 CSM25 : Cottonseed meal containing 25 % crude protein
 CSM30 : Cottonseed meal containing 30 % crude protein

CSM35	:	Cottonseed meal containing 35% crude protein
AI25	:	<i>Acetes indicus</i> meal containing 25% crude protein
AI30	:	<i>Acetes indicus</i> meal containing 30% crude protein
AI35	:	<i>Acetes indicus</i> meal containing 35% crude protein
SF25	:	Sunflower meal containing 25% crude protein
SF30	:	Sunflower meal containing 30% crude protein
SF35	:	Sunflower meal containing 35% crude protein
MM25	:	Mysid meal containing 25% crude protein
MM30	:	Mysid meal containing 30% crude protein
MM35	:	Mysid meal containing 35% crude protein

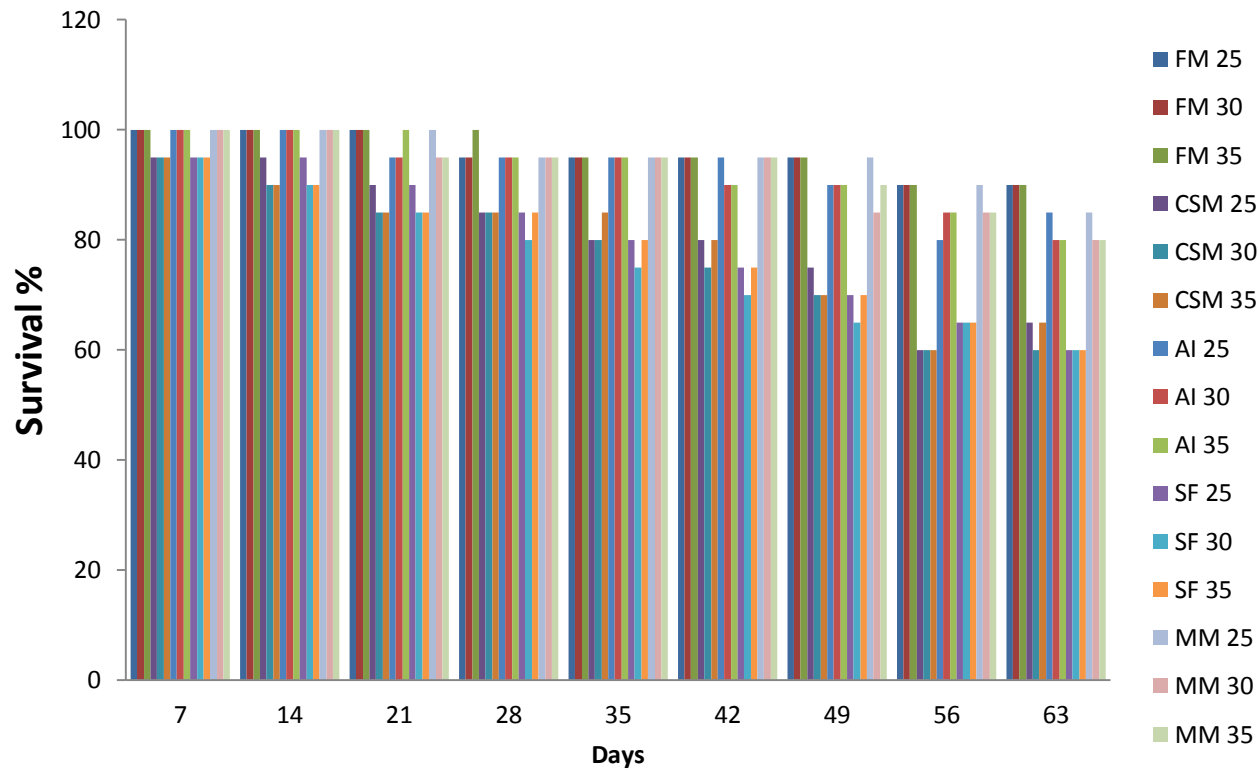
Fig. 17 : The percentage of survival of *L.Vannamei* fed with different experimental diets reared at 0 ppt



Note: FM25 : Fish meal containing 25% crude protein
 FM30 : Fish meal containing 30% crude protein
 FM35 : Fish meal containing 35% crude protein
 CSM25 : Cottonseed meal containing 25 % crude protein
 CSM30 : Cottonseed meal containing 30 % crude protein
 CSM35 : Cottonseed meal containing 35% crude protein

AI25	:	<i>Acetes indicus</i> meal containing 25% crude protein
AI30	:	<i>Acetes indicus</i> meal containing 30% crude protein
AI35	:	<i>Acetes indicus</i> meal containing 35% crude protein
SF25	:	Sunflower meal containing 25% crude protein
SF30	:	Sunflower meal containing 30% crude protein
SF35	:	Sunflower meal containing 35% crude protein
MM25	:	Mysid meal containing 25% crude protein
MM30	:	Mysid meal containing 30% crude protein
MM35	:	Mysid meal containing 35% crude protein

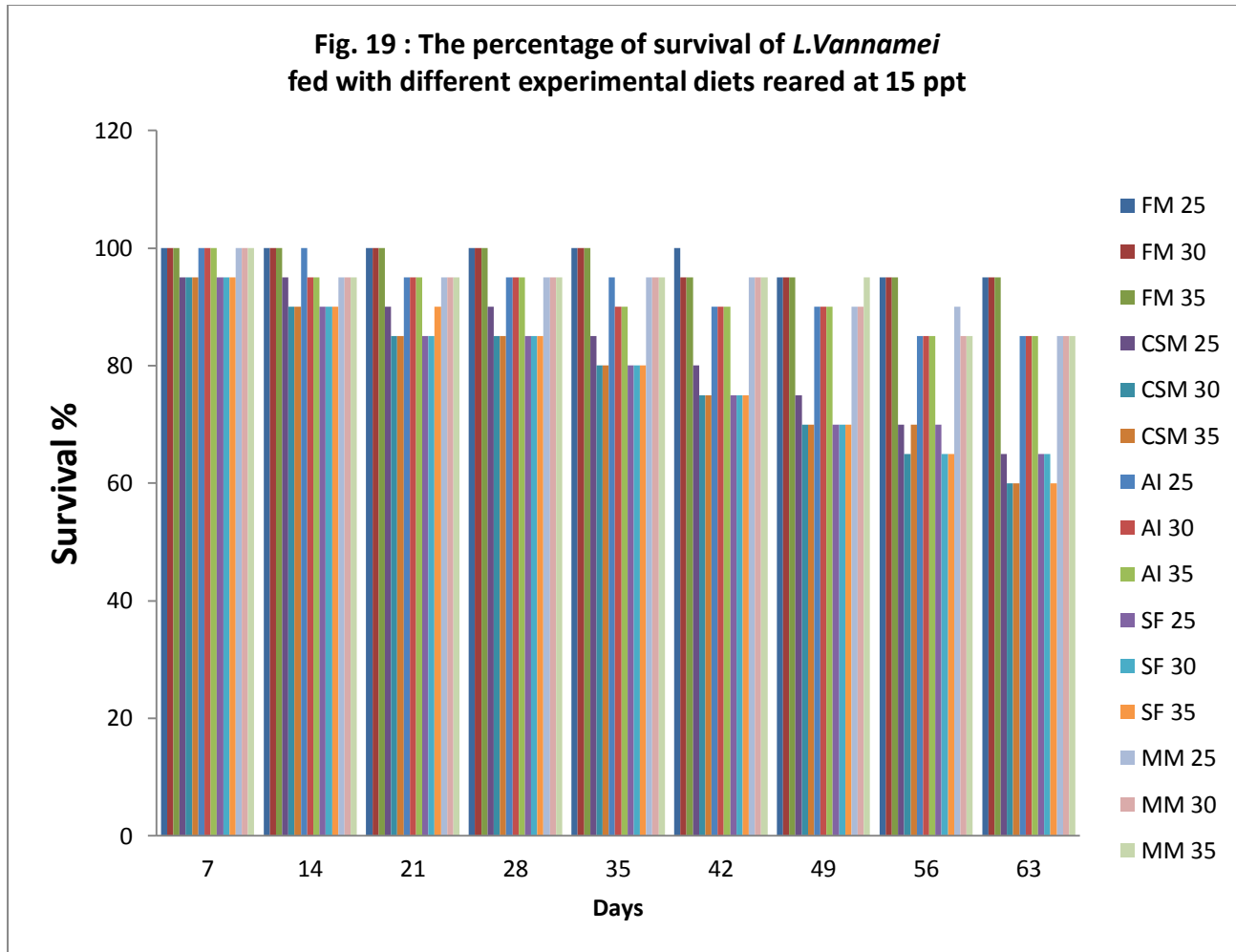
Fig. 18 : The percentage of survival of *L.Vannamei* fed with different experimental diets reared at 5 ppt



Note: FM25 : Fish meal containing 25% crude protein
 FM30 : Fish meal containing 30% crude protein
 FM35 : Fish meal containing 35% crude protein
 CSM25 : Cottonseed meal containing 25 % crude protein
 CSM30 : Cottonseed meal containing 30 % crude protein
 CSM35 : Cottonseed meal containing 35% crude protein

AI25	:	<i>Acetes indicus</i> meal containing 25% crude protein
AI30	:	<i>Acetes indicus</i> meal containing 30% crude protein
AI35	:	<i>Acetes indicus</i> meal containing 35% crude protein
SF25	:	Sunflower meal containing 25% crude protein
SF30	:	Sunflower meal containing 30% crude protein
SF35	:	Sunflower meal containing 35% crude protein
MM25	:	Mysid meal containing 25% crude protein
MM30	:	Mysid meal containing 30% crude protein
MM35	:	Mysid meal containing 35% crude protein

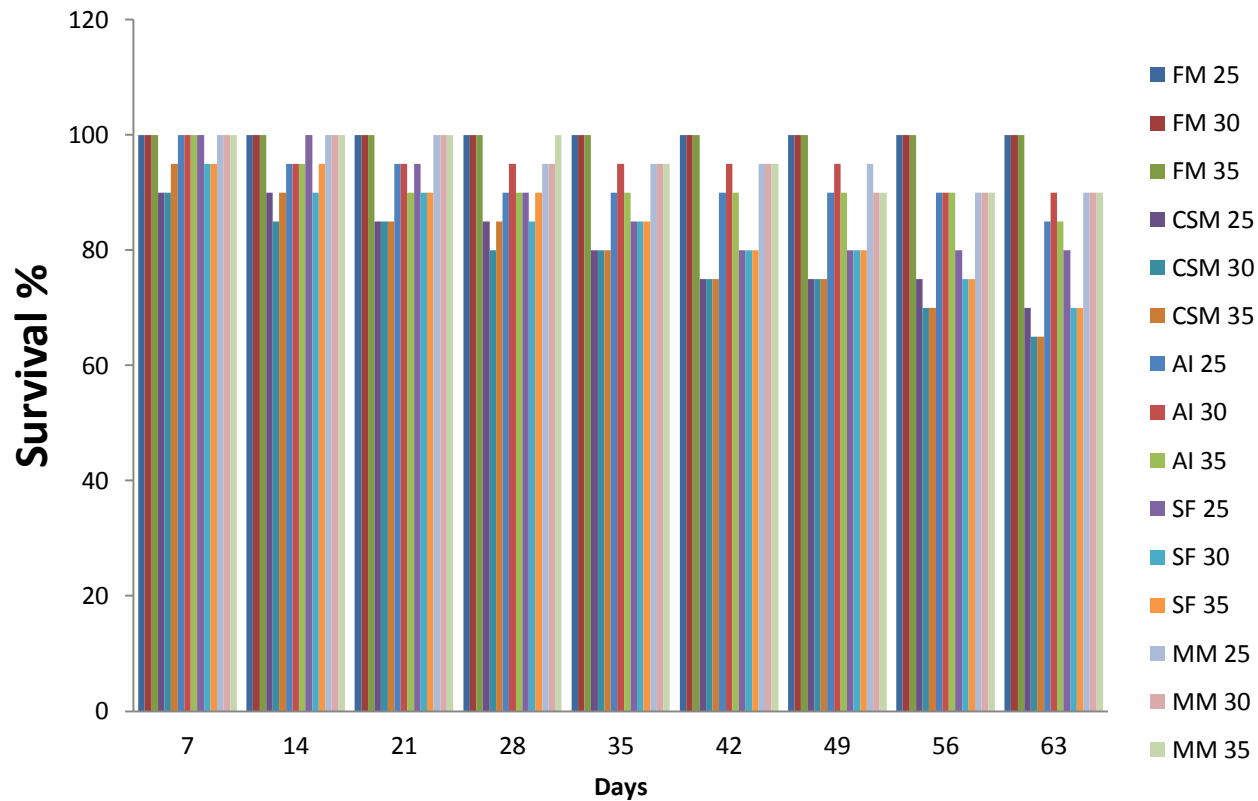
Fig. 19 : The percentage of survival of *L.Vannamei* fed with different experimental diets reared at 15 ppt



Note: FM25 : Fish meal containing 25% crude protein
 FM30 : Fish meal containing 30% crude protein
 FM35 : Fish meal containing 35% crude protein
 CSM25 : Cottonseed meal containing 25 % crude protein

CSM30	:	Cottonseed meal containing 30 % crude protein
CSM35	:	Cottonseed meal containing 35% crude protein
AI25	:	<i>Acetes indicus</i> meal containing 25% crude protein
AI30	:	<i>Acetes indicus</i> meal containing 30% crude protein
AI35	:	<i>Acetes indicus</i> meal containing 35% crude protein
SF25	:	Sunflower meal containing 25% crude protein
SF30	:	Sunflower meal containing 30% crude protein
SF35	:	Sunflower meal containing 35% crude protein
MM25	:	Mysid meal containing 25% crude protein
MM30	:	Mysid meal containing 30% crude protein
MM35	:	Mysid meal containing 35% crude protein

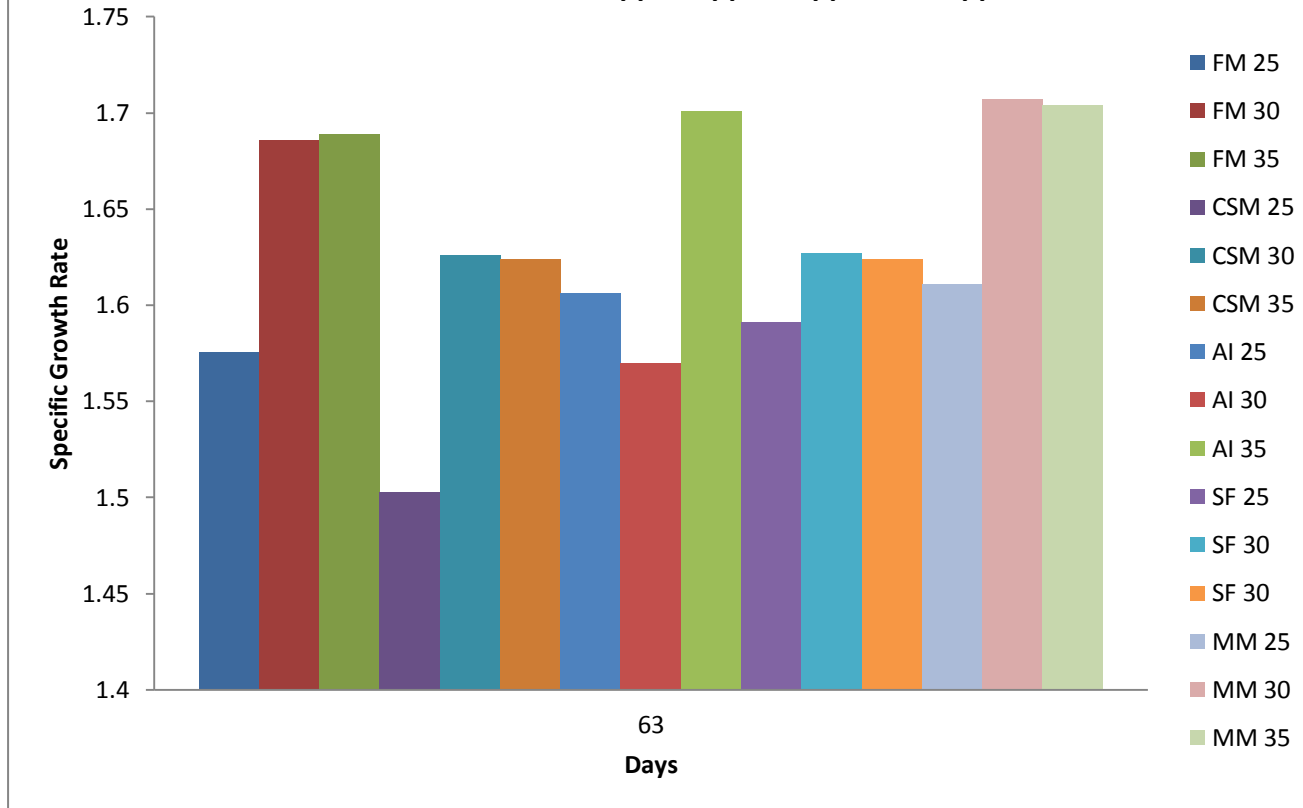
Fig. 20 : The percentage of survival of *L.Vannamei* fed with different experimental diets reared at 25 ppt



Note: FM25 : Fish meal containing 25% crude protein
 FM30 : Fish meal containing 30% crude protein
 FM35 : Fish meal containing 35% crude protein
 CSM25 : Cottonseed meal containing 25 % crude protein
 CSM30 : Cottonseed meal containing 30 % crude protein

CSM35	:	Cottonseed meal containing 35% crude protein
AI25	:	<i>Acetes indicus</i> meal containing 25% crude protein
AI30	:	<i>Acetes indicus</i> meal containing 30% crude protein
AI35	:	<i>Acetes indicus</i> meal containing 35% crude protein
SF25	:	Sunflower meal containing 25% crude protein
SF30	:	Sunflower meal containing 30% crude protein
SF35	:	Sunflower meal containing 35% crude protein
MM25	:	Mysid meal containing 25% crude protein
MM30	:	Mysid meal containing 30% crude protein
MM35	:	Mysid meal containing 35% crude protein

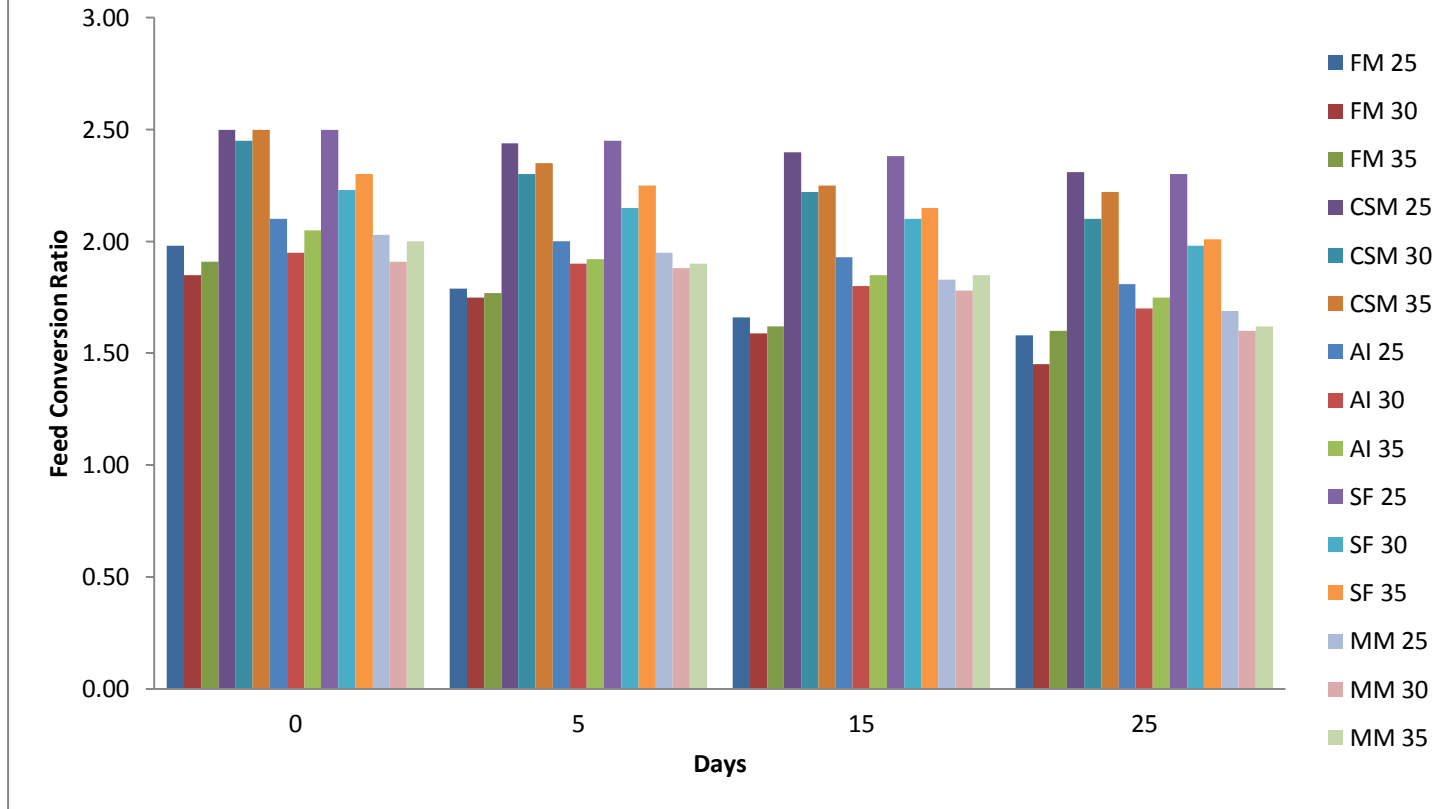
Fig. 21 : Specific Growth Rate (SGR) in Water of Aquaria with *L.vannamei* in different 0 ppt, 5 ppt, 15 ppt and 25 ppt salinities



Note: FM25 : Fish meal containing 25% crude protein
 FM30 : Fish meal containing 30% crude protein
 FM35 : Fish meal containing 35% crude protein
 CSM25 : Cottonseed meal containing 25 % crude protein
 CSM30 : Cottonseed meal containing 30 % crude protein
 CSM35 : Cottonseed meal containing 35% crude protein

AI25	:	<i>Acetes indicus</i> meal containing 25% crude protein
AI30	:	<i>Acetes indicus</i> meal containing 30% crude protein
AI35	:	<i>Acetes indicus</i> meal containing 35% crude protein
SF25	:	Sunflower meal containing 25% crude protein
SF30	:	Sunflower meal containing 30% crude protein
SF35	:	Sunflower meal containing 35% crude protein
MM25	:	Mysid meal containing 25% crude protein
MM30	:	Mysid meal containing 30% crude protein
MM35	:	Mysid meal containing 35% crude protein

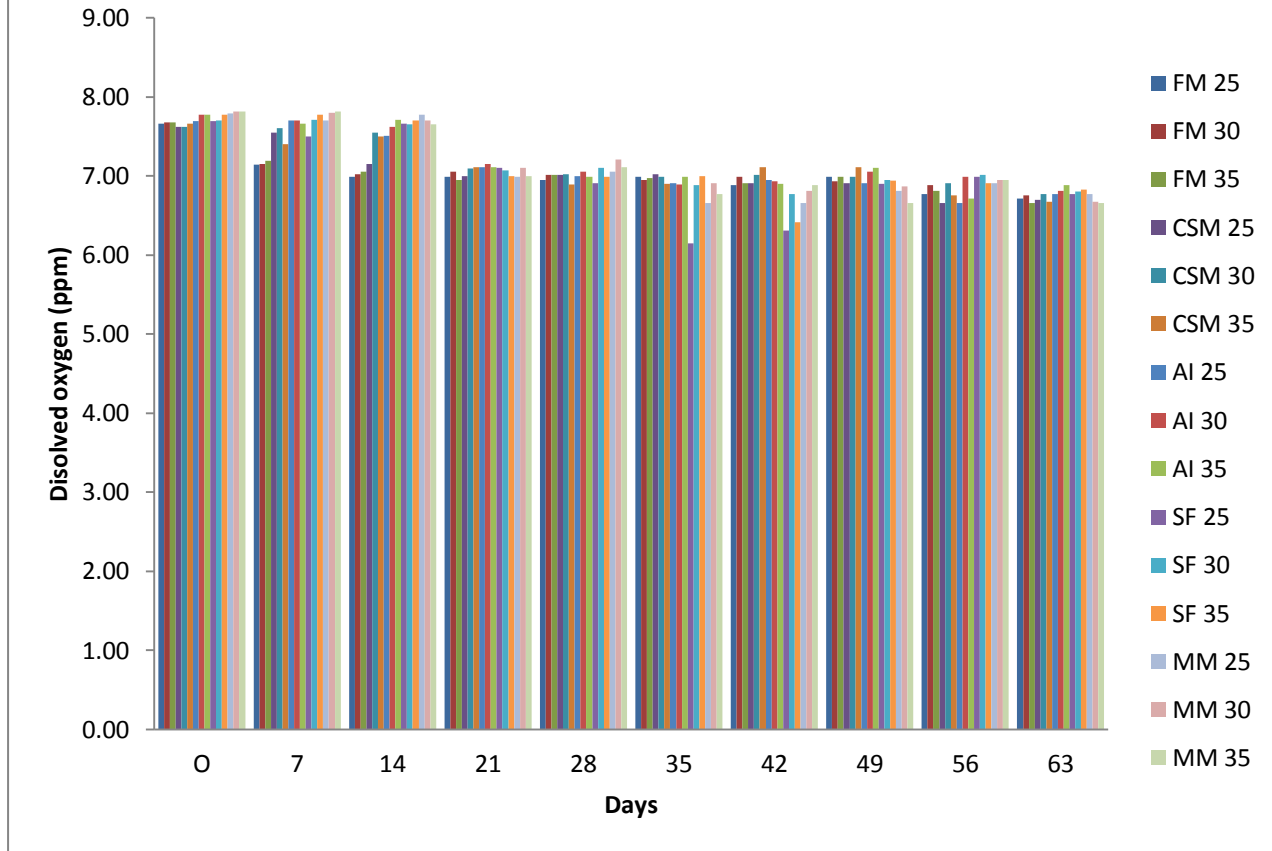
Fig. 22 : Feed Conversion Ratioa of L.vannamei fed with diffent diets in water of aquaria 0 ppt, 5 ppt, 15 ppt and 25 ppt salinities



Note: FM25 : Fish meal containing 25% crude protein
 FM30 : Fish meal containing 30% crude protein
 FM35 : Fish meal containing 35% crude protein
 CSM25 : Cottonseed meal containing 25 % crude protein
 CSM30 : Cottonseed meal containing 30 % crude protein

CSM35	:	Cottonseed meal containing 35% crude protein
AI25	:	<i>Acetes indicus</i> meal containing 25% crude protein
AI30	:	<i>Acetes indicus</i> meal containing 30% crude protein
AI35	:	<i>Acetes indicus</i> meal containing 35% crude protein
SF25	:	Sunflower meal containing 25% crude protein
SF30	:	Sunflower meal containing 30% crude protein
SF35	:	Sunflower meal containing 35% crude protein
MM25	:	Mysid meal containing 25% crude protein
MM30	:	Mysid meal containing 30% crude protein
MM35	:	Mysid meal containing 35% crude protein

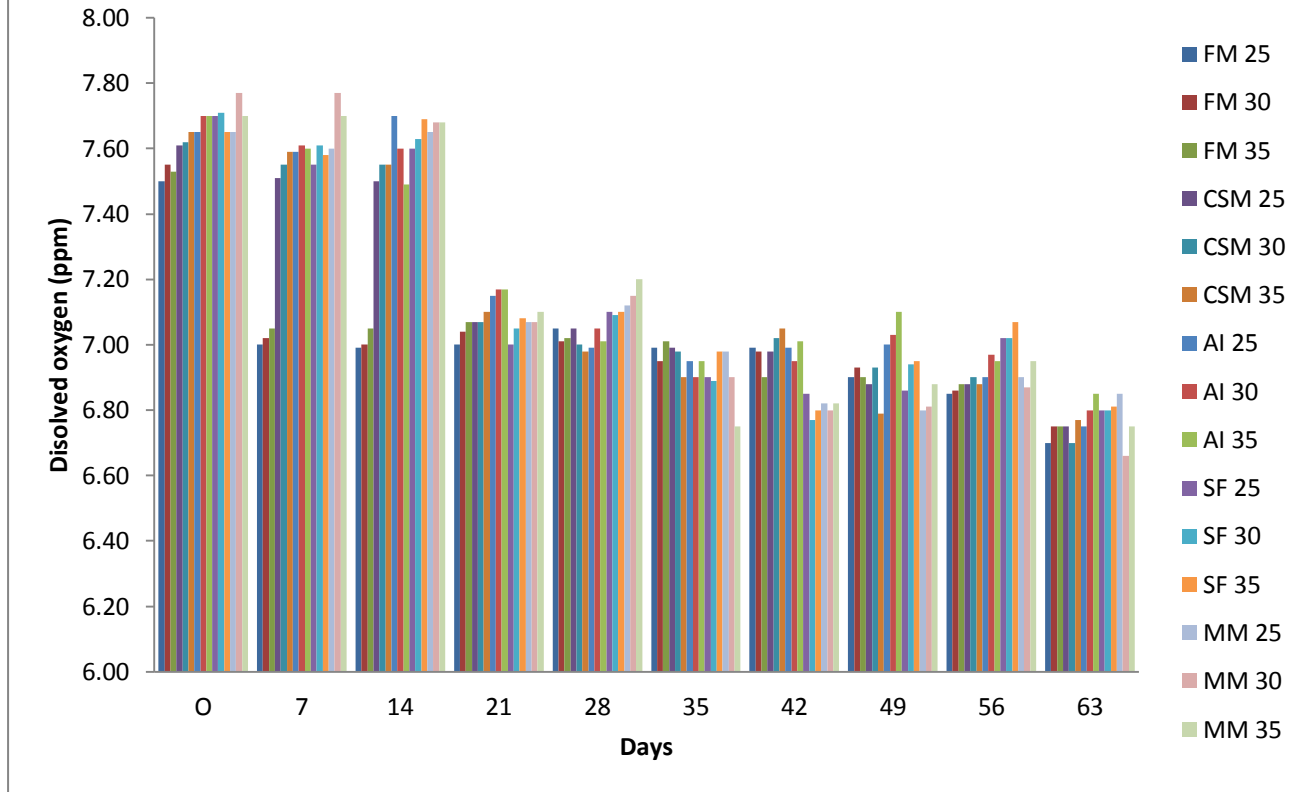
Fig 1 : Dissolved oxygen (mg/l) in the water of aquaria with *L.vannamei* reared at Oppt Salinity :



Note: FM25 : Fish meal containing 25% crude protein
 FM30 : Fish meal containing 30% crude protein
 FM35 : Fish meal containing 35% crude protein
 CSM25 : Cottonseed meal containing 25 % crude protein

CSM30	:	Cottonseed meal containing 30 % crude protein
CSM35	:	Cottonseed meal containing 35% crude protein
AI25	:	<i>Acetes indicus</i> meal containing 25% crude protein
AI30	:	<i>Acetes indicus</i> meal containing 30% crude protein
AI35	:	<i>Acetes indicus</i> meal containing 35% crude protein
SF25	:	Sunflower meal containing 25% crude protein
SF30	:	Sunflower meal containing 30% crude protein
SF35	:	Sunflower meal containing 35% crude protein
MM25	:	Mysid meal containing 25% crude protein
MM30	:	Mysid meal containing 30% crude protein
MM35	:	Mysid meal containing 35% crude protein

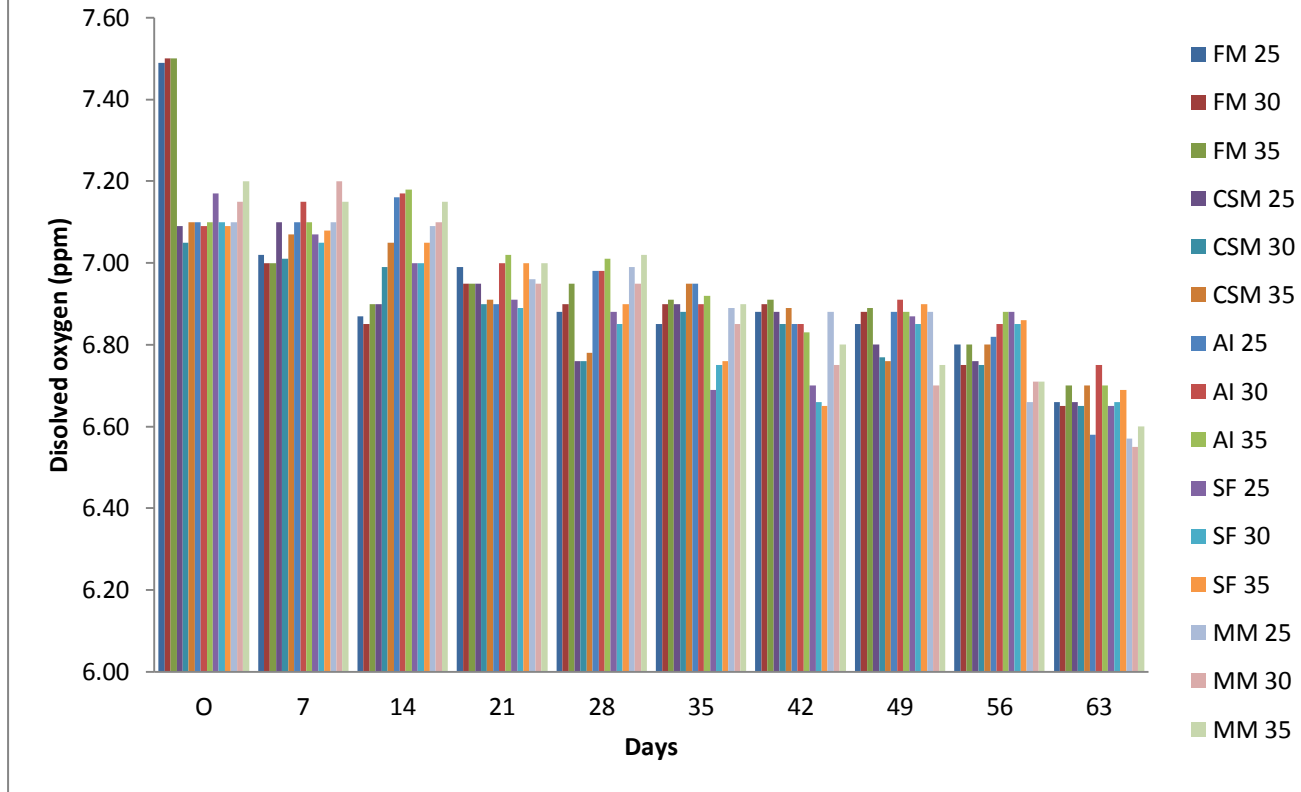
Fig 2 : Dissolved oxygen (mg/l) in the water of aquaria with *L.vannamei* reared at 5ppt Salinity :



Note: FM25 : Fish meal containing 25% crude protein
 FM30 : Fish meal containing 30% crude protein
 FM35 : Fish meal containing 35% crude protein
 CSM25 : Cottonseed meal containing 25 % crude protein
 CSM30 : Cottonseed meal containing 30 % crude protein

CSM35	:	Cottonseed meal containing 35% crude protein
AI25	:	<i>Acetes indicus</i> meal containing 25% crude protein
AI30	:	<i>Acetes indicus</i> meal containing 30% crude protein
AI35	:	<i>Acetes indicus</i> meal containing 35% crude protein
SF25	:	Sunflower meal containing 25% crude protein
SF30	:	Sunflower meal containing 30% crude protein
SF35	:	Sunflower meal containing 35% crude protein
MM25	:	Mysid meal containing 25% crude protein
MM30	:	Mysid meal containing 30% crude protein
MM35	:	Mysid meal containing 35% crude protein

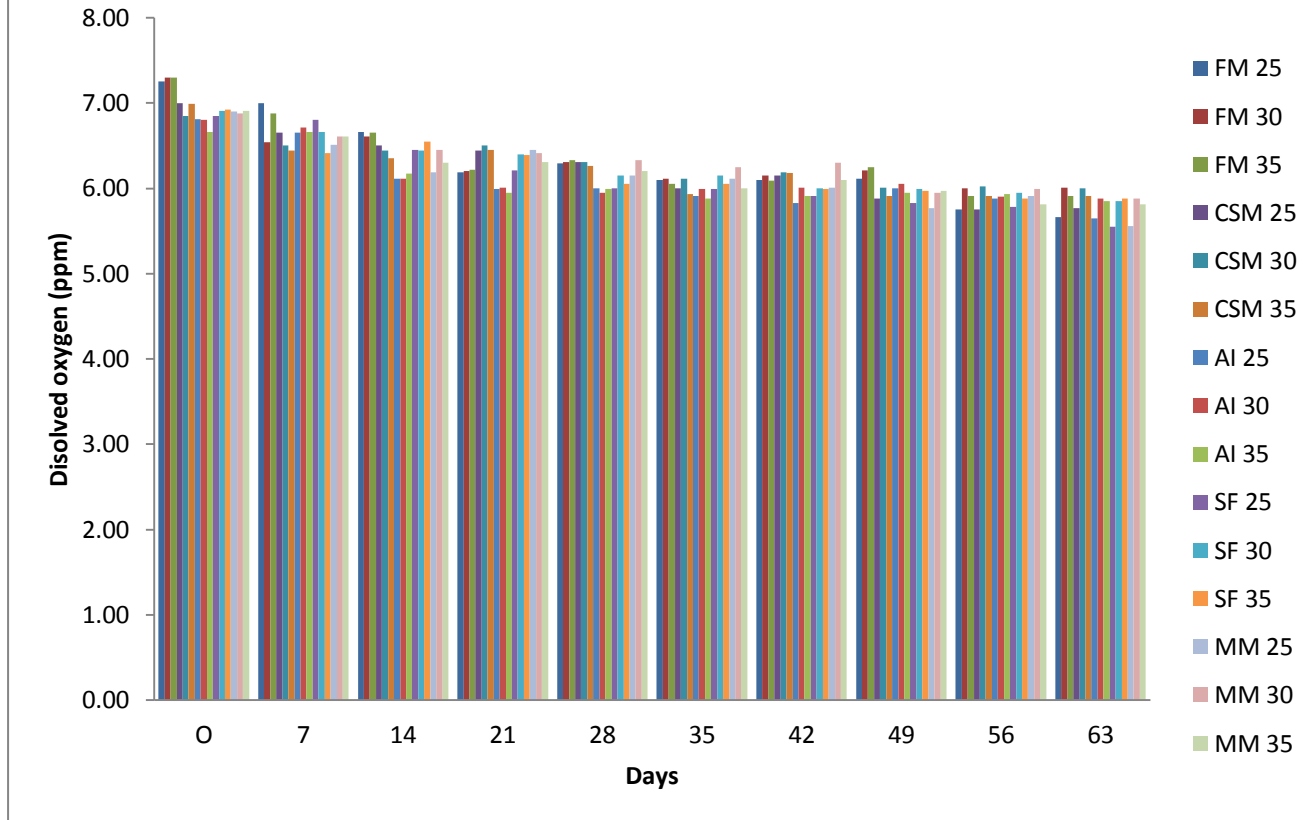
Fig 3 : Dissolved oxygen (mg/l) in the water of aquaria with *L.vannamei* reared at 15ppt Salinity :



Note: FM25 : Fish meal containing 25% crude protein
 FM30 : Fish meal containing 30% crude protein
 FM35 : Fish meal containing 35% crude protein
 CSM25 : Cottonseed meal containing 25 % crude protein
 CSM30 : Cottonseed meal containing 30 % crude protein

CSM35	:	Cottonseed meal containing 35% crude protein
AI25	:	<i>Acetes indicus</i> meal containing 25% crude protein
AI30	:	<i>Acetes indicus</i> meal containing 30% crude protein
AI35	:	<i>Acetes indicus</i> meal containing 35% crude protein
SF25	:	Sunflower meal containing 25% crude protein
SF30	:	Sunflower meal containing 30% crude protein
SF35	:	Sunflower meal containing 35% crude protein
MM25	:	Mysid meal containing 25% crude protein
MM30	:	Mysid meal containing 30% crude protein
MM35	:	Mysid meal containing 35% crude protein

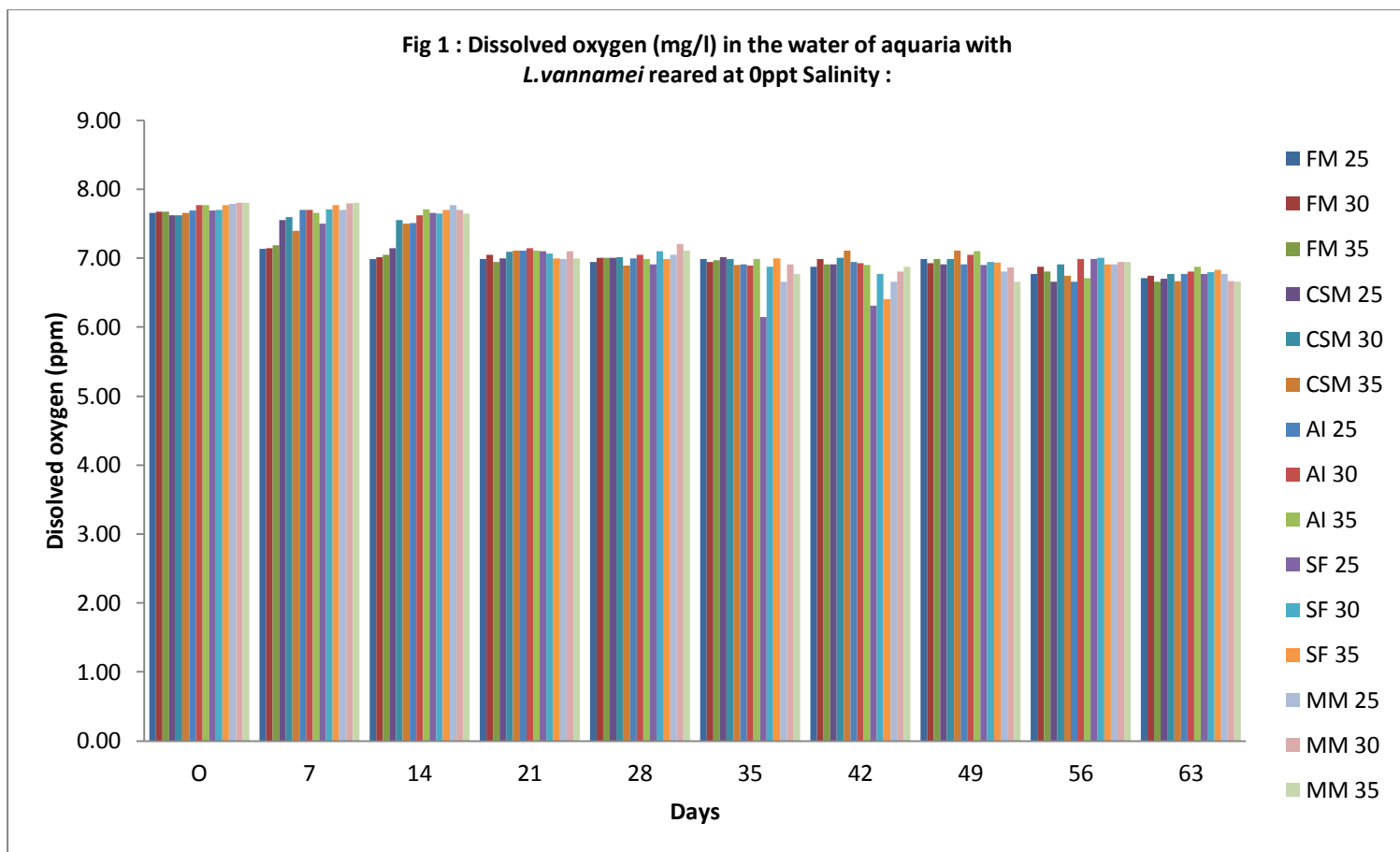
Fig 4 : Dissolved oxygen (mg/l) in the water of aquaria with *L.vannamei* at 25ppt Salinity :



Note: FM25 : Fish meal containing 25% crude protein
 FM30 : Fish meal containing 30% crude protein
 FM35 : Fish meal containing 35% crude protein
 CSM25 : Cottonseed meal containing 25 % crude protein
 CSM30 : Cottonseed meal containing 30 % crude protein
 CSM35 : Cottonseed meal containing 35% crude protein

AI25	:	<i>Acetes indicus</i> meal containing 25% crude protein
AI30	:	<i>Acetes indicus</i> meal containing 30% crude protein
AI35	:	<i>Acetes indicus</i> meal containing 35% crude protein
SF25	:	Sunflower meal containing 25% crude protein
SF30	:	Sunflower meal containing 30% crude protein
SF35	:	Sunflower meal containing 35% crude protein
MM25	:	Mysid meal containing 25% crude protein
MM30	:	Mysid meal containing 30% crude protein
MM35	:	Mysid meal containing 35% crude protein

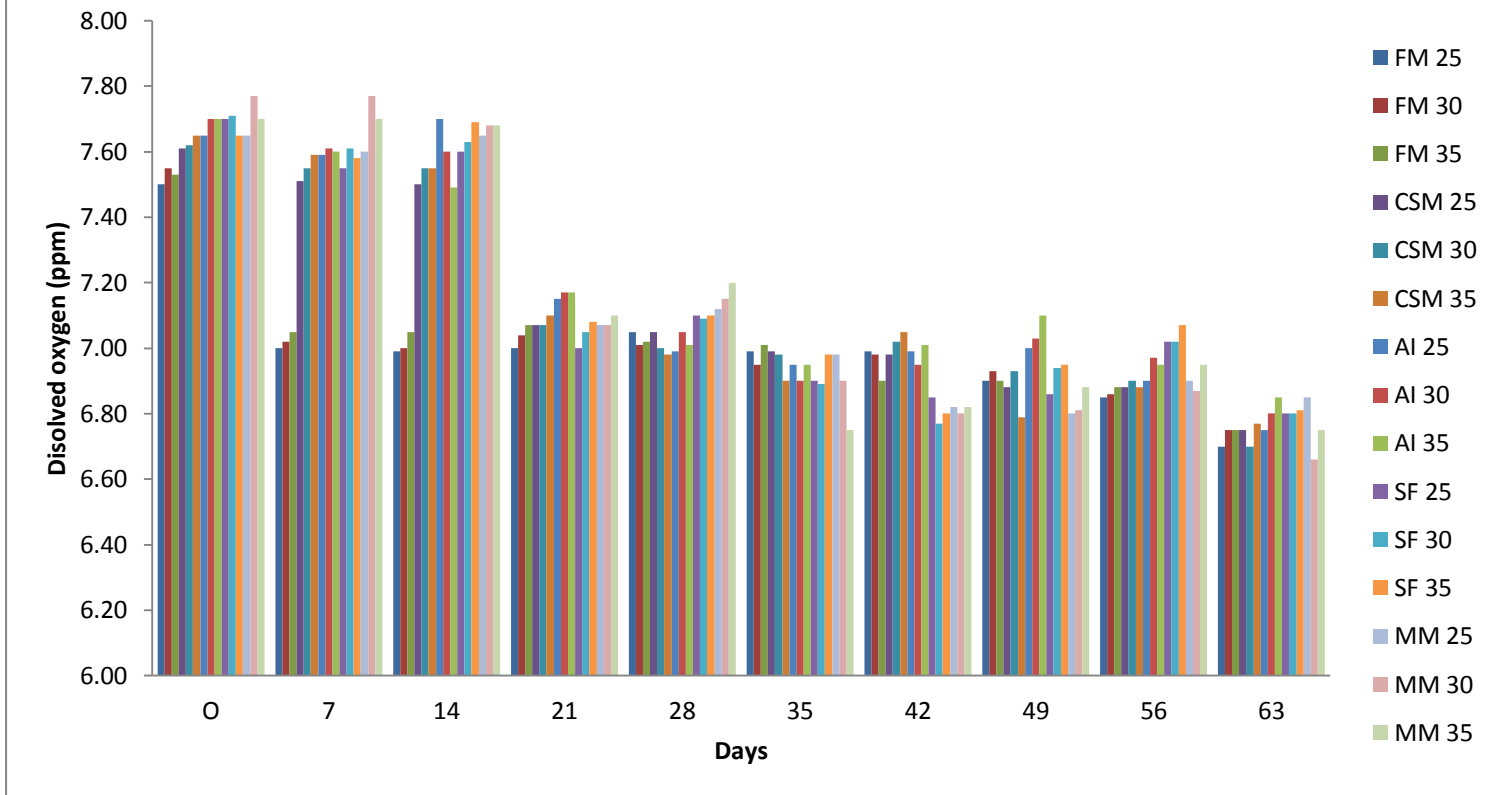
Fig 1 : Dissolved oxygen (mg/l) in the water of aquaria with *L.vannamei* reared at Oppt Salinity :



Note: FM25 : Fish meal containing 25% crude protein
 FM30 : Fish meal containing 30% crude protein
 FM35 : Fish meal containing 35% crude protein
 CSM25 : Cottonseed meal containing 25 % crude protein
 CSM30 : Cottonseed meal containing 30 % crude protein

CSM35	:	Cottonseed meal containing 35% crude protein
AI25	:	<i>Acetes indicus</i> meal containing 25% crude protein
AI30	:	<i>Acetes indicus</i> meal containing 30% crude protein
AI35	:	<i>Acetes indicus</i> meal containing 35% crude protein
SF25	:	Sunflower meal containing 25% crude protein
SF30	:	Sunflower meal containing 30% crude protein
SF35	:	Sunflower meal containing 35% crude protein
MM25	:	Mysid meal containing 25% crude protein
MM30	:	Mysid meal containing 30% crude protein
MM35	:	Mysid meal containing 35% crude protein

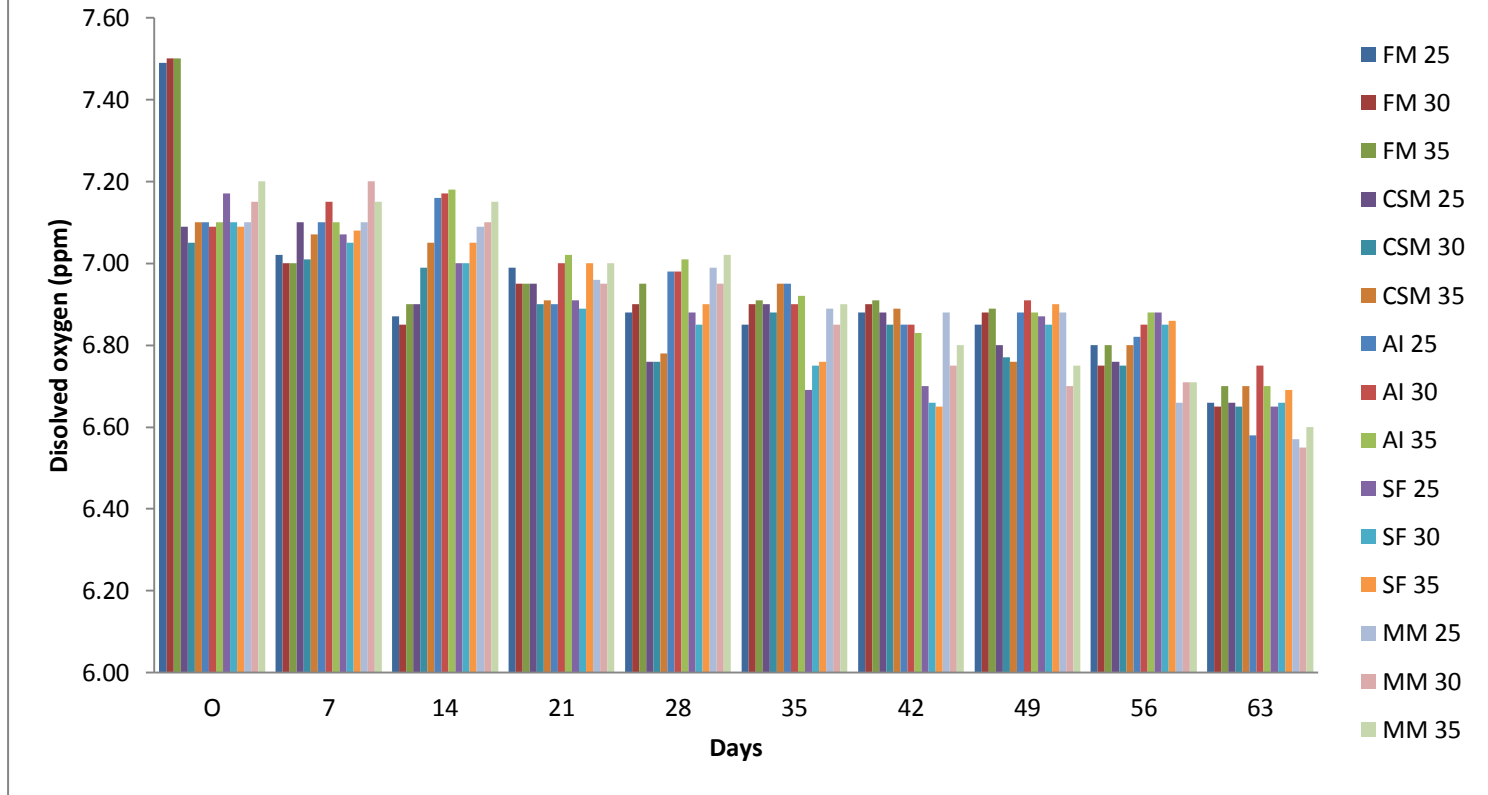
Fig 2 : Dissolved oxygen (mg/l) in the water of aquaria with *L.vannamei* reared at 5ppt Salinity :



Note: FM25 : Fish meal containing 25% crude protein
 FM30 : Fish meal containing 30% crude protein
 FM35 : Fish meal containing 35% crude protein
 CSM25 : Cottonseed meal containing 25 % crude protein
 CSM30 : Cottonseed meal containing 30 % crude protein

CSM35	:	Cottonseed meal containing 35% crude protein
AI25	:	<i>Acetes indicus</i> meal containing 25% crude protein
AI30	:	<i>Acetes indicus</i> meal containing 30% crude protein
AI35	:	<i>Acetes indicus</i> meal containing 35% crude protein
SF25	:	Sunflower meal containing 25% crude protein
SF30	:	Sunflower meal containing 30% crude protein
SF35	:	Sunflower meal containing 35% crude protein
MM25	:	Mysid meal containing 25% crude protein
MM30	:	Mysid meal containing 30% crude protein
MM35	:	Mysid meal containing 35% crude protein

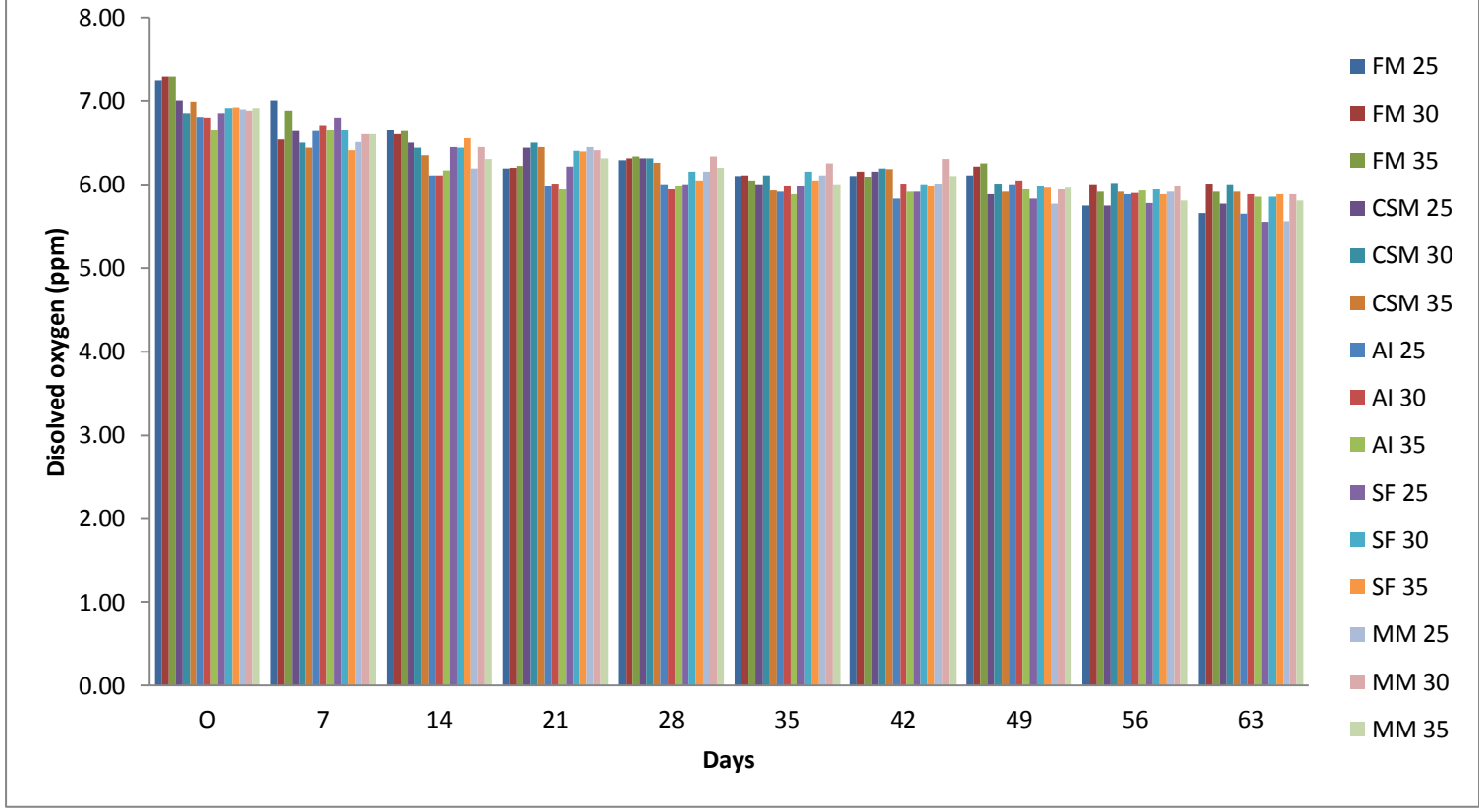
Fig 3 : Dissolved oxygen (mg/l) in the water of aquaria with *L.vannamei* reared at 15ppt Salinity :



Note: FM25 : Fish meal containing 25% crude protein
 FM30 : Fish meal containing 30% crude protein
 FM35 : Fish meal containing 35% crude protein
 CSM25 : Cottonseed meal containing 25 % crude protein
 CSM30 : Cottonseed meal containing 30 % crude protein

CSM35	:	Cottonseed meal containing 35% crude protein
AI25	:	<i>Acetes indicus</i> meal containing 25% crude protein
AI30	:	<i>Acetes indicus</i> meal containing 30% crude protein
AI35	:	<i>Acetes indicus</i> meal containing 35% crude protein
SF25	:	Sunflower meal containing 25% crude protein
SF30	:	Sunflower meal containing 30% crude protein
SF35	:	Sunflower meal containing 35% crude protein
MM25	:	Mysid meal containing 25% crude protein
MM30	:	Mysid meal containing 30% crude protein
MM35	:	Mysid meal containing 35% crude protein

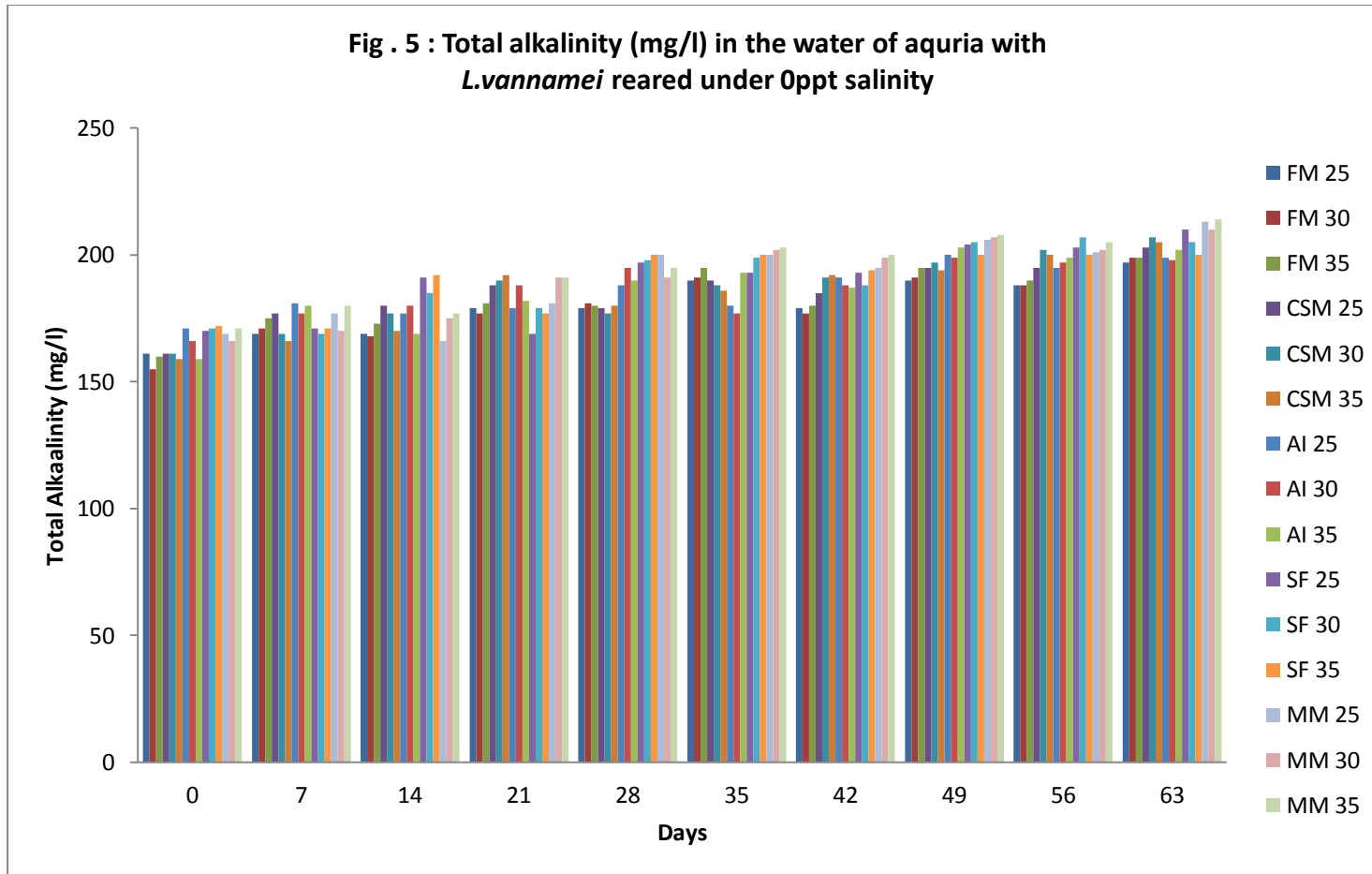
Fig 4 : Dissolved oxygen (mg/l) in the water of aquaria with *L.vannamei* at 25ppt Salinity :



Note: FM25 : Fish meal containing 25% crude protein
 FM30 : Fish meal containing 30% crude protein
 FM35 : Fish meal containing 35% crude protein
 CSM25 : Cottonseed meal containing 25 % crude protein
 CSM30 : Cottonseed meal containing 30 % crude protein
 CSM35 : Cottonseed meal containing 35% crude protein

AI25	:	<i>Acetes indicus</i> meal containing 25% crude protein
AI30	:	<i>Acetes indicus</i> meal containing 30% crude protein
AI35	:	<i>Acetes indicus</i> meal containing 35% crude protein
SF25	:	Sunflower meal containing 25% crude protein
SF30	:	Sunflower meal containing 30% crude protein
SF35	:	Sunflower meal containing 35% crude protein
MM25	:	Mysid meal containing 25% crude protein
MM30	:	Mysid meal containing 30% crude protein
MM35	:	Mysid meal containing 35% crude protein

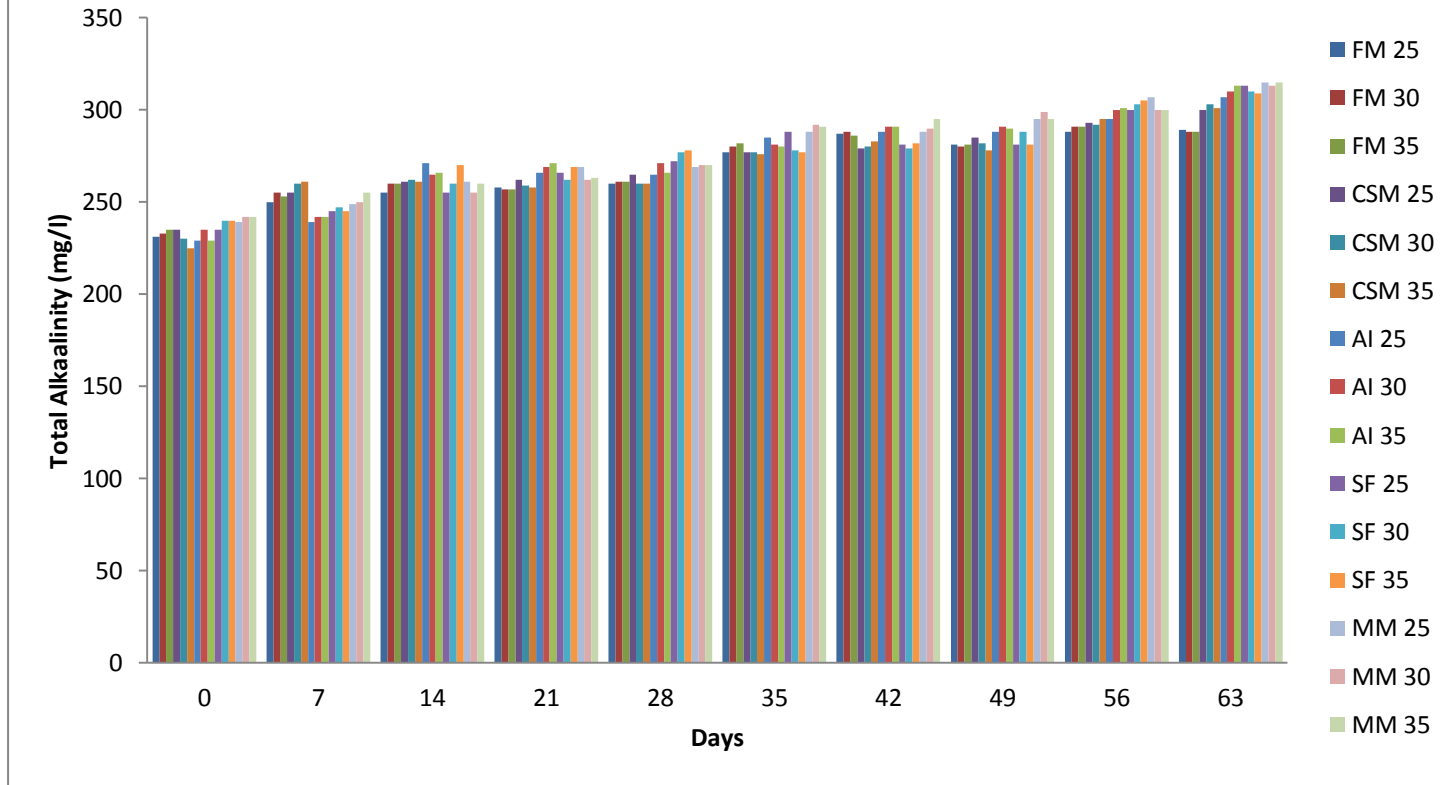
Fig . 5 : Total alkalinity (mg/l) in the water of aquaria with *L.vannamei* reared under 0ppt salinity



Note: FM25 : Fish meal containing 25% crude protein
 FM30 : Fish meal containing 30% crude protein
 FM35 : Fish meal containing 35% crude protein
 CSM25 : Cottonseed meal containing 25 % crude protein

CSM30	:	Cottonseed meal containing 30 % crude protein
CSM35	:	Cottonseed meal containing 35% crude protein
AI25	:	<i>Acetes indicus</i> meal containing 25% crude protein
AI30	:	<i>Acetes indicus</i> meal containing 30% crude protein
AI35	:	<i>Acetes indicus</i> meal containing 35% crude protein
SF25	:	Sunflower meal containing 25% crude protein
SF30	:	Sunflower meal containing 30% crude protein
SF35	:	Sunflower meal containing 35% crude protein
MM25	:	Mysid meal containing 25% crude protein
MM30	:	Mysid meal containing 30% crude protein
MM35	:	Mysid meal containing 35% crude protein

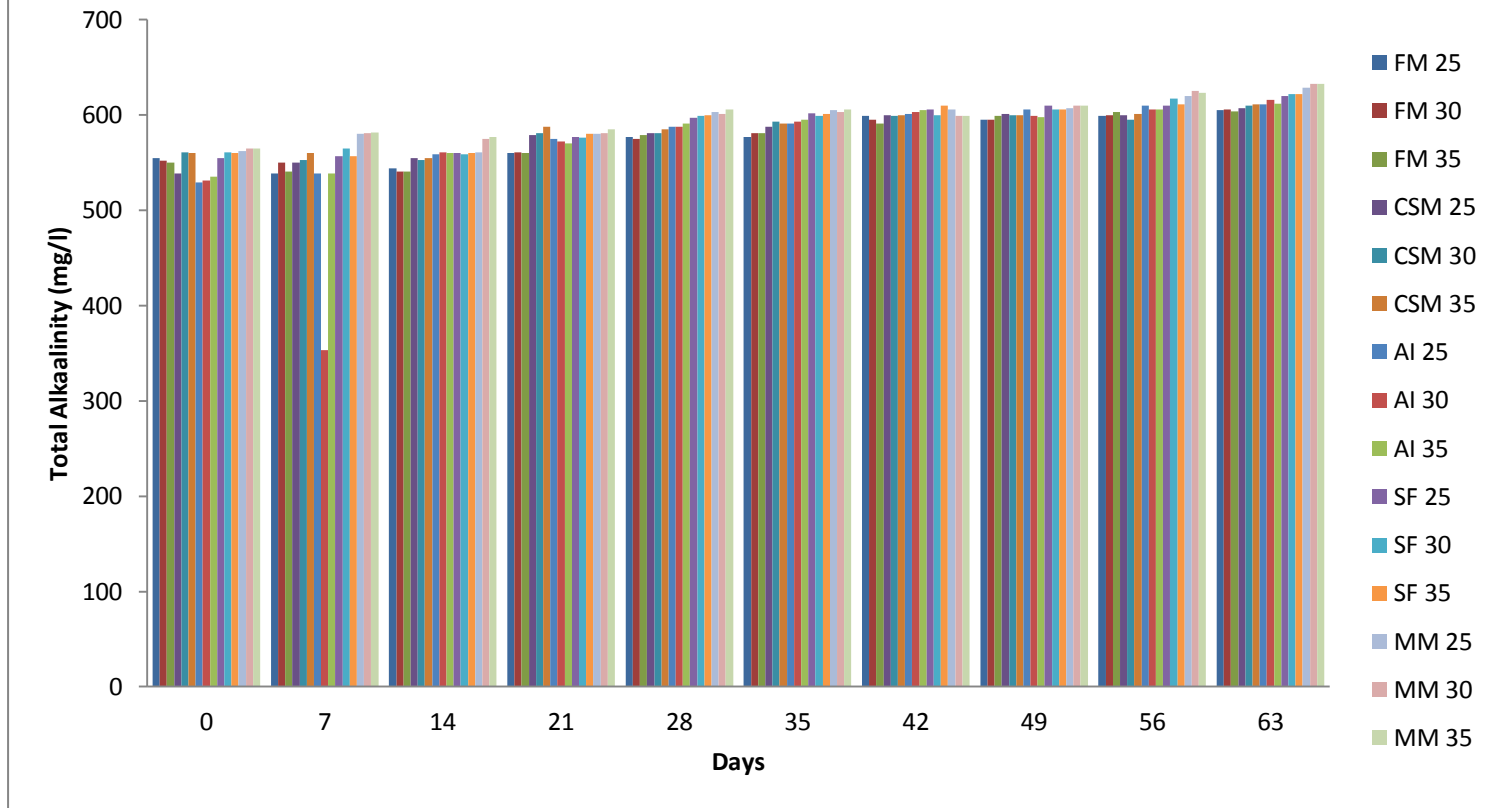
Fig . 6 : Total alkalinity (mg/l) in the water of aquaria with *L.vannamei* reared under 5 ppt salinity



Note: FM25 : Fish meal containing 25% crude protein
 FM30 : Fish meal containing 30% crude protein
 FM35 : Fish meal containing 35% crude protein
 CSM25 : Cottonseed meal containing 25 % crude protein
 CSM30 : Cottonseed meal containing 30 % crude protein

CSM35	:	Cottonseed meal containing 35% crude protein
AI25	:	<i>Acetes indicus</i> meal containing 25% crude protein
AI30	:	<i>Acetes indicus</i> meal containing 30% crude protein
AI35	:	<i>Acetes indicus</i> meal containing 35% crude protein
SF25	:	Sunflower meal containing 25% crude protein
SF30	:	Sunflower meal containing 30% crude protein
SF35	:	Sunflower meal containing 35% crude protein
MM25	:	Mysid meal containing 25% crude protein
MM30	:	Mysid meal containing 30% crude protein
MM35	:	Mysid meal containing 35% crude protein

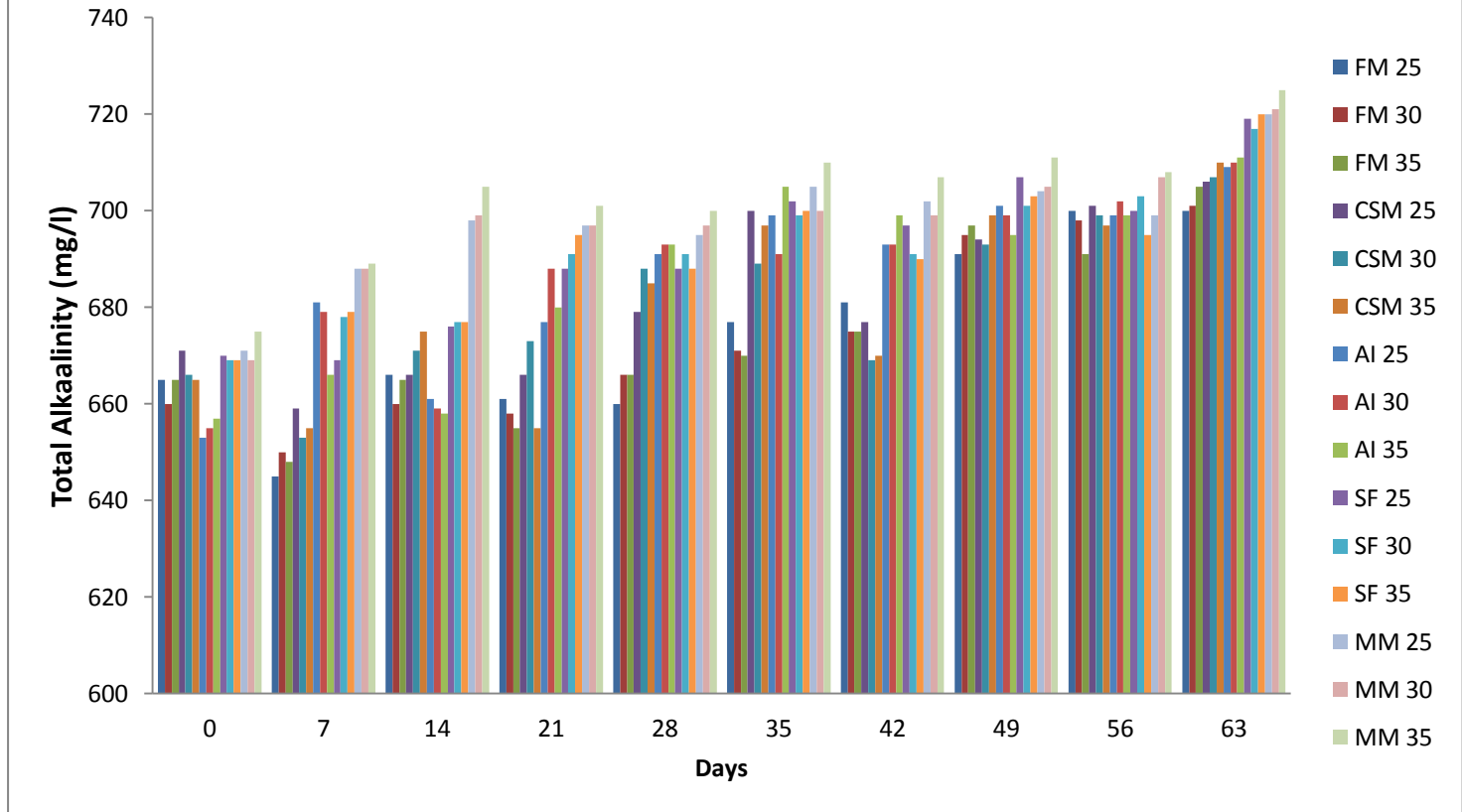
Fig . 7 : Total alkalinity (mg/l) in the water of aquaria with *L.vannamei* reared under 15 ppt salinity



Note: FM25 : Fish meal containing 25% crude protein
 FM30 : Fish meal containing 30% crude protein
 FM35 : Fish meal containing 35% crude protein
 CSM25 : Cottonseed meal containing 25 % crude protein
 CSM30 : Cottonseed meal containing 30 % crude protein
 CSM35 : Cottonseed meal containing 35% crude protein

AI25	:	<i>Acetes indicus</i> meal containing 25% crude protein
AI30	:	<i>Acetes indicus</i> meal containing 30% crude protein
AI35	:	<i>Acetes indicus</i> meal containing 35% crude protein
SF25	:	Sunflower meal containing 25% crude protein
SF30	:	Sunflower meal containing 30% crude protein
SF35	:	Sunflower meal containing 35% crude protein
MM25	:	Mysid meal containing 25% crude protein
MM30	:	Mysid meal containing 30% crude protein
MM35	:	Mysid meal containing 35% crude protein

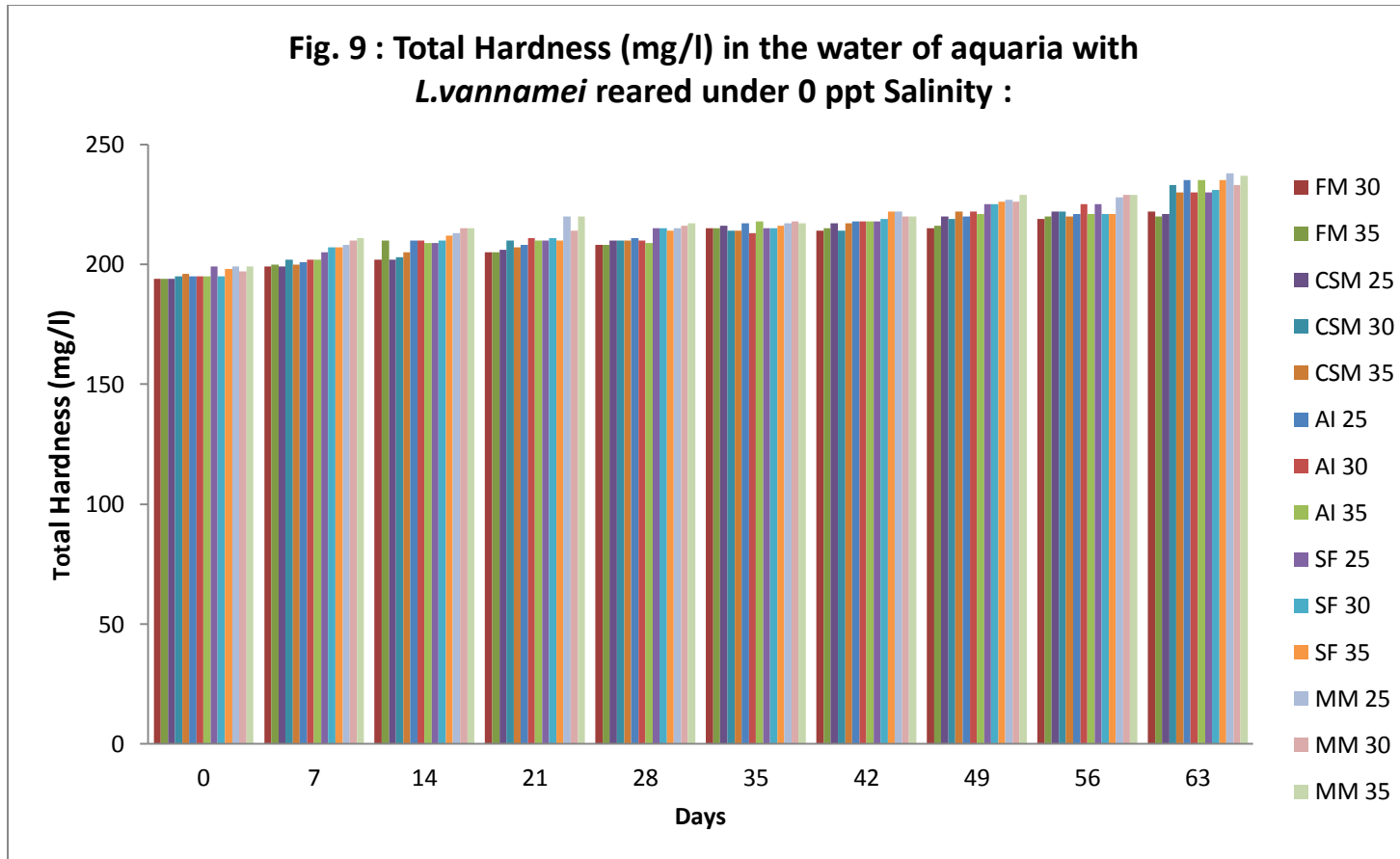
Fig . 8 : Total alkalinity (mg/l) in the water of aquaria with *L.vannamei* reared under 25 ppt salinity



Note: FM25 : Fish meal containing 25% crude protein
 FM30 : Fish meal containing 30% crude protein
 FM35 : Fish meal containing 35% crude protein
 CSM25 : Cottonseed meal containing 25 % crude protein
 CSM30 : Cottonseed meal containing 30 % crude protein

CSM35	:	Cottonseed meal containing 35% crude protein
AI25	:	<i>Acetes indicus</i> meal containing 25% crude protein
AI30	:	<i>Acetes indicus</i> meal containing 30% crude protein
AI35	:	<i>Acetes indicus</i> meal containing 35% crude protein
SF25	:	Sunflower meal containing 25% crude protein
SF30	:	Sunflower meal containing 30% crude protein
SF35	:	Sunflower meal containing 35% crude protein
MM25	:	Mysid meal containing 25% crude protein
MM30	:	Mysid meal containing 30% crude protein
MM35	:	Mysid meal containing 35% crude protein

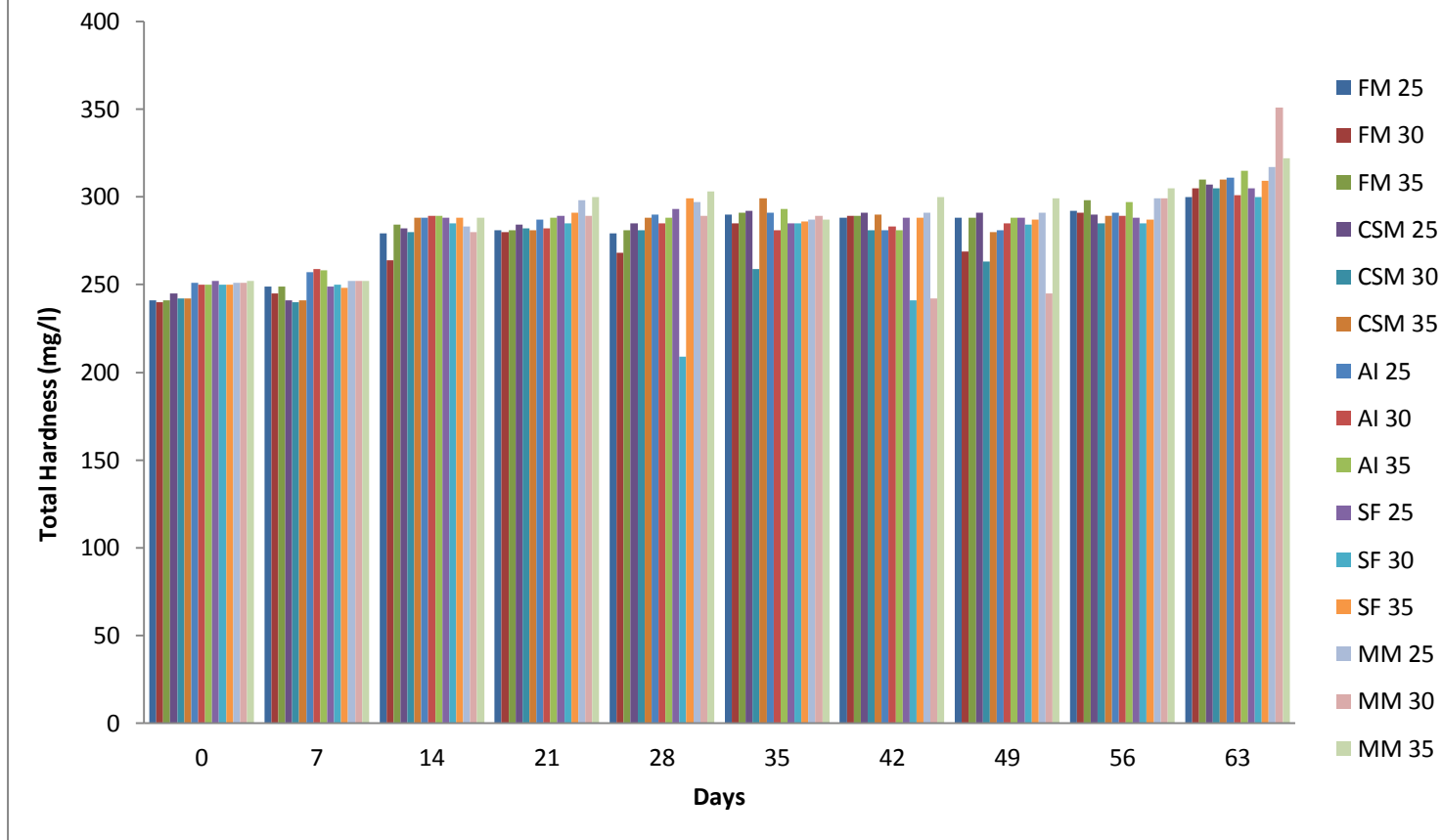
Fig. 9 : Total Hardness (mg/l) in the water of aquaria with *L.vannamei* reared under 0 ppt Salinity :



Note: FM25 : Fish meal containing 25% crude protein
 FM30 : Fish meal containing 30% crude protein
 FM35 : Fish meal containing 35% crude protein
 CSM25 : Cottonseed meal containing 25 % crude protein
 CSM30 : Cottonseed meal containing 30 % crude protein
 CSM35 : Cottonseed meal containing 35% crude protein

AI25	:	<i>Acetes indicus</i> meal containing 25% crude protein
AI30	:	<i>Acetes indicus</i> meal containing 30% crude protein
AI35	:	<i>Acetes indicus</i> meal containing 35% crude protein
SF25	:	Sunflower meal containing 25% crude protein
SF30	:	Sunflower meal containing 30% crude protein
SF35	:	Sunflower meal containing 35% crude protein
MM25	:	Mysid meal containing 25% crude protein
MM30	:	Mysid meal containing 30% crude protein
MM35	:	Mysid meal containing 35% crude protein

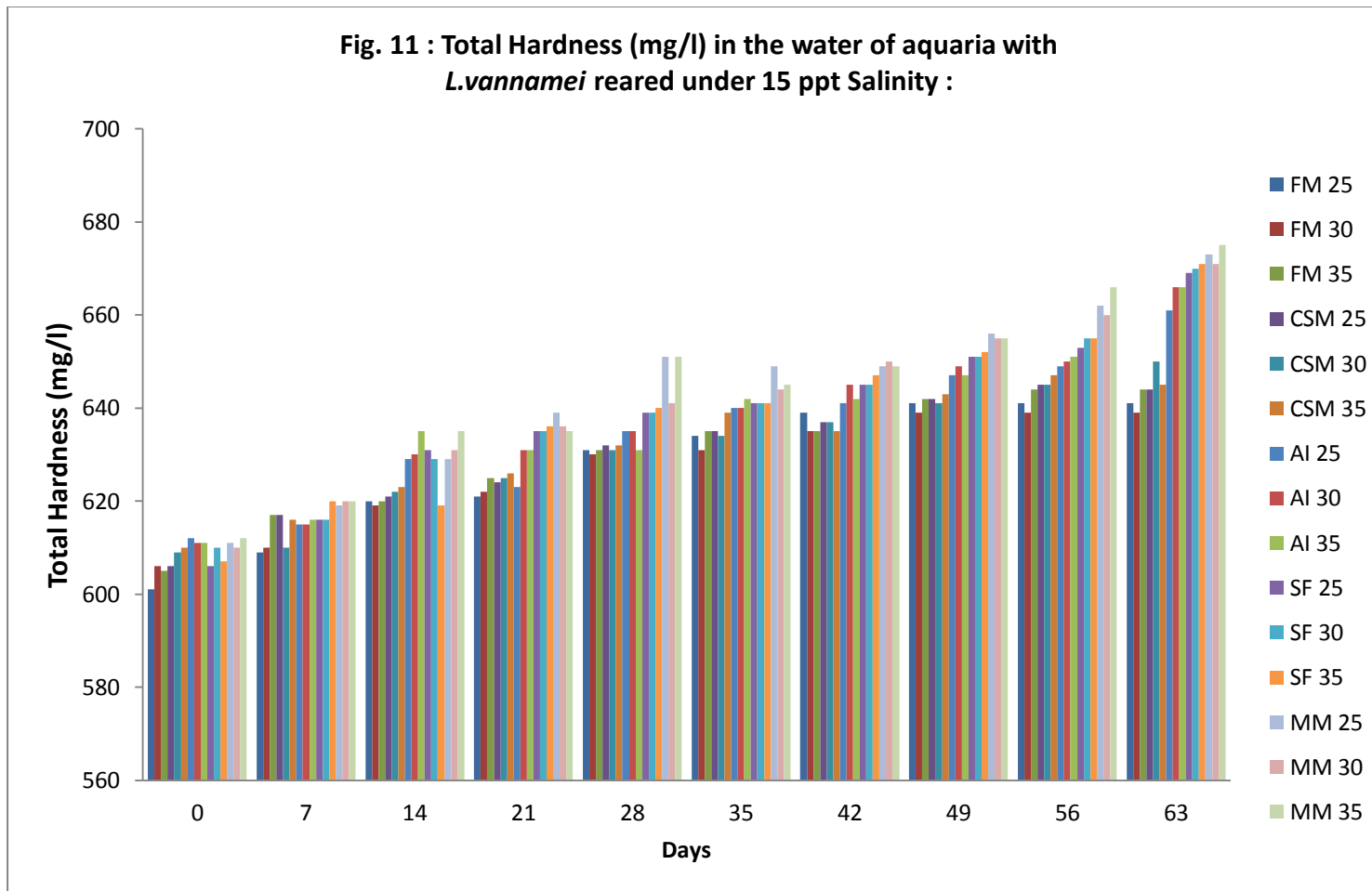
Fig. 10 : Total Hardness (mg/l) in the water of aquaria with *L.vannamei* reared under 5 ppt Salinity :



Note: FM25 : Fish meal containing 25% crude protein
 FM30 : Fish meal containing 30% crude protein

FM35	:	Fish meal containing 35% crude protein
CSM25	:	Cottonseed meal containing 25 % crude protein
CSM30	:	Cottonseed meal containing 30 % crude protein
CSM35	:	Cottonseed meal containing 35% crude protein
AI25	:	<i>Acetes indicus</i> meal containing 25% crude protein
AI30	:	<i>Acetes indicus</i> meal containing 30% crude protein
AI35	:	<i>Acetes indicus</i> meal containing 35% crude protein
SF25	:	Sunflower meal containing 25% crude protein
SF30	:	Sunflower meal containing 30% crude protein
SF35	:	Sunflower meal containing 35% crude protein
MM25	:	Mysid meal containing 25% crude protein
MM30	:	Mysid meal containing 30% crude protein
MM35	:	Mysid meal containing 35% crude protein

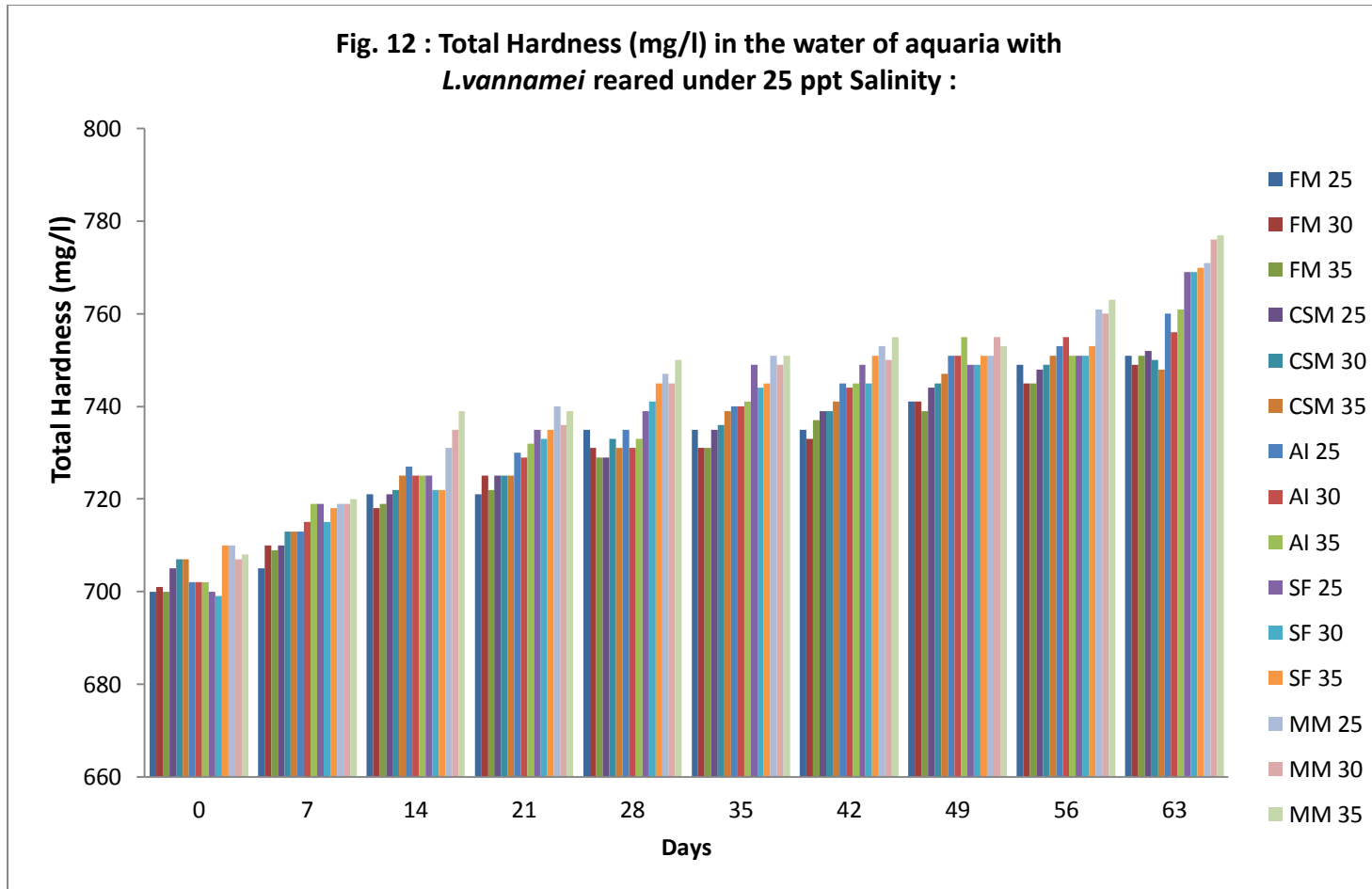
Fig. 11 : Total Hardness (mg/l) in the water of aquaria with *L.vannamei* reared under 15 ppt Salinity :



Note: FM25 : Fish meal containing 25% crude protein
 FM30 : Fish meal containing 30% crude protein
 FM35 : Fish meal containing 35% crude protein

CSM25	:	Cottonseed meal containing 25 % crude protein
CSM30	:	Cottonseed meal containing 30 % crude protein
CSM35	:	Cottonseed meal containing 35% crude protein
AI25	:	<i>Acetes indicus</i> meal containing 25% crude protein
AI30	:	<i>Acetes indicus</i> meal containing 30% crude protein
AI35	:	<i>Acetes indicus</i> meal containing 35% crude protein
SF25	:	Sunflower meal containing 25% crude protein
SF30	:	Sunflower meal containing 30% crude protein
SF35	:	Sunflower meal containing 35% crude protein
MM25	:	Mysid meal containing 25% crude protein
MM30	:	Mysid meal containing 30% crude protein
MM35	:	Mysid meal containing 35% crude protein

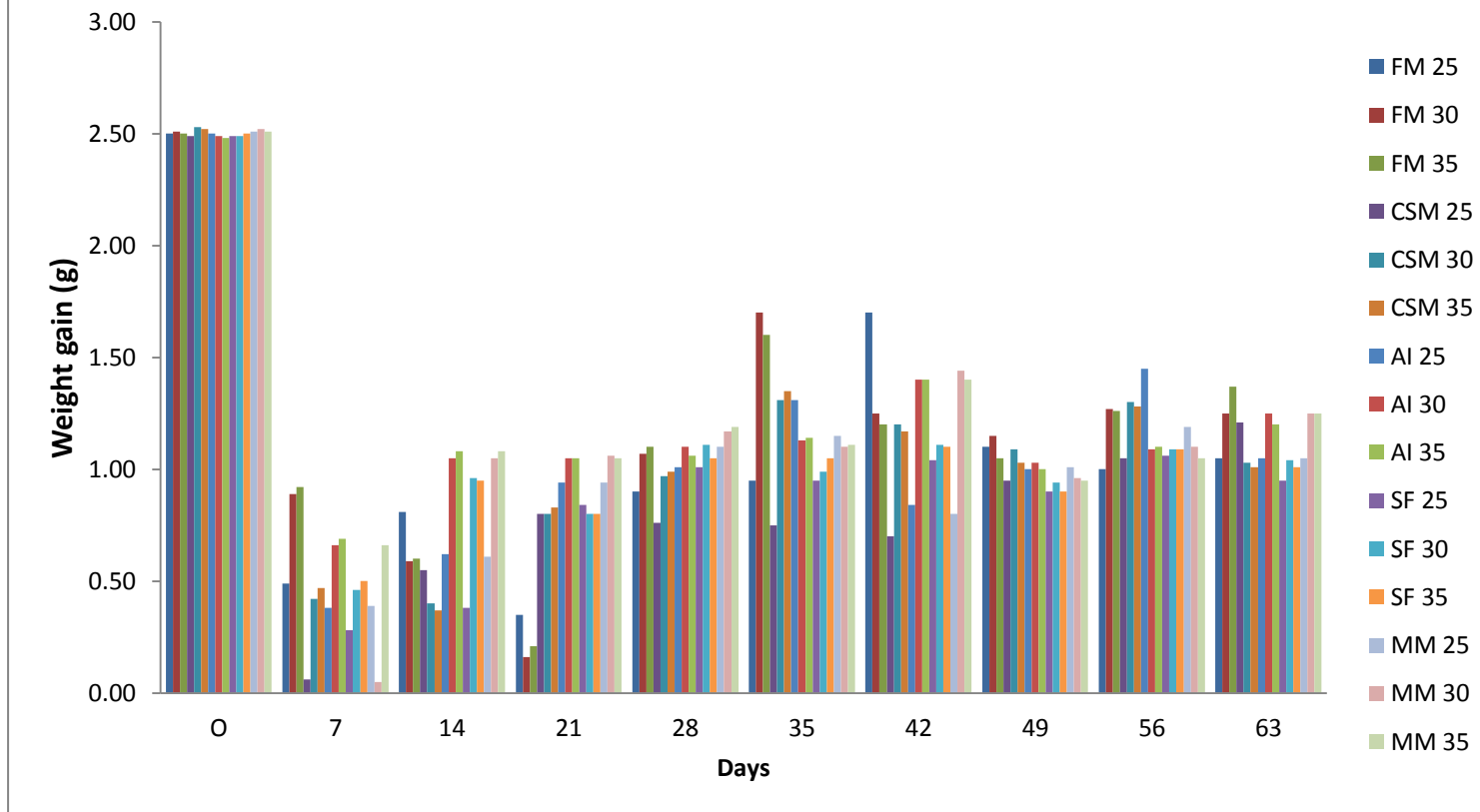
Fig. 12 : Total Hardness (mg/l) in the water of aquaria with *L.vannamei* reared under 25 ppt Salinity :



Note: FM25 : Fish meal containing 25% crude protein
 FM30 : Fish meal containing 30% crude protein
 FM35 : Fish meal containing 35% crude protein
 CSM25 : Cottonseed meal containing 25 % crude protein
 CSM30 : Cottonseed meal containing 30 % crude protein

CSM35	:	Cottonseed meal containing 35% crude protein
AI25	:	<i>Acetes indicus</i> meal containing 25% crude protein
AI30	:	<i>Acetes indicus</i> meal containing 30% crude protein
AI35	:	<i>Acetes indicus</i> meal containing 35% crude protein
SF25	:	Sunflower meal containing 25% crude protein
SF30	:	Sunflower meal containing 30% crude protein
SF35	:	Sunflower meal containing 35% crude protein
MM25	:	Mysid meal containing 25% crude protein
MM30	:	Mysid meal containing 30% crude protein
MM35	:	Mysid meal containing 35% crude protein

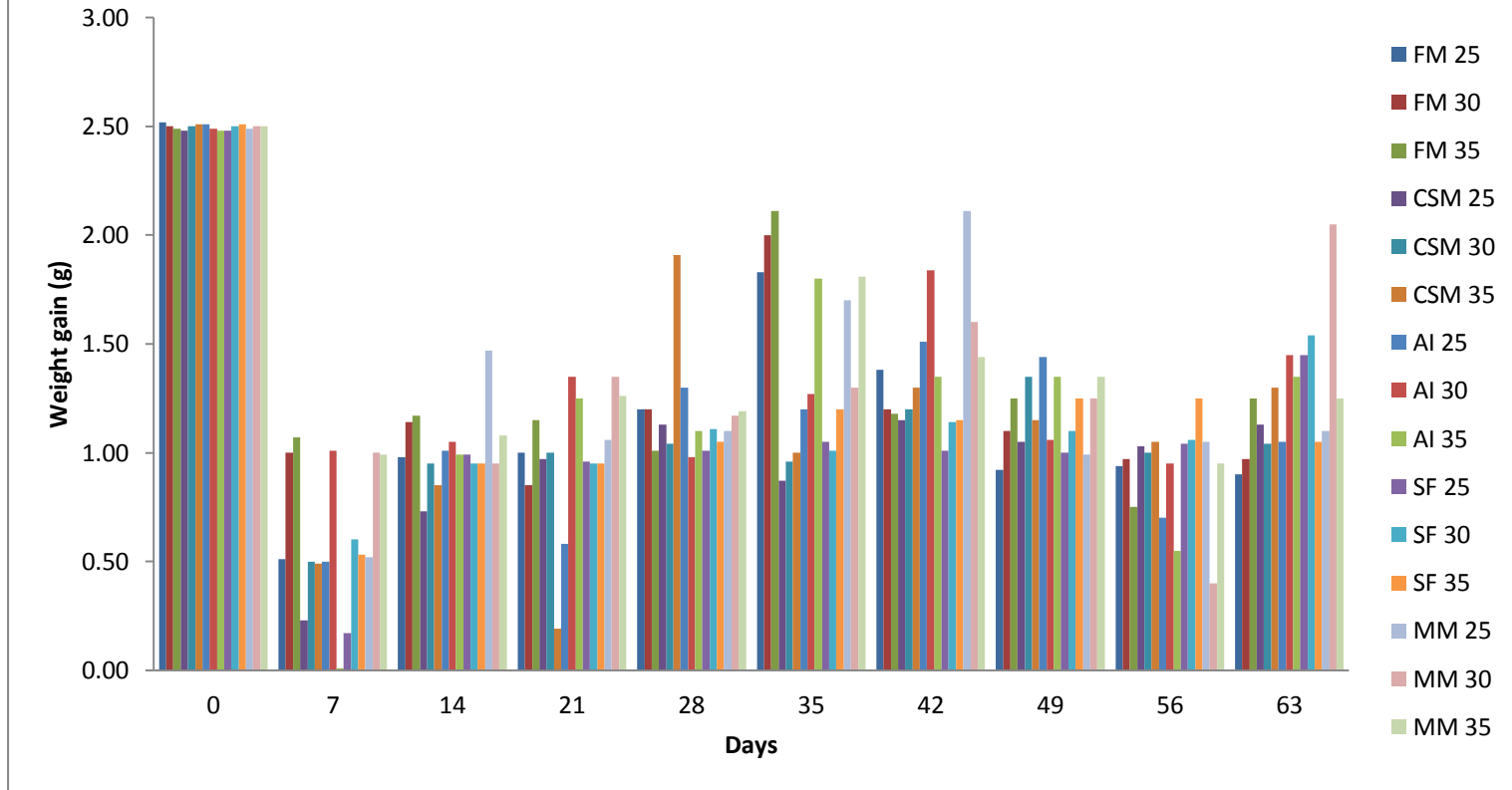
Fig .13: Weight Gain in *L.vennamei* fed with different experimental diets reared at 0 ppt salinity :



Note: FM25 : Fish meal containing 25% crude protein
 FM30 : Fish meal containing 30% crude protein
 FM35 : Fish meal containing 35% crude protein
 CSM25 : Cottonseed meal containing 25 % crude protein
 CSM30 : Cottonseed meal containing 30 % crude protein

CSM35	:	Cottonseed meal containing 35% crude protein
AI25	:	<i>Acetes indicus</i> meal containing 25% crude protein
AI30	:	<i>Acetes indicus</i> meal containing 30% crude protein
AI35	:	<i>Acetes indicus</i> meal containing 35% crude protein
SF25	:	Sunflower meal containing 25% crude protein
SF30	:	Sunflower meal containing 30% crude protein
SF35	:	Sunflower meal containing 35% crude protein
MM25	:	Mysid meal containing 25% crude protein
MM30	:	Mysid meal containing 30% crude protein
MM35	:	Mysid meal containing 35% crude protein

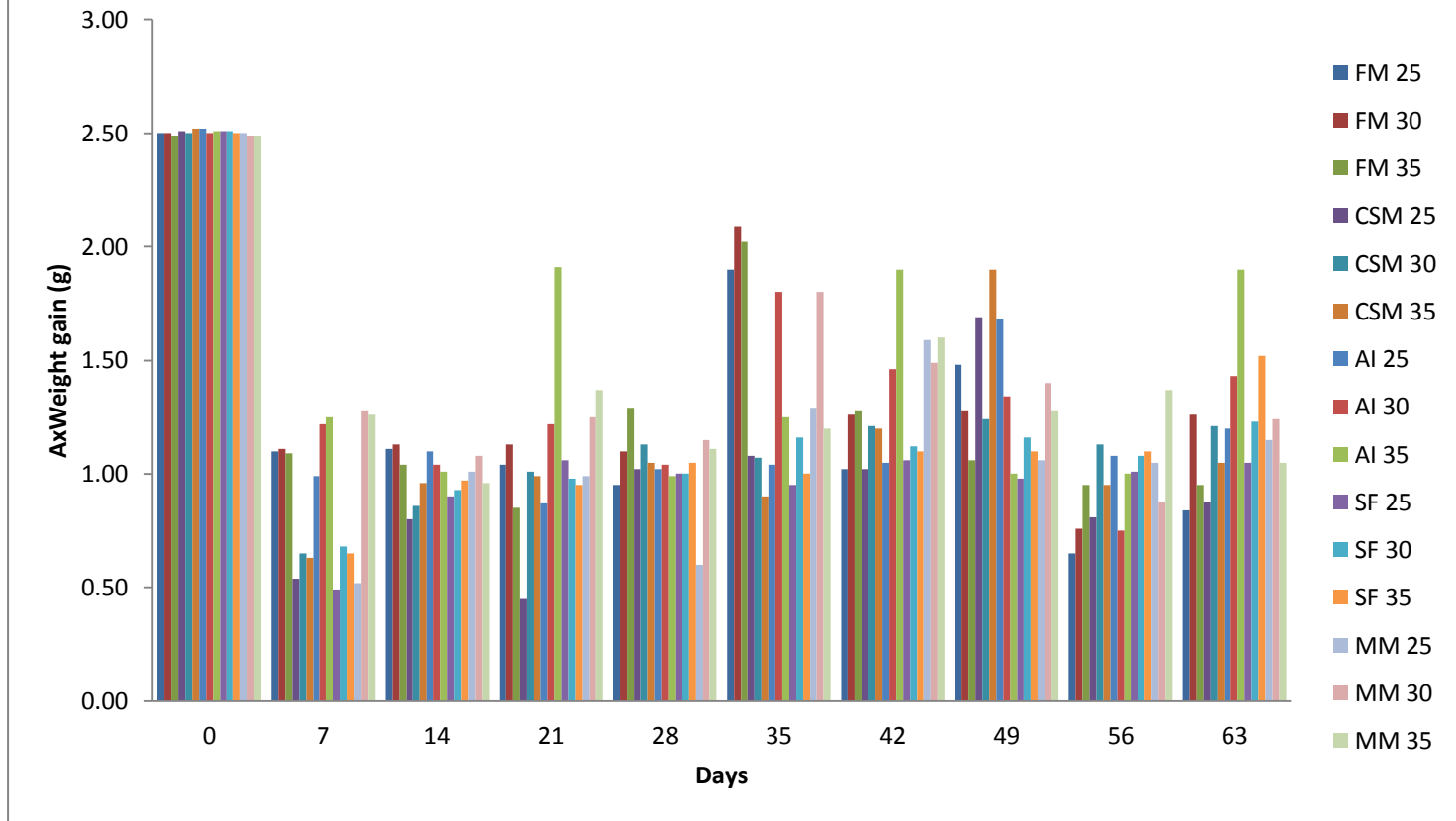
Fig . 14: Weight Gain in *L.vennamei* fed with different experimental diets reared at 5 ppt salinity :



Note: FM25 : Fish meal containing 25% crude protein
 FM30 : Fish meal containing 30% crude protein
 FM35 : Fish meal containing 35% crude protein
 CSM25 : Cottonseed meal containing 25 % crude protein
 CSM30 : Cottonseed meal containing 30 % crude protein
 CSM35 : Cottonseed meal containing 35% crude protein

AI25	:	<i>Acetes indicus</i> meal containing 25% crude protein
AI30	:	<i>Acetes indicus</i> meal containing 30% crude protein
AI35	:	<i>Acetes indicus</i> meal containing 35% crude protein
SF25	:	Sunflower meal containing 25% crude protein
SF30	:	Sunflower meal containing 30% crude protein
SF35	:	Sunflower meal containing 35% crude protein
MM25	:	Mysid meal containing 25% crude protein
MM30	:	Mysid meal containing 30% crude protein
MM35	:	Mysid meal containing 35% crude protein

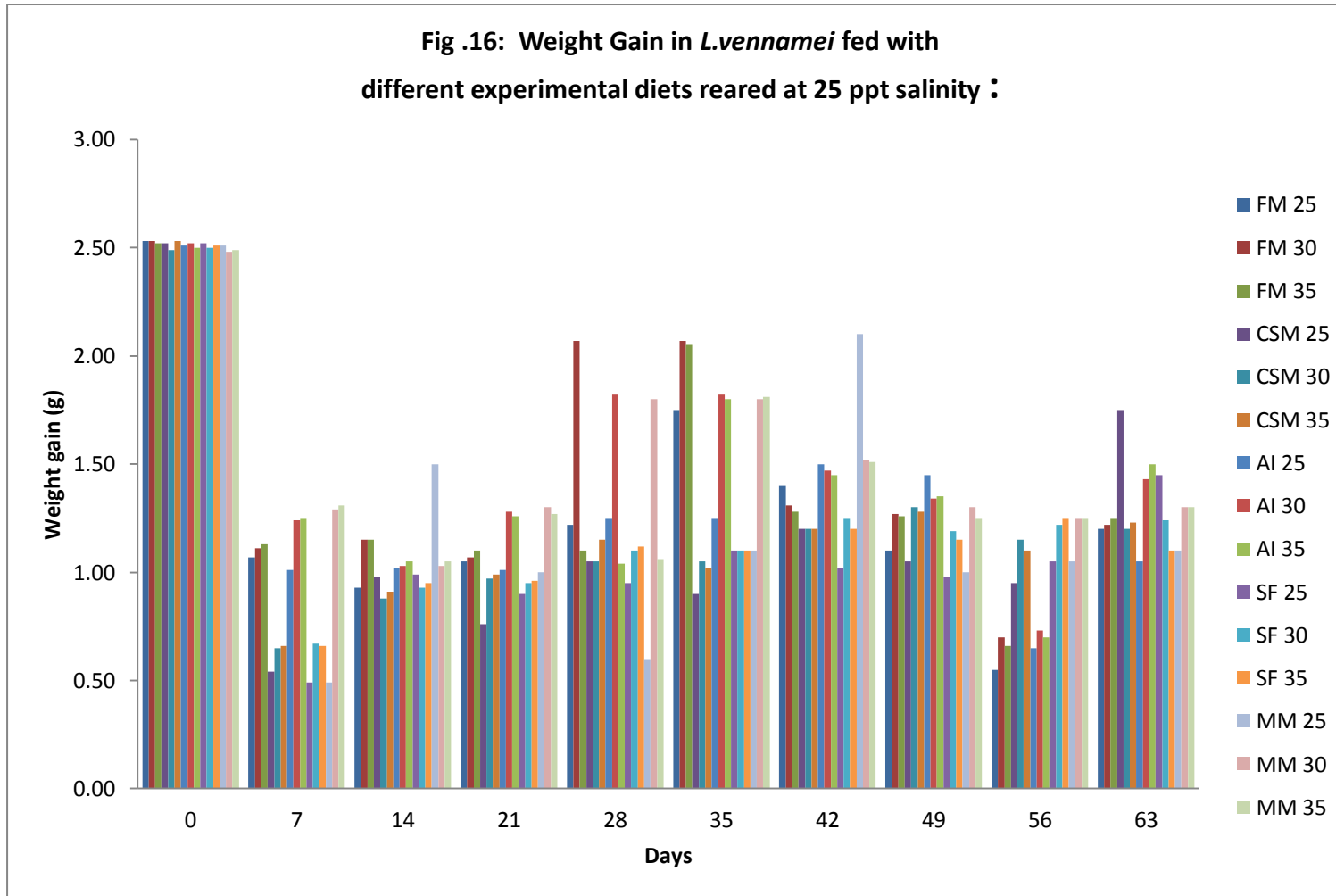
Fig . 15 : Weight Gain in *L.vennamei* fed with different experimental diets reared at 15 ppt salinity :



Note: FM25 : Fish meal containing 25% crude protein
 FM30 : Fish meal containing 30% crude protein
 FM35 : Fish meal containing 35% crude protein
 CSM25 : Cottonseed meal containing 25 % crude protein
 CSM30 : Cottonseed meal containing 30 % crude protein

CSM35	:	Cottonseed meal containing 35% crude protein
AI25	:	<i>Acetes indicus</i> meal containing 25% crude protein
AI30	:	<i>Acetes indicus</i> meal containing 30% crude protein
AI35	:	<i>Acetes indicus</i> meal containing 35% crude protein
SF25	:	Sunflower meal containing 25% crude protein
SF30	:	Sunflower meal containing 30% crude protein
SF35	:	Sunflower meal containing 35% crude protein
MM25	:	Mysid meal containing 25% crude protein
MM30	:	Mysid meal containing 30% crude protein
MM35	:	Mysid meal containing 35% crude protein

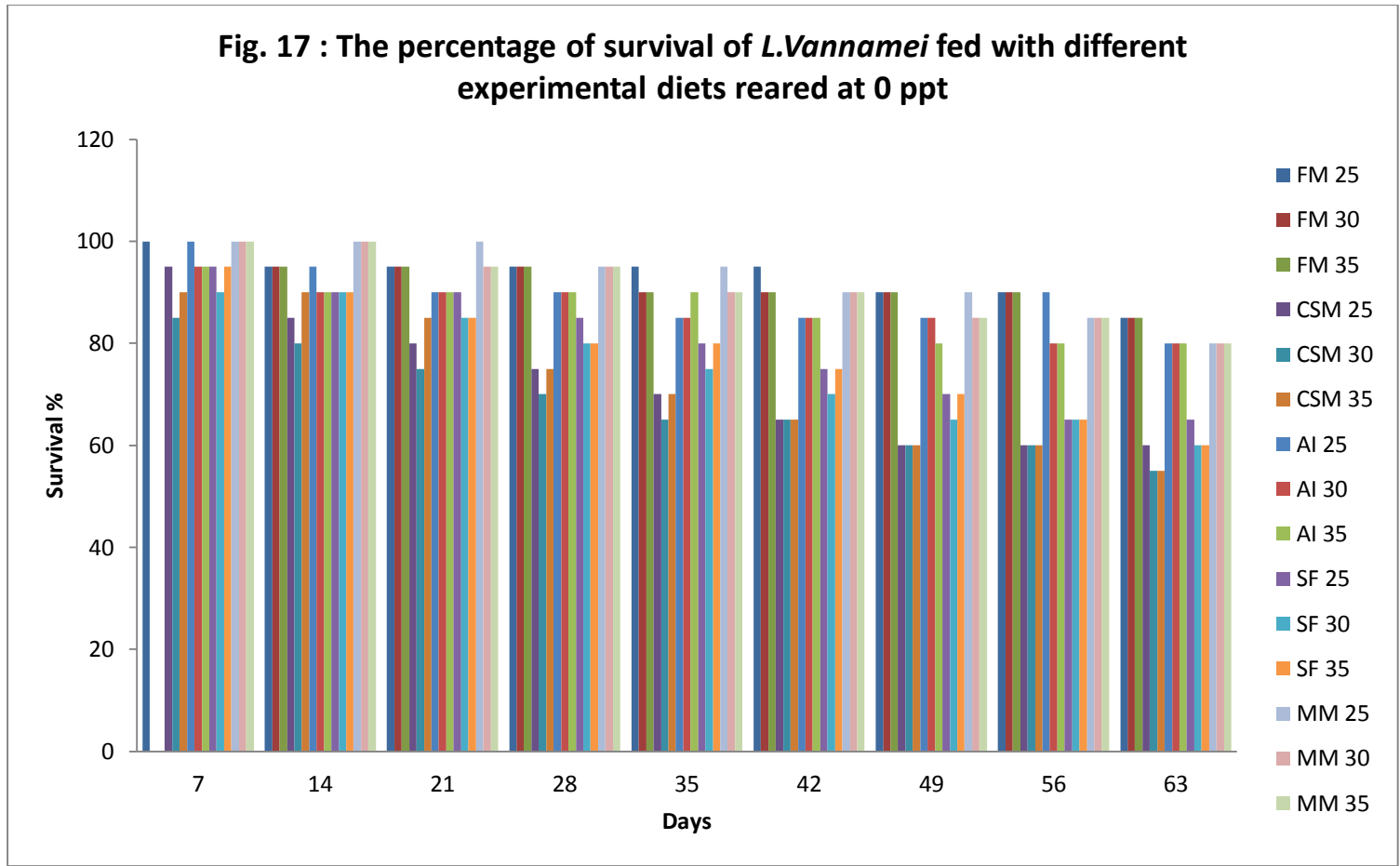
Fig .16: Weight Gain in *L.vennamei* fed with different experimental diets reared at 25 ppt salinity :



Note: FM25 : Fish meal containing 25% crude protein
 FM30 : Fish meal containing 30% crude protein
 FM35 : Fish meal containing 35% crude protein
 CSM25 : Cottonseed meal containing 25 % crude protein

CSM30	:	Cottonseed meal containing 30 % crude protein
CSM35	:	Cottonseed meal containing 35% crude protein
AI25	:	<i>Acetes indicus</i> meal containing 25% crude protein
AI30	:	<i>Acetes indicus</i> meal containing 30% crude protein
AI35	:	<i>Acetes indicus</i> meal containing 35% crude protein
SF25	:	Sunflower meal containing 25% crude protein
SF30	:	Sunflower meal containing 30% crude protein
SF35	:	Sunflower meal containing 35% crude protein
MM25	:	Mysid meal containing 25% crude protein
MM30	:	Mysid meal containing 30% crude protein
MM35	:	Mysid meal containing 35% crude protein

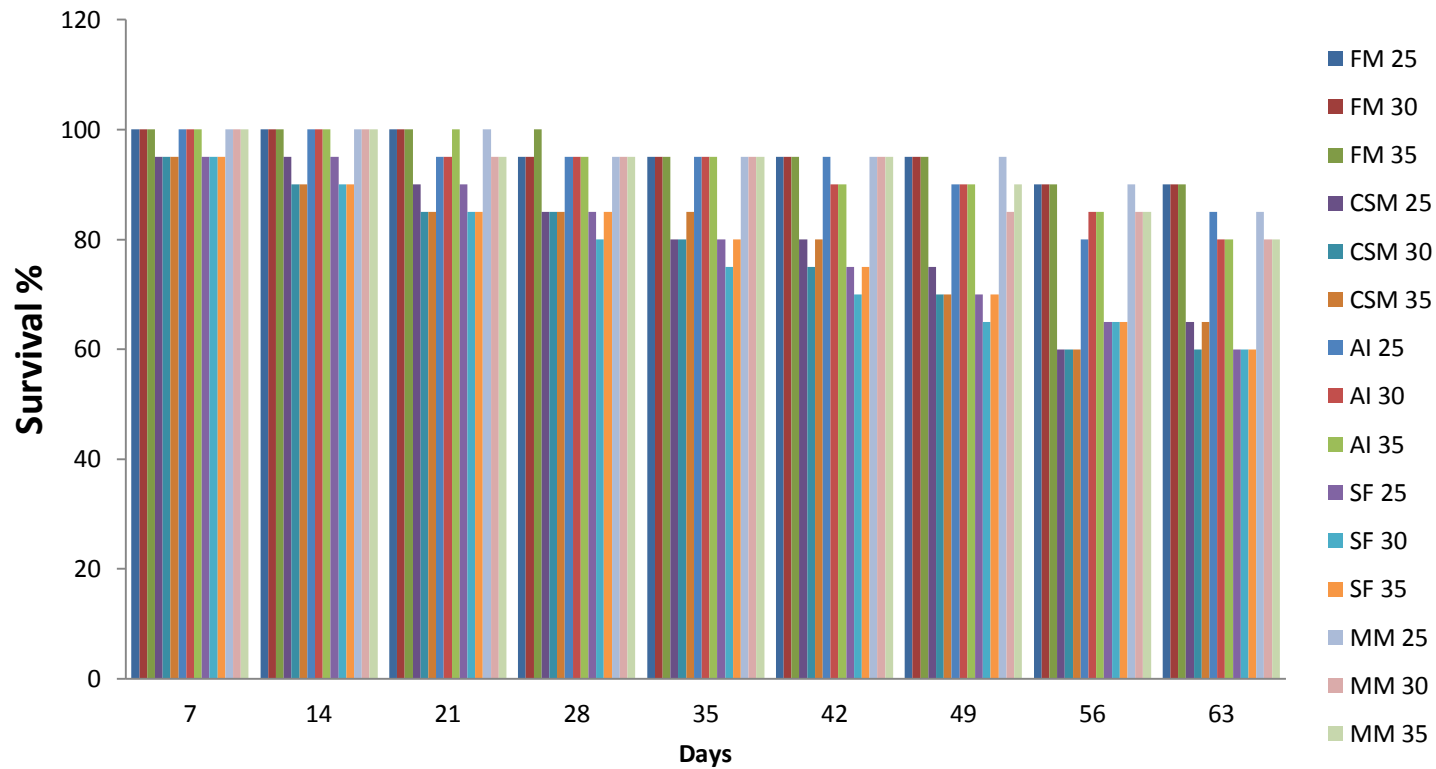
Fig. 17 : The percentage of survival of *L.Vannamei* fed with different experimental diets reared at 0 ppt



Note: FM25 : Fish meal containing 25% crude protein
 FM30 : Fish meal containing 30% crude protein
 FM35 : Fish meal containing 35% crude protein
 CSM25 : Cottonseed meal containing 25 % crude protein
 CSM30 : Cottonseed meal containing 30 % crude protein
 CSM35 : Cottonseed meal containing 35% crude protein

AI25	:	<i>Acetes indicus</i> meal containing 25% crude protein
AI30	:	<i>Acetes indicus</i> meal containing 30% crude protein
AI35	:	<i>Acetes indicus</i> meal containing 35% crude protein
SF25	:	Sunflower meal containing 25% crude protein
SF30	:	Sunflower meal containing 30% crude protein
SF35	:	Sunflower meal containing 35% crude protein
MM25	:	Mysid meal containing 25% crude protein
MM30	:	Mysid meal containing 30% crude protein
MM35	:	Mysid meal containing 35% crude protein

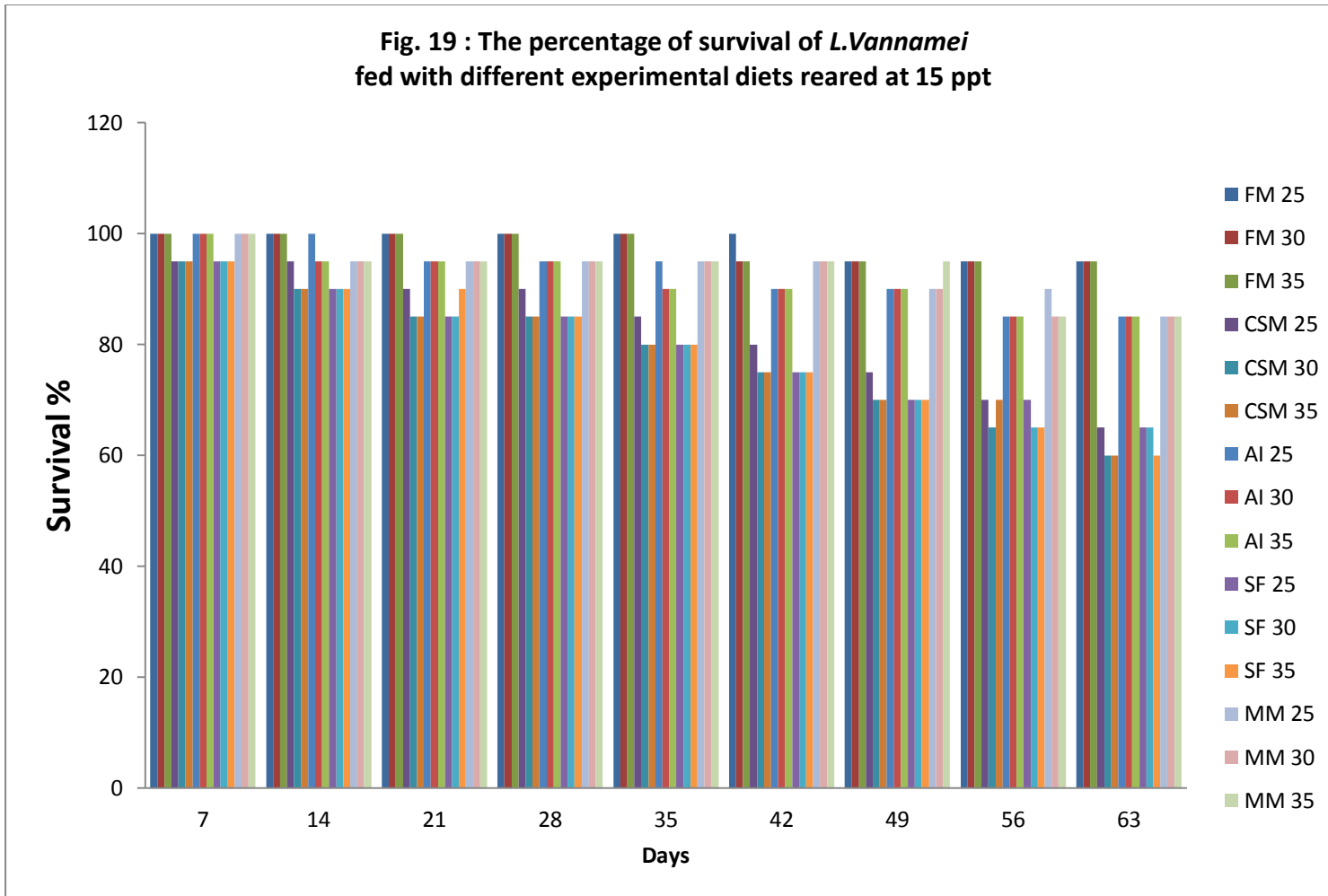
Fig. 18 : The percentage of survival of *L.Vannamei* fed with different experimental diets reared at 5 ppt



Note: FM25 : Fish meal containing 25% crude protein
 FM30 : Fish meal containing 30% crude protein
 FM35 : Fish meal containing 35% crude protein
 CSM25 : Cottonseed meal containing 25 % crude protein
 CSM30 : Cottonseed meal containing 30 % crude protein
 CSM35 : Cottonseed meal containing 35% crude protein

AI25	:	<i>Acetes indicus</i> meal containing 25% crude protein
AI30	:	<i>Acetes indicus</i> meal containing 30% crude protein
AI35	:	<i>Acetes indicus</i> meal containing 35% crude protein
SF25	:	Sunflower meal containing 25% crude protein
SF30	:	Sunflower meal containing 30% crude protein
SF35	:	Sunflower meal containing 35% crude protein
MM25	:	Mysid meal containing 25% crude protein
MM30	:	Mysid meal containing 30% crude protein
MM35	:	Mysid meal containing 35% crude protein

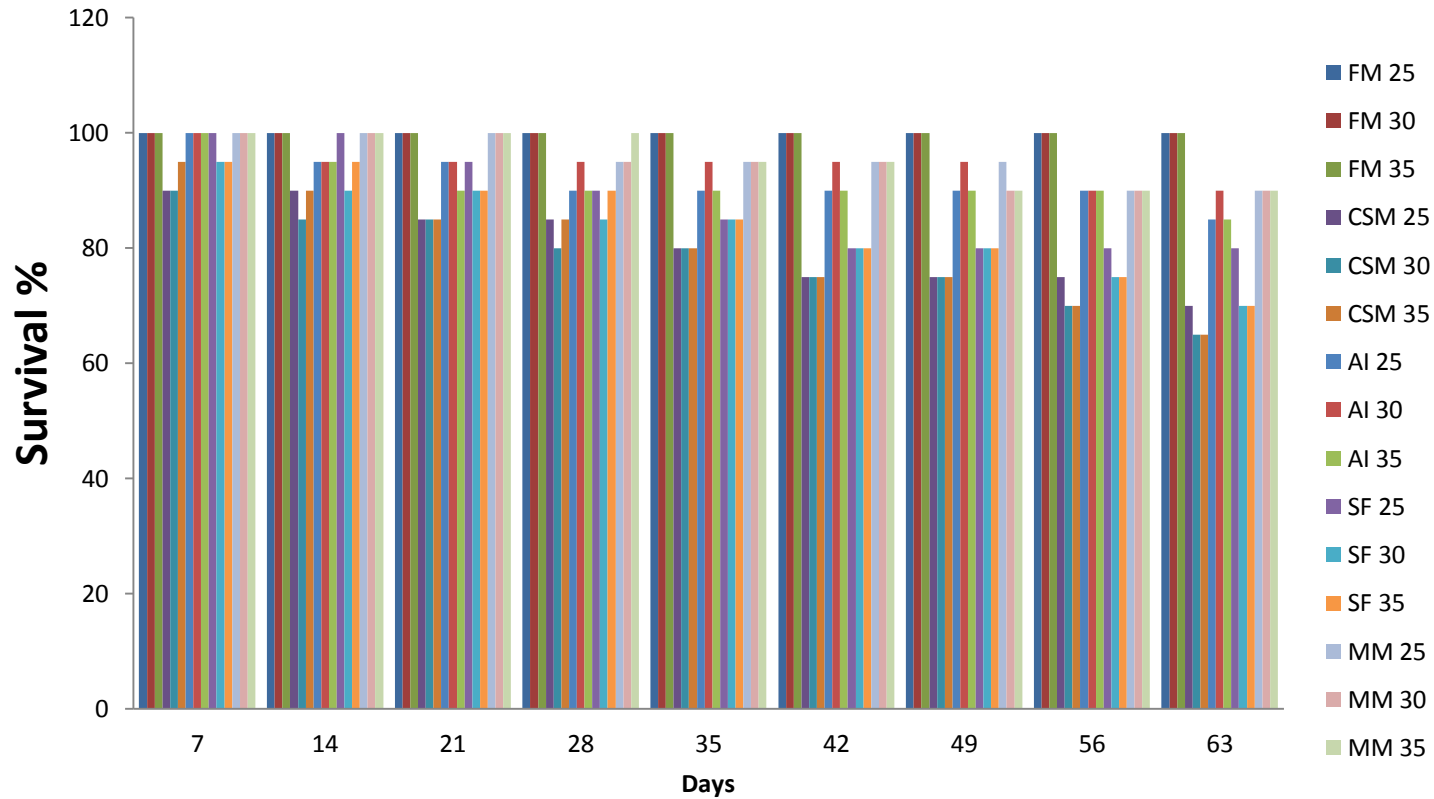
Fig. 19 : The percentage of survival of *L.Vannamei* fed with different experimental diets reared at 15 ppt



Note: FM25 : Fish meal containing 25% crude protein
 FM30 : Fish meal containing 30% crude protein
 FM35 : Fish meal containing 35% crude protein
 CSM25 : Cottonseed meal containing 25 % crude protein

CSM30	:	Cottonseed meal containing 30 % crude protein
CSM35	:	Cottonseed meal containing 35% crude protein
AI25	:	<i>Acetes indicus</i> meal containing 25% crude protein
AI30	:	<i>Acetes indicus</i> meal containing 30% crude protein
AI35	:	<i>Acetes indicus</i> meal containing 35% crude protein
SF25	:	Sunflower meal containing 25% crude protein
SF30	:	Sunflower meal containing 30% crude protein
SF35	:	Sunflower meal containing 35% crude protein
MM25	:	Mysid meal containing 25% crude protein
MM30	:	Mysid meal containing 30% crude protein
MM35	:	Mysid meal containing 35% crude protein

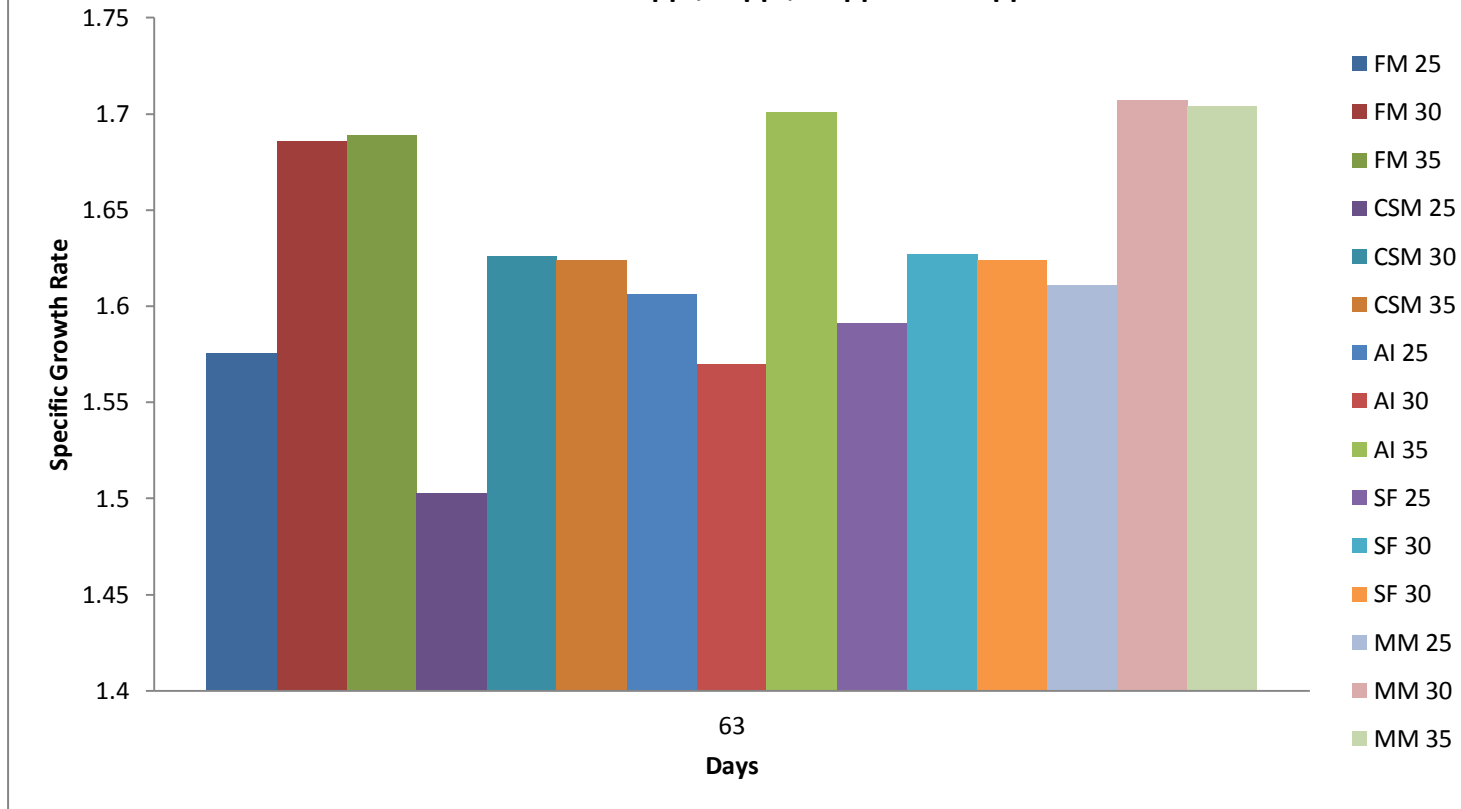
Fig. 20 : The percentage of survival of *L.Vannamei* fed with different experimental diets reared at 25 ppt



Note: FM25 : Fish meal containing 25% crude protein
 FM30 : Fish meal containing 30% crude protein
 FM35 : Fish meal containing 35% crude protein
 CSM25 : Cottonseed meal containing 25 % crude protein
 CSM30 : Cottonseed meal containing 30 % crude protein

CSM35	:	Cottonseed meal containing 35% crude protein
AI25	:	<i>Acetes indicus</i> meal containing 25% crude protein
AI30	:	<i>Acetes indicus</i> meal containing 30% crude protein
AI35	:	<i>Acetes indicus</i> meal containing 35% crude protein
SF25	:	Sunflower meal containing 25% crude protein
SF30	:	Sunflower meal containing 30% crude protein
SF35	:	Sunflower meal containing 35% crude protein
MM25	:	Mysid meal containing 25% crude protein
MM30	:	Mysid meal containing 30% crude protein
MM35	:	Mysid meal containing 35% crude protein

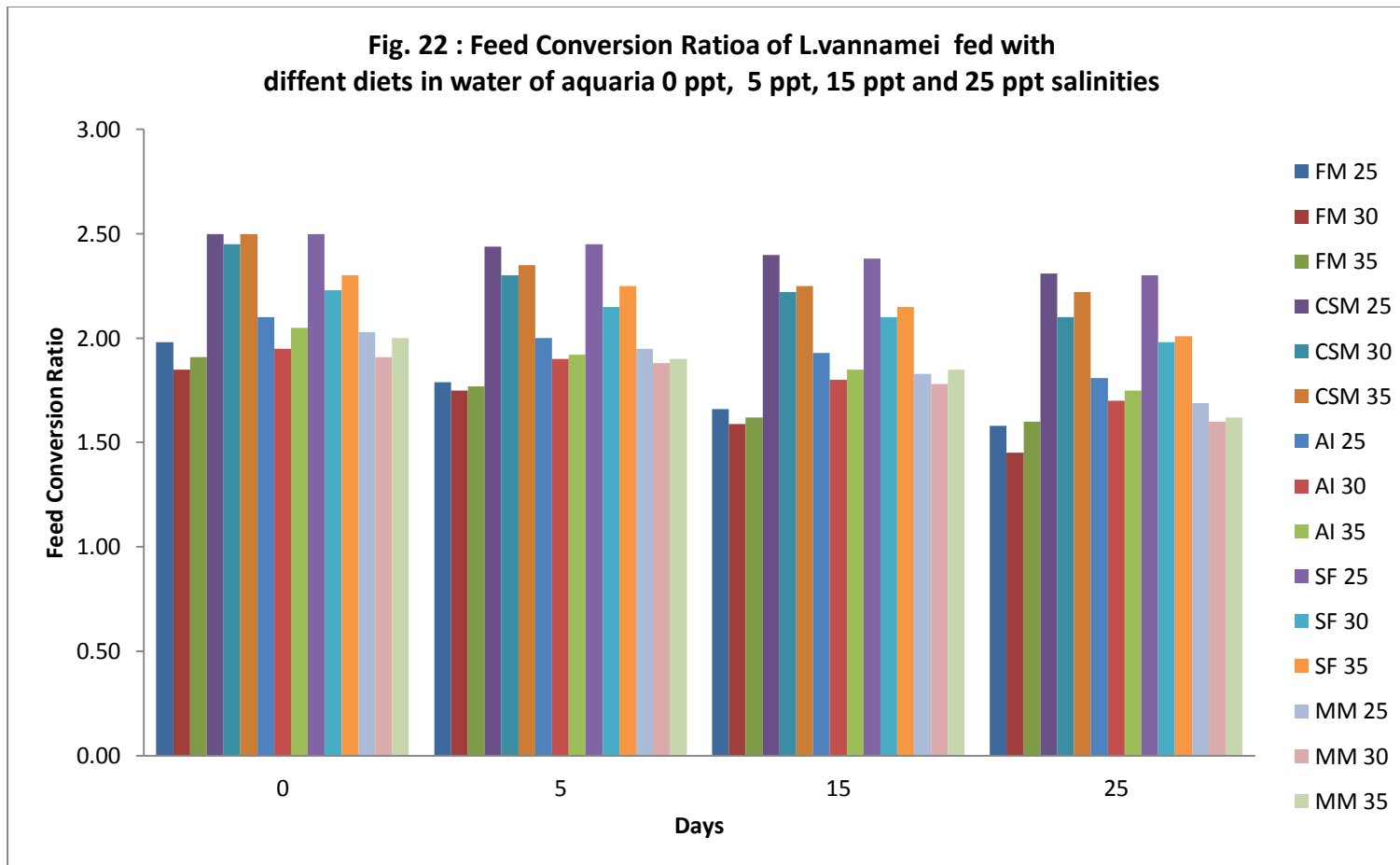
Fig. 21 : Specific Growth Rate (SGR) in Water of Aquaria with *L.vannamei* in different 0 ppt, 5 ppt, 15 ppt and 25 ppt salinities



Note: FM25 : Fish meal containing 25% crude protein
 FM30 : Fish meal containing 30% crude protein
 FM35 : Fish meal containing 35% crude protein
 CSM25 : Cottonseed meal containing 25 % crude protein
 CSM30 : Cottonseed meal containing 30 % crude protein
 CSM35 : Cottonseed meal containing 35% crude protein

AI25	:	<i>Acetes indicus</i> meal containing 25% crude protein
AI30	:	<i>Acetes indicus</i> meal containing 30% crude protein
AI35	:	<i>Acetes indicus</i> meal containing 35% crude protein
SF25	:	Sunflower meal containing 25% crude protein
SF30	:	Sunflower meal containing 30% crude protein
SF35	:	Sunflower meal containing 35% crude protein
MM25	:	Mysid meal containing 25% crude protein
MM30	:	Mysid meal containing 30% crude protein
MM35	:	Mysid meal containing 35% crude protein

Fig. 22 : Feed Conversion Ratio of L.vannamei fed with different diets in water of aquaria 0 ppt, 5 ppt, 15 ppt and 25 ppt salinities



Note: FM25 : Fish meal containing 25% crude protein
 FM30 : Fish meal containing 30% crude protein
 FM35 : Fish meal containing 35% crude protein
 CSM25 : Cottonseed meal containing 25 % crude protein
 CSM30 : Cottonseed meal containing 30 % crude protein

CSM35	:	Cottonseed meal containing 35% crude protein
AI25	:	<i>Acetes indicus</i> meal containing 25% crude protein
AI30	:	<i>Acetes indicus</i> meal containing 30% crude protein
AI35	:	<i>Acetes indicus</i> meal containing 35% crude protein
SF25	:	Sunflower meal containing 25% crude protein
SF30	:	Sunflower meal containing 30% crude protein
SF35	:	Sunflower meal containing 35% crude protein
MM25	:	Mysid meal containing 25% crude protein
MM30	:	Mysid meal containing 30% crude protein
MM35	:	Mysid meal containing 35% crude protein

5. Discussion

5.1. WATER:

Temperature is the key factor affecting growth and production of shrimp (Teichert-Coddington and Rodriguez, 1995) et al 1994, Jackson and wang 1998) Water temperature is probably the most important environmental variables in shrimp culture, because it directly affects metabolism, oxygen consumption, growth, moulting, and survival. In a general, a sudden change of (Gunalan *et al.*, 2013) temperature affects the shrimp immune system. The

ideal temperature range for *L.vannamei* is reported to be between 28.5-32.1⁰ c (Nunes 2000) In the present study optimum range of temperature for *L.vannamei* was between 29.5 to 31.7 °C as observed in earlier studies.

The optimal temperature for growth 27-30°C (Ponce-Palafox *et al.*, 1997). *L. vannamei* grew more slowly when the temperature was 22°C or below, with an optimal growth temperature of 23-34°C. Dissolved oxygen levels remained > 5 mg/lit. pH and salinity levels of the water were measured as 8.0-8.2 and 38-39.4‰. (Metin- Kumulu *et al.*, 2011).

Gunalan *et al.*, 2013, recommended the favorable pH between 7.9 – 8.8 in the morning and 8.2-9.1 in the evening for *L.vannamei*. The pH of pond water is influenced by many factors, including pH of source water, acidity of bottom soil and shrimp culture inputs and biological activity and recommended the favorable pH range of 7.6-8.6 for *L. vannamei*.

The ideal pH for the farming of *L.vannamei* ranges from 8.1 to 8.5 (Hernandez and Nunes, 2001). According to Nunes 2002, the responsible Aquaculture Program of the GAA (Global Aquaculture Alliance) proposes a pH of 6 and 9. According to (Boyd 2000 and 2003), the concentration of dissolved oxygen is the variable that most influences the well being of aquatic organisms. In the Responsible Aquaculture Program of the Global Aquaculture Alliance (GAA), reported that the initial norm for the minimal concentration of oxygen in marine shrimp farming is 3 mg.L⁻¹, with a goal of 4 mg.L⁻¹.

Dissolved oxygen plays an important role on growth and production through its direct effect on feed consumption and maturation. Oxygen affects the solubility and availability of many nutrients. Low levels of dissolved oxygen can cause damages in oxidation state of substances from the oxidized to the

reduced form. Lack of dissolved oxygen is directly harmful to shrimps and causes a substantial increase in the level of toxic metabolites. Low-level of oxygen tension hampers metabolic performances in shrimp and can reduce growth and moulting and cause mortality (Gilles Le Molluae, 2001).

The Brazilian National Environmental Council – CONAMA establishes that this value should not be less than 5 mgL⁻¹ (Nunes, 2002). The DO for the experiment in the present study was ranging 5.66 and 7.81 mg/L.

Brock and Main, (1994) reported that dissolved oxygen should be maintained above 4 mg/l. Oxygen solubility in water is affected by temperature, salinity, organic matter content, and rate of photosynthesis (Zendejas 1999). Dissolved oxygen concentrations of 0-1.5 mg/l are lethal for shrimp and concentrations of 1.7- 3.0 mg/l result in low feed conversion and slow growth. Aragon and Garcia (1996) recommend that the minimum oxygen concentration for optimal shrimp growth be approximately 5 mg/l.

Seidman and Lawrence (1985) reported the critical DO point for shrimp as being below 2 mg/l. Aeration is commonly used to improve water quality in ponds and enhance production of farmed aquatic animals. Dissolved oxygen is a major factor in intensive production and its directly related to a aeration rate (Hopkins *et al.*, 1991). Mc Graw *et al.*,(2001) working with *L.vannamei* and *L. stylostris*, showed that increasing minimum dissolved oxygen by aeration resulted in higher survival, yield, and net income, although no significant differences occurred in final weight .(Ruiz- Velasco *et al.*, 2010)

In the present study the mean temperature, dissolved oxygen, pH and other water quality parameters were well within the appropriate ranges for *L. vannamei* culture as it correlates with the (Treece 2000), (Audelo-Naranjo) *et al.*, 2012 And (Pramoda malli) 2014. The values of water quality parameters reveal that all these are in the acceptable range for survival and growth for *L.vannamei*.

5.2. PROTEIN:

The quantitative requirement of protein has not been determined for penaeid shrimp, several studies have evaluated the effect of variation in dietary protein level on growth and feed conversion. Colvin and Brand (1977) studied post larval and juvenile of *L.vannamei* semi-purified diets of 25, 30, 35 and 40 % crude protein over a four week period. Feed conversion was found to be significantly lower for shrimp fed the 25% crude protein diet and the dietary protein requirement by post larval shrimp as 30-35%, juveniles 30%. Respectively.

The growth response of three sizes of *L.vannamei* (4.0, 9.8 and 20.8) fed diets containing 22, 29 and 36% crude protein for a period of 30 days and Results indicated that a dietary protein level in excess of 36% would be required to yield a maximum growth rate for the 4.0g shrimp.(Smith et al 1984).

Another study conducted by Khureshy and Davis, 2000, juveniles shrimp exhibited significantly higher weight gain, percent weight gain, when fed the 32% protein diet as compared with the 16% protein diet The poor growth response seen with the 16% protein diet, probably primarily due to larger quantity of feed which must be consumed to meet daily nutrient requirements. The poor performance of shrimp offered the 25% diet is probably due to the inability of the shrimp effectively assimilate sufficient protein, resulting in a limitation of nutrient intake.

Teichert-Coddington and Rodriguez, 1995. conducted an experiment in Honduras indicate that 30% protein feed did not result in increased shrimp production compared to 20% protein feed (Teichert-Coddington *et al.*, 1997). Reported shrimp yields and growth were similar when fed with 20% or 40% dietary protein

No significant in shrimp yield was reported when a 29 or 37% protein feed was offered to *L.vannamei* stocked in earthen ponds at four to eight shrimp m⁻².(Teichert-Coddington and Arrue, 1988)

Generally, animal proteins have been associated with a hypercholesterolemic effect, plant proteins a hypocholesterolemic effect. Polyunsaturated fatty acids are also essential for the growth of crustaceans. They have been reported to improve the growth performance of fresh water prawns (*Macrobrachium rosenbergii*; D'Abramo and Sheen, 1993) and various other prawns, such as *P.chinensis* (Xu *et al.*, 1993), *P.japonicus* (Kanazawa *et al.*, 1979), *P.indicus* (Read, 1981), and *P.duodorum* (Sick and Andrews, 1973 Cheng and Hardy, 2004). In the present study, the animal proteins gave almost similar growth to that of FM than the plant proteins. It can be suggested that the total replacement of FM can be done with animal protein. *L.vannamei* growth was significantly decreased when fed diets containing 25% protein indicating that essential amino acids were already limiting at this protein level. However, it is important to remember that determination of the optimum dietary protein level is also affected by the level of other components in feed (Andrews *et al.*, 1972; Sedgwick, 1979), quality of the protein (Smith *et al.*, 1985), water quality (Shiau *et al.*, 1991; Robertson *et al.*, 1993a; Wyban *et al.*, 1995), and experimental factors such as stocking density, daily ration size, and feeding frequency (Teshima and Kanazawa, 1984; Robertson *et al.*, 1993b), which makes comparison of results difficult.(Velasco *et al.*, 2000).

In the present study, both animal and plant dietary protein 30% showed better growth than 25% and 35%, which almost coincides with the investigation of Khureshy and Davis, (2000), Pramoda malli G (2014)

5.3. REPLACEMENT OF FISH MEAL:

One of the options proposed to significantly reduce the production costs of the shrimp industry is to replace fish meal with vegetable protein sources in the formulation, therefore reducing the cost of feeds. Fish meal has been completely replaced in production diets for several species of fish e.g., catfish, tilapia and crustaceans. e.g., *Macrobrachium rosenbergii*.; however, replacement of marine protein sources in practical diets for *L. vannamei* has been less successful. (Davis and Arnold, 2000).

Although fishmeal is commonly the most costly ingredient in shrimp feeds, and therefore receives the most attention in searching for alternative sources, identifying alternatives for other components of feeds will broaden the scope of selection for feed formulators. It remains for feed formulators to locate sources of these and similar ingredients for inclusion in shrimp feed on a least cost basis. (Forester *et al.*, 2010) Amaya *et al.*, (2007a) confirms that commercial extrusion may have an important role in improving the overall nutritional quality of shrimp diets including high levels of plant protein sources.

Luis *et al.*, (2006) studied the comparative effects on growth efficiency and energy budget of *L.vannamei* post larvae and early juveniles-previously weaned on inert particles-while being exposed to two types of diets: one composed of vegetable protein and carbohydrates and another containing animal protein sources. In the present study also attempt had been made to evaluate the effect of plant protein source like cottonseed meal Sunflower meal and animal protein source like *Acetes indicus* meal, mysid meal which are locally available and cheaper than fish meal.

5.4 COTTON SEED MEAL:

It has been reported that a number of crustacean species, especially those having low food chain such as omnivorous *L.vannamei* diets performed well on diets with minimal level of or containing no marine animal proteins. Cottonseed meal has a number of benefits as a potential fishmeal replacement. It is a safe, heavy metal-free plant protein source that is high in protein, less expensive than fishmeal and generally less expensive than soybean meal. However, normal glanded cottonseed meal contains gossypol, a compound that is toxic to aquatic animals, including shrimp. The ability to effectively replace fishmeal protein with glandless cottonseed meal protein would provide a cost savings to shrimp farmers and help produce a more sustainable product than fishmeal-based feeds.

Venero *et al.*, 2008 stated that The cost of the different sources of plant proteins, on unit protein basis, is quite often lower than that of fish meal. However, prices will vary depending on the region, shipping costs as well as import tariffs. Even if prices are equal, plant proteins are often less variable in terms of nutrient profile, more shelf-life stable, and easier to handle and incorporate into diet formulations. Robinson *et al.*, 1994 Inclusion of cottonseed meal in an all-plant protein feed may be desirable not only for economic reasons but it may also improve palatability. Currently, tests are being conducted using a diet without animal proteins or fish meal (Davis *et al.*, 2004b).

Watanabe *et al.*, 1995; Regost *et al.*, 1999; Soltan *et al.*, 2008 reported that Individually cottonseed meal plant by-product meals are fairly rich in protein and favorable essential amino acid profiles, and contained various quantities of anti-nutritional factors. Some studies have also stressed that a mixture of plant protein source is more appropriate to obtain adequate amino acid profile compared to the incorporation of a single plant protein source.

Cottonseed meal may be related to the high-crude fiber, presence of identified or unidentified anti-nutritional factors and poor palatability of PPM which reduce FI and adversed FCR and PER (Luo *et al.*, 2006). Free gossypol, when present in large quantity in the diet, has been shown to be toxic to mono

–gastric animals including fish. Cottonseed meal usually contains 0.4 to 1.7% gossypol. Consequently, the increased levels of PPM in the diets may reduce growth, feed intake, feed and protein utilization, digestibilities of the different nutrients and histological changes in the liver and kidney. Feeding cost in fish production is about 50% of the total production costs. All other costs in the present study are constant, therefore, the feeding costs required to produce one kg gain in weight could be used to compare the different experimental treatments. The calculated figures showed that, the cost of one ton feed mixture was reduced in all replacing levels of FM by PPM and the replacing level 45% reduced feeding costs by 11.4% and decreased feed costs/kg weight gain by 6.74%. (Soltan *et al.*, 2008), where as in the present study also the feed cost reduced by 40-50% in all the salinities.

The solvent-extracted cottonseed meal can be used to replace 40% of the marine protein mix. Higher levels of replacement with cottonseed meal resulted in reduced performance of the shrimp, presumably because of the gossypol content of cottonseed meal, however, higher levels of replacements resulted in reduced growth. Lim (1996)

Phytic acid negatively affects the utilization of minerals which can be seen by its ability to bind up to 75% of all phosphorus. It can chelate di- and tri-valent metals including calcium, magnesium, zinc and iron into compounds that are less easily absorbed in the intestine. Phytic acid inhibits activities of some digestive enzymes such as pepsin, trypsin and alpha-amylase. (Soltan *et al.*, 2008).

A good alternative protein source to fish meal is glandless cottonseed meal (GCSM). It is relatively high in protein, renewable, and, if current development efforts reach fruition, these meals should be generally less expensive per unit of protein than fish meal. Richardson, (2013) studies showed the ability of GCSM, produced from naturally occurring mutant plants, to effectively replace 67-100% of fish meal in shrimp diets with no reduction in growth performance or survival.

The high apparent digestibility values obtained in this study demonstrated glandless cottonseed meal can be effectively utilized by feed formulators to replace more-expensive protein sources in *Litopenaeus vannamei* diets. These findings suggested glandless cottonseed meal has the ability to replace significant amounts of fishmeal in research and commercial diets. (Anthony J.Siccardi III, 2012)

(Wang 2010) showed that increasing replacement level of fish meal protein by cottonseed meal up to 24%, the protein efficiency ratio and phosphorus content in whole body and muscle were significantly decreased ($P < 0.05$). and is concluded that cottonseed meal can replace of 24-30% of the fish meal protein of *Litopenaeus vannamei* without compromising growth, feed intake and protein utilization.

Iron supplementation has been suggested to compensate for gossypol toxicity by formation of an iron-gossypol complex in the intestinal tract, preventing gossypol absorption (Wenegaertner, 1981; Yildirim *et al.*, 2003; Meric *et al.*, 2010). This beneficial effect of iron supplementation has been reported in a number of studies. In the iron-supplemented 30% oilcake diet, growth parameters were indeed slightly increased compared to the unsupplemented 30% group. Still, at 45% replacement, no effect of iron was observed.

The performance of shrimp was adversely affected when diets containing more than 26.5% cottonseed meal, or 1,100 ppm free gossypol, were fed. Shrimp fed the diet with 39.8% cottonseed meal or 1,600 ppm free gossypol had depressed weight gain, reduced feed intake and high mortality. The groups receiving the two highest dietary levels of cottonseed meal lost weight by the end of week 4 and all shrimp in these treatments died within 6 to 8 weeks. These adverse effects were probably due to the toxicity of free gossypol. At 1% inclusion of iron in the CSM 20,25,30 feed because in this study total replacement of fish meal has been done.

Shrimp appeared to accumulate gossypol in the body as evidenced by light yellow-green coloration in shrimp fed diets containing cottonseed meal. (Lim and Chhorn, 1996). But in the present study, this accumulation of gossypol was not observed at all in all the salinities except survival rate was affected. And it might be due the CSM feed with the supplementation of iron.

Furthermore this study provided important evidence that animal protein sources can be completely removed from shrimp feeds without negatively affecting shrimp growth. Although the all-plant diet produced good shrimp performance, this diet may be marginal and further studies are recommended to evaluate potential limiting nutrients as well as the efficacy of cottonseed meal. Additional studies with plant based diets at a larger scale under commercial pond conditions are also suggested.

5.5 SUNFLOWER MEAL:

Economic analysis indicated that fishmeal replacement by vegetable protein ingredients was more advantageous. Sunflower meal has been reported to contain a lot of endogenous anti-nutritional factors, such as a protease inhibitor, an arginase inhibitor and the polyphenolic tannin chlorogenic acid (Tacon *et al.*, 1984). It has relatively high crude fiber content, which can reduce the pelleting quality and protein digestibility of the feed included at high levels (Kamarudin *et al.*, 1989). Sunflower meal also contains low levels of lysine. Samocha *et al.* (2004) did not find significant differences in weight gain, survival and feed efficiency of *L. vannamei* fed 32% CP (crude protein) practical diets, where up to 100% of fish meal was replaced with co-extruded soybean poultry by-product meal with egg supplement.

Dayal J.S. et al., (2011) stated that 20% replacement of Fish meal and sunflower meal can reduce 1 Rs/KG but in the present study 100% replacement of FM with plant origin protein sunflower meal could save total feed cost by 40% and almost to same growth performance. except the survival percentage. One of the options proposed based on the results from the study to significantly reduce the production costs of the shrimp industry is to replace fish meal with vegetable protein sources in the formulation, therefore reducing the cost of the feeds.

5.6 *Acetes indicus*:

The replacement level of fish meal by alternative proteins in shrimp feeds is partially dependent on the amount of fish meal used in the basal feed (Wang *et al.*, 2005), in addition to experimental conditions (Hernandez *et al.*, 2008). The highest average weight of prawn was achieved in the groups that were fed on FM protein was replaced by extracted *Acetes indicus*. Moreover, the FM used in the study was obtained from a locally available dried fish, Bombay duck, (*Herpodon nehereus*). The protein availability of FM was assumed to reduce due to un-hygienic storage condition and insect infestation during storage (Abul Farah 2009).

The presence of chitin interferes with the determination of crude protein in crustacean meal because chitin contains nitrogen that is calculated as protein nitrogen. Cruz-Suarez *et al.*, (1993) reported that via by-product meal of Pacific white shrimp contains 11% chitin and Calvo-Carrillo *et al.*, (1995) determined that red crab meal (RCM) contains about 9% chitin. If these different contents of chitin are considered to calculate real protein content, then the crude protein content for the red crab and shrimp meals are overestimated by 1%. Villarreal *et al.*, (1994) reported a significantly better growth of *L.vannamei* and they also reported an increase in feed consumption of diet contained red crab meal. Fox *et al.*, (1994) observed that the inclusion of shrimp head meal in diets for juvenile *Penaeus monodon* significantly improved palatability, when compared to a diet based on fish meal. Like other crustacean

meals, *Acetes indicus* meal contains nitrogenous compounds such as amino acids, peptides and nucleotides that have been identified as feeding stimulants (Cruz-Suarez *et al.*, 1993; Harpaz, 1997; Lee and Meyers, 1997). Montemayor *et al.*, (1998) included red crab soluble and also an attractant in feeds for several crustaceans and obtained results comparable to those obtained with commercial attractants for shrimp.

Fatty acids play important roles in the nutrition of crustaceans. Their main functions are as a source of energy and for the maintenance of the functional integrity of bio-membranes (Reigh and Stickney 1989). The highly unsaturated fatty acid content of artificial diets has been found to have a strong impact on the survival, growth, feed conversion, fecundity, egg hatchability, and molting and stress tolerance of *Penaeus* and *Macrobrachium* species. Particularly, 18:2n-6 (linoleic acid), 18:3n-3 (linolenic acid), 20:5n-3 (EPA) and 22:6n-3 (DHA) fatty acids are essential to increase the growth and survival of larvae and juveniles of these genera (Querijero, *et. al.*, 1997; D Abramo 1998; Roustaian, Kamarudin, Omar, Saad & Ahmad 1999; Mitra *et al.*, 2005).

The results of the Anh *et al.*, 2009 study indicates that the growth performance of a prawn *M. rosenbergii* PL fed an FM control diet was inferior to prawn fed *Acetes indicus* diets. Feed intake was not determined in this study; the *Acetes indicus* diets are apparently more attractive to the prawns than the FM control diets. This may result in better feed satiation of the prawns and hence survival and growth performance. From a nutritional view point in terms of fatty acids, most levels of important fatty acids such as n-3 and n-6 PUFA in the diets containing 50% or more *Acetes indicus* protein significantly higher than those in the FM control diets, which may have contributed to the better survival and growth of PL s in their experiment. Overall, the formulated diets containing *Acetes indicus* biomass ensured that the essential nutrient requirements for *M.rosenbergii* PL were met and resulted in excellent growth

performance and a high survival. Among the experimental diets, total replacement of FM protein with frozen *Acetes indicus* protein seems to be the best for prawn PL.

The results of this study are in agreement with those of Abelin, Trackaert and Sorgeloos (1980), who reported that PL 30-45 of *P.monodon* and PL15 of *L.vannamei* fed a diet containing a freeze dried *Acetes indicus* meal, had a significantly better growth as compared with the FM-based diet. but in the present investigation the *Acetes indicus* meal .

Naegel and Rodriguez-Astudillo (2004) demonstrated that feeding *L.vannamei* shrimp PL with dried *Acetes indicus* biomass resulted in a significantly higher survival. . (Anh *et al.*, 2009)

Until now, this product has been not efficiently utilized. Therefore, use of *Acetes indicus* biomass as a protein source for feeding aquatic species or other animals may not only help the *Acetes indicus* producers to improve the cost effectiveness of their operations but may also contribute to reduce the use of FM in aqua diets. The results of this study moreover demonstrated that survival of *L.vannamei* fed the *Acetes indicus* diets was higher than prawn fed the FM diet and so that *Acetes indicus* diet can replace totally FM fish meal cost effecting the growth. Feed Conversion Ratio and protein efficiency ratio were also improved by the inclusion of *Acetes indicus* meal in the diets, possibly as an effect of increased palatability and protein digestibility .A high palatability minimizes the time the feed remains uneaten and thereby minimizes nutrient losses through leaching (Tacon *et al.*, 2000). But in the that time taken for total consumption was not considered FCR values obtained in the present investigation (1.45-2.50) are better than those reported by Villarreal *et al.*, (1994) and Civera *et al.*, (1994) for juvenile *F.californiensis* and *L.vannamei* fed practical diets under similar conditions.

5.7 MYSID MEAL:

Among the experimental diets, total replacement of FM protein with MM protein seems to be the best for *L.vannamei*. Until now, this product has been not efficiently utilized. Therefore, use of MM biomass as a protein source for feeding aquatic species or other animals may not only help the MM producers ie. small fishermen to improve the cost-effectiveness of their operations but may also contribute to reduce the use of FM in aquafeeds. Red crab meal which is a crustacean meal has been evaluated as a replacement of FM in crustacean diet (Vanolst et al 1976 and Villarreal et al 2004 Civera *et al.*,2000). In the present investigation also MM which is also a crustacean selected for the replacement of FM. Like other crustacean meals,MM also contains nitrogenous compounds such as amino acids,peptides and nucleotides that have been identified as feeding stimulants for several sps. (Cruz-Suarez et al.,1993; Harpaz,1997; Lee and Meyers, 1997).Mon-temayor et al. (1998) included red crab soluble fluid from crushed red crab (*P.planipes*) and also as an attractant in feeds for several crustaceans and obtained results comparable to those obtained with commercial attractants for shrimp.

Feed conversion ratio (FCR) and protein efficiency ratio (PER) were also improved by the inclusion of MM in the diets,possibly as an effect of increased palatability and protein digestibility. A high palatability minimizes the time the feed remains uneaten and thereby with the determination of crude protein in crustacean meal because chitin contain nitrogen that is calculated as protein nitrogen. Cruz-Suarez *et al.*, (1993) reported that a by-product meal of pacific white shrimp contained chitin and Calvo-Carrillo et.al., (1995) determined that RCM contain about 9% chitin. If this different contents of chitin are considered to calculate real protein content, than the crude protein content for the red crab and shrimp-head meals are over estimated by 1%. In the present investigation the mysid meal fed shrimp showing better growth than fish meal shrimp in all the salinities and different protein percentage diets.

5.8 SALINITIES:

Salinity is an important parameter to control growth and survival of shrimps. *L.vannamei* may have the ability to survive in a wide range of salinities but its growth can vary significantly among different salinity conditions. The ability to grow *L.vannamei* in fresh water may also be advantage in the USA market, based on results of a consumer acceptance test run by the UF/IFAS Food Science and Human Nutrition Department of the University of Florida. This study concluded that USA consumers preferred fresh water prawn *L.vannamei* over those grown in brackish water or salt water or harvested from the sea. This was due to better aroma, appearance, flavour and texture characteristics of freshwater grown shrimp. They stated that there was a strong consumer demand in the USA for a higher quality product than that currently available (UF/IFAS, 2003).

Bray *et al.*, 1994 found that higher growth in 5 and 15ppt treatments were compared to treatments of 25, 35, and 49ppt. No significant difference in survival was observed among different treatments. Laramore *et al.*, 2001 recorded that there was a significant difference in growth for post larval shrimp that were kept in 2 and 3ppt treatments as compared to 30ppt treatments. Not only growth was significantly lower for the shrimp grown in the lower salinities they also showed decreased survival. The shrimp in the 2ppt treatments had a 20% survival while in 30ppt treatment had 80% survival and there was no survival of shrimp in treatments below 2ppt.

In yet another study, results showed the best growth at salinities above 20ppt and temperatures between 20 and 30°C (Ponce-Palafox *et al.*, 1997). At high salinity the shrimp will grow slowly but they are healthy and resistance to diseases. Van Wyk *et al.*, 1999 reported that salinities less than 0.5 ppt put this species at its physiological limit and cause a large proportion of its energy to be used in osmoregulation, therefore limiting growth and preventing it from reaching commercial sizes. A reduction in salinity can significantly affect the survival and growth of penaeid shrimps, although they

possess the ability to tolerate low salinities (Huang 1983, Bray *et al.*, 1994). Galli & Stern (1998) reported that the survival of *L. vannamei* was drastically reduced when salinity decreased below 0.18 ppt. Currently, no shrimps survived in tap water (about 0.2 ppt, salinity), the survival increased with increasing salinity from 1 ppt to 2 ppt, and they grew well in salinities 4 ppt, indicating the limited capability for *L. vannamei* juveniles to tolerate very low salinities. It was apparent from the experiment; however, that salinity had significant effects on both growth and food efficiency.

A reduction in salinity may modify the osmotic and ionic balances in shrimp. To recover the normal balances, the shrimp may have to expend a considerable amount of energy, resulting in less energy to spare for growth (Bindu and Diwan 2002; Zhang and Dong 2002). Rosas *et al.*, (2001a) reported that *L. vannamei* juveniles maintained at 15 ppt salinities consumed 1.69 times more food than those at 5 ppt.

The results obtained (Rosas *et al.*, 2001 a) in the work provide evidence that the growth differences between salinities are primarily the consequence of variations in both food consumption and energy allocation. Food consumption and energy gain (the percentage of energy invested in growth) of *L. vannamei* juveniles appeared to increase with increased salinity within the range of 1-16 ppt, whereas total metabolic expenditure (the percentages of energy lost to respiration and excretion) displayed inverse trends with salinity.

Thus, the enhanced growth of *L. vannamei* juveniles with increasing salinity from 1 ppt to 16 ppt was mainly due to the significantly increased food consumption associated with the significantly decreased proportion of energetic cost for osmotic regulation. At 16 ppt, the food consumption was highest of all while the proportion of energy losses was least of all, and consequently, the growth rate was highest of all. (Cao Mei, 2004).

Results from the 62 day trial in this study indicated that both PL₈ and PL₁₀ exhibited better survival rates at a salinity level of 3 ppt than at 1 ppt. Similar results were reported by McGraw *et al.* (2002) and Davis *et al.* (2002), who demonstrated that during a 48-hour acclimation period, PL₁₀ had greater survival rates at salinities in excess of 4 ppt than at salinities of 2 ppt and lower. McGraw and Scarpa (2004) found that at 3 ppt, the survival rates of shrimp of both PL₈ and PL₁₀ were significantly higher than those with a salinity level of 1 ppt in the groups with the same stocking density (Limhang *et al.*, 2010). Larvae *et al.* 2001 found survival was 80% at 30 ppt salinity where as that 2 ppt the survival was 20% only. In The present investigation, it was found that the survival rate decrease as the salinity decreases, irrespective of the meal. The survival is as low as 55% in case of CSM at 0 PPT and as high as 100% in case of FM at 25 ppt. The results of the present study are not coincided with the studies of (Sowers and Tomasso 2006) and (Atwood *et al.* 2003), pramoda malli 2014, The result of (Bray *et al.* 1994), (Samocha *et al.* 1998a) indicated that shrimp grow better at lower salinities 2-10ppt . The present results are considering with the (ponee – palafore *et al.* , 1997) observation where the best growth achieved above 20 ppt salinity. And it may be due to the *L. vannamei* utilizes large portion of energy to be used in osmoregulation, therefore limiting the growth at the lower salinities

The present study results conforming that the *L. vannamei* also been cultured in low salinities, freshwater in addition to brackish water. And also this will be the alternative species for *M. rosenbergii* in future. White shrimp culture in freshwater could reduce pressure on coastal areas and provide disease incidence below that of marine culture systems. If culture systems are established in rural areas it could be increase the employment opportunities and aid in diversifying production.

6. Summary

The experiment was conducted in the Wet Laboratory of the Department of Aquaculture, College of Fishery Science, Sri Venkateswara Veterinary University, Muthukur to study “ **Study on effects of different plant and animal protein sources as replacements to fishmeal in vannamei (*Litopenaeus vannamei*) reared under varying water salinities** ” *L. vannamei* (500 numbers) PLs were obtained from CP Hatchery and reared in farmer’s pond till it reaches approximately weight of 2.5g for 25 days at 30 ppt salinity. Then the seed were acclimatized to the required salinity, i.e., 0 ppt, 5 ppt and 15 ppt, 25 ppt by slowly adding fresh water at the rate of reduction of 2 salinity per hour. They are fed on different diets prepared with Fish meal at 25%, 30%, 35% protein levels, Cottonseed meal at 25%, 30%, 35% protein levels *Acetes indicus* meal at 25%, 30%, 35% sunflower meal 25%, 30%, 35 and Mysid meal 25%, 30%, 35 protein inclusion levels maintained at four different salinities such as 0 ppt, 5 ppt, 15 ppt and 25 ppt salinities. Shrimp were fed with Cottonseed meal *Acetes indicus* meal diets sunflower meal , and Mysid meal and compared with shrimp fed on fish meal diets for growth and survival rates at three different salinities 0, 5, 15 and 25 ppt.

The duration of the experiment was 63 days and the sampling was done weekly. Triplicates were maintained for all the treatments. The results obtained were subjected to statistical analysis.

The results obtained in the present study on growth, survival, feed conversion ratio of *L. vannamei* is summarized as follows.

- Proximate analysis of the feeds has confirmed that mixing of various ingredients at three inclusion levels of 25%, 30% and 35% met the nutrient requirements of *L. vannamei*.
- Important water quality parameters such as dissolved oxygen, temperature, pH, total alkalinity and total hardness were analyzed.
- The DO was found to be in the range of 7.84ppm and 6.31ppm at 0ppt, 7.77ppm to 6.08 ppm at 5ppt and 7.50ppm to 6.55ppm at 15ppt and 7.30ppm to 5.55ppm at 25ppt salinity.
- The pH ranged from 8.2 to 8.3 at 0ppt, 8.2 to 8.3 at 5ppt , 8.3 to 8.4 at 15ppt and 8.4 to 8.5 at 25ppt during the period of experiment.
- The range of total alkalinity varied between 214 mg/l to 155 mg/l at 0ppt salinity, 315mg/l to 229 mg/l at 5ppt salinity , 625mg/l to 535mg/l at 15ppt and 725mg/l to 645 mg/l at 25ppt salinity.
- Total hardness of water ranged between 238 mg/l to 191 mg/l at 0ppt salinity, 322mg/l to 240 mg/l at 5ppt salinity , 675mg/l to 601mg/l at 15ppt salinity and 777 mg/ l to 700 mg/l at 25 ppt .
- Weekly sampling for 63 days was done to study Growth, Survival, and Feed Conversion Ratio.
- Overall after 63 days, the highest average body weight of 14.01 gms (MM 30) and the lowest average body weight of 9.26 gms (CSM 25) at 15 PPT salinity and 0 PPT respectively.
- The Analysis of variance for growth performance (at both levels) showed significant difference among the treatments at all salinities.

- The SGR for *L. vannamei* treated with different diets reared under different salinities were regard between 0.05 to 2.11 for the entire experimental period. At 0 ppt the highest value was recorded 1.7 (FM 35) another lowest value of 0.05 (MM 30); at 5 ppt the highest value was 2.11 (FM 35) and the lowest value was 0.01 (AI 35), at 15 ppt the highest value was 2.02 (FM 35) another lowest value was 0.49 (SF 25) and finally at 25 ppt the highest value was 2.07 (FM 30) and the lowest value was 0.49 (SF 25, MM 25) recorded respectively. Presented at table number 14 figures number 21.
- The highest survival of 100% (FM25,30,35 at 25 ppt) and lowest survival of 55% (CSM30,35, at 0 ppt) were recorded respectively at 0 ppt salinity.
- Analysis of variance for survival revealed significant differences among the treatments for survival at all salinities.
- The Feed Conversion Ratio in different experiments of *L. vannamei* groups reared at varying salinities were calculated and presented in the table number 15 and figure number 22. The highest and lowest values were recorded 2.50 (CSM 25, CSM 35, SF 25) at 0 salinity and 1.45 (FM 30) at 25 salinity respectively. At 0 salinity the highest value recorded CSM 25, CSM 35, SF 25 (2.50) and the lowest value for 1.85 (FM 30), where as at 5 ppt the highest value for 2.45 (SF 25) and the lowest value for 1.75 (FM 30), for 15 ppt the highest (2.40 – CSM 25) and lowest (1.59 – FM 30) and finally at 25 ppt the highest (2.30 – SF 25) and the lowest (1.45 – FM 30) respectively.
- Shrimps fed on all the test diets in the present study performed better and almost equal to the shrimp fed on fish meal diets.

It can be concluded that Cottonseed meal *Acetes indicus* meal, sunflower meal and Mysid meal can be included in the diets of *L. vannamei* up to 30% protein inclusion for the total replacement of fish meal.

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**Table No. 5 : Dissolved oxygen (mg/l) in the water of aquaria with
L.vannamei (White leg shrimp) reared at 0 PPT, 5 PPT, 15 PPT and 25 PPT Salinity :**

Treatment Days		FM			CSM			AI			SF			MM		
		FM 25	FM 30	FM 35	CSM 25	CSM 30	CSM 35	AI 25	AI 30	AI 35	SF 25	SF 30	SF 35	MM 25	MM 30	MM 35
0	0	7.66	7.68	7.68	7.62	7.62	7.66	7.69	7.77	7.77	7.69	7.70	7.77	7.79	7.81	7.81
	5	7.50	7.55	7.53	7.61	7.62	7.65	7.65	7.70	7.70	7.70	7.71	7.65	7.65	7.77	7.70
	15	7.49	7.50	7.50	7.09	7.05	7.10	7.10	7.09	7.10	7.17	7.10	7.09	7.10	7.15	7.20
	25	7.25	7.30	7.30	7.00	6.85	6.99	6.81	6.80	6.66	6.85	6.91	6.92	6.90	6.88	6.91
7	0	7.14	7.15	7.19	7.55	7.60	7.40	7.70	7.70	7.66	7.50	7.71	7.77	7.70	7.80	7.81
	5	7.00	7.02	7.05	7.51	7.55	7.59	7.59	7.61	7.60	7.55	7.61	7.58	7.60	7.77	7.70
	15	7.02	7.00	7.00	7.10	7.01	7.07	7.10	7.15	7.10	7.07	7.05	7.08	7.10	7.20	7.15
	25	7.00	6.54	6.88	6.65	6.50	6.44	6.65	6.71	6.66	6.80	6.66	6.41	6.51	6.61	6.61
14	0	6.99	7.02	7.05	7.15	7.55	7.50	7.51	7.62	7.71	7.66	7.65	7.70	7.77	7.70	7.65
	5	6.99	7.00	7.05	7.50	7.55	7.55	7.70	7.60	7.49	7.60	7.63	7.69	7.65	7.68	7.68
	15	6.87	6.85	6.90	6.90	6.99	7.05	7.16	7.17	7.18	7.00	7.00	7.05	7.09	7.10	7.15
	25	6.66	6.61	6.65	6.50	6.44	6.35	6.11	6.11	6.17	6.45	6.44	6.55	6.19	6.45	6.30
21	0	6.99	7.05	6.95	7.00	7.09	7.11	7.11	7.15	7.11	7.10	7.07	7.00	6.99	7.10	7.00
	5	7.00	7.04	7.07	7.07	7.07	7.10	7.15	7.17	7.17	7.00	7.05	7.08	7.07	7.07	7.10
	15	6.99	6.95	6.95	6.95	6.90	6.91	6.90	7.00	7.02	6.91	6.89	7.00	6.96	6.95	7.00
	25	6.19	6.20	6.22	6.44	6.50	6.45	5.99	6.01	5.95	6.21	6.40	6.39	6.45	6.41	6.31
28	0	6.95	7.01	7.01	7.01	7.02	6.89	7.00	7.05	6.99	6.91	7.10	6.99	7.05	7.21	7.11
	5	7.05	7.01	7.02	7.05	7.00	6.98	6.99	7.05	7.01	7.10	7.09	7.10	7.12	7.15	7.20
	15	6.88	6.90	6.95	6.76	6.76	6.78	6.98	6.98	7.01	6.88	6.85	6.90	6.99	6.95	7.02
	25	6.29	6.31	6.33	6.31	6.31	6.26	6.00	5.95	5.99	6.00	6.15	6.05	6.15	6.33	6.20
35	0	6.99	6.95	6.97	7.02	6.99	6.90	6.91	6.89	6.99	6.15	6.88	7.00	6.66	6.91	6.77
	5	6.99	6.95	7.01	6.99	6.98	6.90	6.95	6.90	6.95	6.90	6.89	6.98	6.98	6.90	6.75
	15	6.85	6.90	6.91	6.90	6.88	6.95	6.95	6.90	6.92	6.69	6.75	6.76	6.89	6.85	6.90
	25	6.10	6.11	6.05	6.00	6.11	5.93	5.91	5.99	5.88	5.99	6.15	6.05	6.11	6.25	6.00
42	0	6.88	6.99	6.91	6.91	7.01	7.11	6.95	6.93	6.90	6.31	6.77	6.41	6.66	6.81	6.88
	5	6.99	6.98	6.90	6.98	7.02	7.05	6.99	6.95	7.01	6.85	6.77	6.80	6.82	9.80	6.82
	15	6.88	6.90	6.91	6.88	6.85	6.89	6.85	6.85	6.83	6.70	6.66	6.65	6.88	6.75	6.80
	25	6.10	6.15	6.09	6.15	6.19	6.18	5.83	6.01	5.91	5.91	6.00	5.99	6.01	6.30	6.10
49	0	6.99	6.93	6.99	6.91	6.99	7.11	6.91	7.05	7.10	6.90	6.95	6.94	6.81	6.87	6.66

	5	6.90	6.93	6.90	6.88	6.93	6.79	7.00	7.03	7.10	6.86	6.94	6.95	6.80	6.81	6.88
	15	6.85	6.88	6.89	6.80	6.77	6.76	6.88	6.91	6.88	6.87	6.85	6.90	6.88	6.70	6.75
	25	6.11	6.21	6.25	5.88	6.01	5.91	6.00	6.05	5.95	5.83	5.99	5.97	5.77	5.95	5.97
56	0	6.77	6.88	6.81	6.66	6.91	6.75	6.66	6.99	6.71	6.99	7.01	6.91	6.91	6.95	6.95
	5	6.85	6.86	6.88	6.88	6.90	6.88	6.90	6.97	6.95	7.02	7.02	7.07	6.90	6.87	6.95
	15	6.80	6.75	6.80	6.76	9.75	6.80	6.82	6.85	6.88	6.88	6.85	6.86	6.66	6.71	6.71
	25	5.75	6.00	5.91	5.75	6.02	5.91	5.88	5.90	5.93	5.78	5.95	5.88	5.91	5.99	5.81
63	0	6.71	6.75	6.66	6.70	6.77	6.67	6.77	6.81	6.88	6.77	6.80	6.83	6.77	6.67	6.66
	5	6.70	6.75	6.75	6.75	6.70	6.77	6.75	6.80	6.85	6.80	6.80	6.81	6.85	6.66	6.75
	15	6.66	6.65	6.70	6.66	6.65	6.70	6.58	6.75	6.70	6.65	6.66	6.69	6.57	6.55	6.60
	25	5.66	6.01	5.91	5.77	6.00	5.91	5.65	5.88	5.85	5.55	5.85	5.88	5.56	5.88	5.81

**Table No. 6 : Total alkalinity (mg/l) in the water of aquaria with
L.vannamei (white leg shrimp) reared under 0 PPT, 5 PPT, 15 PPT and 25 PPT salinity**

Treatment Days	FM			CSM			AI			SF			MM			
	FM 25	FM 30	FM 35	CSM 25	CSM 30	CSM 35	AI 25	AI 30	AI 35	SF 25	SF 30	SF 35	MM 25	MM 30	MM 35	
0	0	161	155	160	161	161	159	171	166	159	170	171	172	169	166	171
	5	231	233	235	235	230	225	229	235	229	235	240	240	239	242	242
	15	555	552	550	539	561	560	529	531	535	555	561	560	562	565	565
	25	665	660	665	671	666	665	653	655	657	670	669	669	671	669	675
7	0	169	171	175	177	169	166	181	177	180	171	169	171	177	170	180
	5	250	255	253	255	260	261	239	242	242	245	247	245	249	250	255
	15	539	550	541	550	553	560	539	353	539	557	565	557	580	581	582
	25	645	650	648	659	653	655	681	679	666	669	678	679	688	688	689
14	0	169	168	173	180	177	170	177	180	169	191	185	192	166	175	177
	5	255	260	260	261	262	261	271	265	266	255	260	270	261	255	260
	15	544	541	541	555	553	555	559	561	560	560	559	560	561	575	577
	25	666	660	665	666	671	675	661	659	658	676	677	677	698	699	705
21	0	179	177	181	188	190	192	179	188	182	169	179	177	181	191	191
	5	258	257	257	262	259	258	266	269	271	266	262	269	269	262	263
	15	560	561	560	579	581	588	575	572	570	577	576	580	580	581	585
	25	661	658	655	666	673	655	677	688	680	688	691	695	697	697	701
28	0	179	181	180	179	177	180	188	195	190	197	198	200	200	191	195
	5	260	261	261	265	260	260	265	271	266	272	277	278	269	270	270
	15	577	575	579	581	581	585	588	588	591	597	599	600	603	601	606
	25	660	666	666	679	688	685	691	693	693	688	691	688	695	697	700
35	0	190	191	195	190	188	186	180	177	193	193	199	200	200	202	203
	5	277	280	282	277	277	276	285	281	280	288	278	277	288	292	291
	15	577	581	581	588	593	591	591	593	595	602	599	601	605	603	606
	25	677	671	670	700	689	697	699	691	705	702	699	700	705	700	710
42	0	179	177	180	185	191	192	191	188	187	193	188	194	195	199	200
	5	287	288	286	279	280	283	288	291	291	281	279	282	288	290	295
	15	599	595	591	600	599	600	601	603	605	606	600	610	606	599	599
	25	681	675	675	677	669	670	693	693	699	697	691	690	702	699	707
49	0	190	191	195	195	197	194	200	199	203	204	205	200	206	207	208
	5	281	280	281	285	282	278	288	291	290	281	288	281	295	299	295
	15	595	595	599	601	600	600	606	599	598	610	606	606	607	610	610
	25	691	695	697	694	693	699	701	699	695	707	701	703	704	705	711

56	0	188	188	190	195	202	200	195	197	199	203	207	200	201	202	205
	5	288	291	291	293	292	295	295	300	301	300	303	305	307	300	300
	15	599	600	603	600	595	601	610	606	606	610	617	611	620	625	623
	25	700	698	691	701	699	697	699	702	699	700	703	695	699	707	708
63	0	197	199	199	203	207	205	199	198	202	210	205	200	213	210	214
	5	289	288	288	300	303	301	307	310	313	313	310	309	315	313	315
	15	605	606	604	607	610	611	611	616	612	620	622	622	629	633	633
	25	700	701	705	706	707	710	709	710	711	719	717	720	720	721	725

**Table. No. 9 : Weight Gain (grams) in Water of Aquaria with
Litopenaeus Vannamei reared in 0 PPTs Salinity**

Treatment Days		FM			CSM			AI			SF			MM		
		FM 25	FM 30	FM 35	CSM 25	CSM 30	CSM 35	AI 25	AI 30	AI 35	SF 25	SF 30	SF 35	MM 25	MM 30	MM 35
0	0	2.50	2.51	2.50	2.49	2.53	2.52	2.50	2.49	2.48	2.49	2.49	2.50	2.51	2.52	2.51
	5	2.52	2.50	2.49	2.48	2.50	2.51	2.51	2.49	2.48	2.48	2.50	2.51	2.49	2.50	2.50
	15	2.50	2.50	2.49	2.51	2.50	2.52	2.52	2.50	2.51	2.51	2.51	2.50	2.50	2.49	2.49
	25	2.53	2.53	2.52	2.52	2.49	2.53	2.51	2.52	2.50	2.52	2.50	2.51	2.51	2.48	2.49
7	0	0.49	0.89	0.92	0.06	0.42	0.47	0.38	0.66	0.69	0.28	0.46	0.50	0.39	0.05	0.66
	5	0.51	1.00	1.07	0.23	0.50	0.49	0.50	1.01	0.01	0.17	0.60	0.53	0.52	1.00	0.99
	15	1.10	1.11	1.09	0.54	0.65	0.63	0.99	1.22	1.25	0.49	0.68	0.65	0.52	1.28	1.26
	25	1.07	1.11	1.13	0.54	0.65	0.66	1.01	1.24	1.25	0.49	0.67	0.66	0.49	1.29	1.31
14	0	0.81	0.59	0.60	0.55	0.40	0.37	0.62	1.05	1.08	0.38	0.96	0.95	0.61	1.05	1.08
	5	0.98	1.14	1.17	0.73	0.95	0.85	1.01	1.05	0.99	0.99	0.95	0.95	1.47	0.95	1.08
	15	1.11	1.13	1.04	0.80	0.86	0.96	1.10	1.04	1.01	0.90	0.93	0.97	1.01	1.08	0.96
	25	0.93	1.15	1.15	0.98	0.88	0.91	1.02	1.03	1.05	0.99	0.93	0.95	1.50	1.03	1.05
21	0	0.35	0.16	0.21	0.80	0.80	0.83	0.94	1.05	1.05	0.84	0.80	0.80	0.94	1.06	1.05
	5	1.00	0.85	1.15	0.97	1.00	0.19	0.58	1.35	1.25	0.96	0.95	0.95	1.06	1.35	1.26
	15	1.04	1.13	0.85	0.45	1.01	0.99	0.87	1.22	1.91	1.06	0.98	0.95	0.99	1.25	1.37
	25	1.05	1.07	1.10	0.76	0.97	0.99	1.01	1.28	1.26	0.90	0.95	0.96	1.00	1.30	1.27
28	0	0.90	1.07	1.10	0.76	0.97	0.99	1.01	1.10	1.06	1.01	1.11	1.05	1.10	1.17	1.19
	5	1.20	1.20	1.01	1.13	1.04	1.91	1.30	0.98	1.10	1.01	1.11	1.05	1.10	1.17	1.19
	15	0.95	1.10	1.29	1.02	1.13	1.05	1.02	1.04	0.99	1.00	1.00	1.05	0.60	1.15	1.11
	25	1.22	2.07	1.10	1.05	1.05	1.15	1.25	1.82	1.04	0.95	1.10	1.12	0.60	1.80	1.06
35	0	0.95	1.7	1.6	0.75	1.31	1.35	1.31	1.13	1.14	0.95	0.99	1.05	1.15	1.10	1.11
	5	1.83	2.00	2.11	0.87	0.96	1.00	1.20	1.27	1.80	1.05	1.01	1.20	1.70	1.30	1.81
	15	1.90	2.09	2.02	1.08	1.07	0.90	1.04	1.80	1.25	0.95	1.16	1.00	1.29	1.80	1.20
	25	1.75	2.07	2.05	0.90	1.05	1.02	1.25	1.82	1.80	9.10	1.10	1.10	1.10	1.80	1.81
42	0	1.70	1.25	1.20	0.70	1.20	1.17	0.84	1.40	1.40	1.04	1.11	1.10	0.80	1.44	1.40
	5	1.38	1.20	1.18	1.15	1.20	1.30	1.51	1.84	1.35	1.01	1.14	1.15	2.11	1.60	1.44
	15	1.02	1.26	1.28	1.02	1.21	1.20	1.05	1.46	1.90	1.06	1.12	1.10	1.59	1.49	1.60
	25	1.40	1.31	1.28	1.20	1.20	1.20	1.50	1.47	1.45	1.02	1.25	1.20	2.10	1.52	1.51
49	0	1.10	1.15	1.05	0.95	1.09	1.03	1.00	1.03	1.00	0.90	0.94	0.90	1.01	0.96	0.95
	5	0.92	1.10	1.25	1.05	1.35	1.15	1.44	1.06	1.35	1.00	1.10	1.25	0.99	1.25	1.35
	15	1.48	1.28	1.06	1.69	1.24	1.90	1.68	1.34	1.00	0.98	1.16	1.10	1.06	1.40	1.28
	25	1.10	1.27	1.26	1.05	1.30	1.28	1.45	1.34	1.35	0.98	1.19	1.15	1.00	1.30	1.25

56	0	1.00	1.27	1.26	1.05	1.30	1.28	1.45	1.09	1.10	1.06	1.09	1.09	1.19	1.10	1.05
	5	0.94	0.97	0.75	1.03	1.00	1.05	0.70	0.95	0.55	1.04	1.06	1.25	1.05	0.40	0.95
	15	0.65	0.76	0.95	0.81	1.13	0.95	1.08	0.75	1.00	1.01	1.08	1.10	1.05	0.88	1.37
	25	0.55	0.70	0.66	0.95	1.15	1.10	0.65	0.73	0.70	1.05	1.22	1.25	1.05	1.25	1.25
63	0	1.05	1.25	1.37	1.21	1.03	1.01	1.05	1.25	1.20	0.95	1.04	1.01	1.05	1.25	1.25
	5	0.90	9.97	1.25	1.13	1.04	1.30	1.05	1.45	1.35	1.45	1.54	1.05	1.10	2.05	1.25
	15	0.84	1.26	0.95	0.88	1.21	1.05	1.20	1.43	1.90	1.05	1.23	1.52	1.15	1.24	1.05
	25	1.20	1.22	1.25	1.75	1.20	1.23	1.05	1.43	1.50	1.45	1.24	1.10	1.10	1.30	1.30

Treatment Days		FM			CSM			AI			SF			MM		
		FM 25	FM 30	FM 35	CSM 25	CSM 30	CSM 35	AI 25	AI 30	AI 35	SF 25	SF 30	SF 35	MM 25	MM 30	MM 35
0	0	2.50	2.51	2.50	2.49	2.53	2.52	2.50	2.49	2.48	2.49	2.49	2.50	2.51	2.52	2.51
	5	2.52	2.50	2.49	2.48	2.50	2.51	2.51	2.49	2.48	2.48	2.50	2.51	2.49	2.50	2.50
	15	2.50	2.50	2.49	2.51	2.50	2.52	2.52	2.50	2.51	2.51	2.51	2.50	2.50	2.49	2.49
	25	2.53	2.53	2.52	2.52	2.49	2.53	2.51	2.52	2.50	2.52	2.50	2.51	2.51	2.48	2.49
7	0	2.99	3.40	3.44	2.55	2.95	2.99	2.88	3.15	3.17	2.77	2.95	3.00	2.90	3.17	3.17
	5	3.01	3.51	3.56	2.75	3.00	3.00	3.01	3.50	3.49	2.65	3.10	3.10	3.00	3.50	3.49
	15	3.60	3.62	3.58	3.05	3.15	3.15	3.51	3.75	3.76	3.00	3.19	3.15	3.02	3.77	3.75
	25	3.60	3.63	3.65	3.06	3.17	3.19	3.52	3.74	3.75	3.01	3.17	3.17	3.00	3.77	3.80
14	0	3.80	3.99	4.04	3.10	3.35	3.36	3.50	4.20	4.25	3.15	3.91	3.95	3.51	4.22	4.25
	5	4.11	4.65	4.60	3.55	3.95	3.96	4.12	4.55	4.50	3.55	4.05	4.07	4.01	4.45	4.45
	15	4.58	4.75	4.75	3.78	4.01	4.00	4.52	4.78	4.75	3.99	4.12	4.10	4.49	4.85	4.83
	25	4.53	4.78	4.80	4.04	4.05	4.10	4.54	4.77	4.80	4.00	4.10	4.12	4.50	4.80	4.85
21	0	4.15	4.15	4.25	3.90	4.15	4.19	4.44	5.25	5.30	3.99	4.71	4.75	4.45	5.28	5.30
	5	5.15	5.50	5.45	4.00	4.95	4.95	4.99	5.90	5.91	4.61	5.00	5.02	5.00	5.80	5.82
	15	5.58	5.88	5.90	4.75	5.02	5.01	5.50	6.00	6.00	4.95	5.10	5.05	5.55	6.10	6.09
	25	5.58	5.85	5.90	4.80	5.02	5.05	5.55	6.05	6.06	4.90	5.05	5.08	5.05	6.10	6.12
28	0	5.05	5.20	5.45	4.75	5.29	5.30	5.35	6.35	6.36	5.00	5.82	5.80	5.55	6.45	6.49
	5	6.10	6.70	6.69	5.02	5.99	6.00	6.01	6.88	6.85	5.40	6.00	6.00	6.01	6.95	7.00
	15	6.78	6.90	6.91	5.88	6.13	6.10	6.80	7.15	7.10	5.95	6.19	6.10	6.15	7.20	7.20
	25	6.80	6.95	7.00	5.85	6.15	6.20	6.80	7.09	7.10	5.85	6.15	6.20	6.10	7.15	7.18
35	0	6.00	6.90	7.05	5.50	6.60	6.65	6.66	7.48	7.50	5.95	6.81	6.85	6.70	7.55	7.60
	5	8.00	8.70	8.71	6.10	6.95	6.90	7.05	8.15	8.10	6.35	7.01	7.00	7.30	8.25	8.20
	15	8.61	8.99	9.02	6.75	7.20	7.10	8.00	8.95	8.90	7.00	7.35	7.30	7.85	9.00	9.01
	25	8.55	9.02	9.05	6.75	7.20	7.22	8.05	8.91	8.90	6.95	7.25	7.30	7.80	8.95	8.93
42	0	7.10	8.15	8.25	6.20	7.80	7.82	7.50	8.88	8.90	6.99	7.92	7.95	7.50	8.99	9.00
	5	9.02	9.95	9.99	7.12	8.15	8.10	8.10	9.99	10.00	7.41	8.15	8.10	8.89	9.85	9.80
	15	9.99	10.25	10.20	7.90	8.41	8.40	9.51	10.41	10.25	8.01	8.55	8.45	9.96	10.49	10.45
	25	9.95	10.33	10.33	7.95	8.40	8.42	9.55	10.38	10.35	7.97	8.50	8.50	9.90	10.47	10.50
49	0	8.20	9.30	9.30	7.15	8.89	8.85	8.50	9.91	9.90	7.89	8.86	8.85	8.51	9.95	9.95
	5	10.50	11.05	11.05	8.81	9.50	9.50	9.75	11.05	11.00	8.39	9.25	9.20	9.95	11.10	11.08
	15	10.91	11.53	11.45	8.95	9.65	9.55	10.95	11.75	11.60	9.01	9.71	9.70	10.95	11.89	11.80
	25	10.95	11.60	11.59	9.00	9.70	9.70	11.00	11.72	11.70	8.95	9.69	9.65	10.90	11.77	11.75

56	0	9.20	10.70	10.63	8.05	9.95	9.94	9.65	11.00	11.00	9.40	9.95	9.94	9.70	11.05	11.00
	5	11.15	12.02	12.00	9.62	10.50	10.45	10.80	12.00	12.00	10.05	10.31	10.30	11.00	12.50	12.45
	15	11.85	12.29	12.20	9.98	10.78	10.60	11.65	12.50	12.15	10.00	10.97	10.95	12.00	12.77	12.75
	25	11.50	12.30	12.25	9.95	10.85	10.80	11.65	12.45	12.40	9.90	10.91	10.90	11.95	12.65	12.60
63	0	10.25	11.95	12.00	9.26	10.98	10.95	10.70	12.25	12.20	10.45	10.99	10.95	10.75	12.30	12.25
	5	11.99	12.99	12.95	10.50	11.55	11.50	12.00	13.45	13.40	11.50	11.85	11.82	12.15	13.55	13.50
	15	12.75	13.55	13.45	11.11	11.99	11.90	12.70	13.93	13.50	11.45	12.20	12.00	13.10	14.01	14.00
	25	12.70	13.52	13.50	11.70	12.05	12.03	12.69	13.90	13.90	11.45	12.15	12.00	13.05	13.95	13.90

Average body weight (gms) of *L.vannamei* fed with different experimental diets reared at 0 PPT, 5 PPT, 15 PPT and 25 PPT salinity

Treatment		FM			CSM			AI			SF			MM	
		FM 25	FM 30	FM 3.5	CSM 25	CSM 30	CSM 35	AI 25	AI 30	AI 35	SF 25	SF 30	SF 35	MM 25	MM 30
0	0	2.50	2.51	2.50	2.49	2.53	2.52	2.50	2.49	2.48	2.49	2.49	2.50	2.51	2.52
	5	2.52	2.50	2.49	2.48	2.50	2.51	2.51	2.49	2.48	2.48	2.50	2.51	2.49	2.50
	15	2.50	2.50	2.49	2.51	2.50	2.52	2.52	2.50	2.51	2.51	2.51	2.50	2.50	2.49
	25	2.53	2.53	2.52	2.52	2.49	2.53	2.51	2.52	2.50	2.52	2.50	2.51	2.51	2.48
7	0	2.99	3.40	3.44	2.55	2.95	2.99	2.88	3.15	3.17	2.77	2.95	3.00	2.90	3.17
	5	3.01	3.51	3.56	2.75	3.00	3.00	3.01	3.50	3.49	2.65	3.10	3.10	3.00	3.50
	15	3.60	3.62	3.58	3.05	3.15	3.15	3.51	3.75	3.76	3.00	3.19	3.15	3.02	3.77
	25	3.60	3.63	3.65	3.06	3.17	3.19	3.52	3.74	3.75	3.01	3.17	3.17	3.00	3.77
14	0	3.80	3.99	4.04	3.10	3.35	3.36	3.50	4.20	4.25	3.15	3.91	3.95	3.51	4.22
	5	4.11	4.65	4.60	3.55	3.95	3.96	4.12	4.55	4.50	3.55	4.05	4.07	4.01	4.45
	15	4.58	4.75	4.75	3.78	4.01	4.00	4.52	4.78	4.75	3.99	4.12	4.10	4.49	4.85
	25	4.53	4.78	4.80	4.04	4.05	4.10	4.54	4.77	4.80	4.00	4.10	4.12	4.50	4.80
21	0	4.15	4.15	4.25	3.90	4.15	4.19	4.44	5.25	5.30	3.99	4.71	4.75	4.45	5.28
	5	5.15	5.50	5.45	4.00	4.95	4.95	4.99	5.90	5.91	4.61	5.00	5.02	5.00	5.80
	15	5.58	5.88	5.90	4.75	5.02	5.01	5.50	6.00	6.00	4.95	5.10	5.05	5.55	6.10
	25	5.58	5.85	5.90	4.80	5.02	5.05	5.55	6.05	6.06	4.90	5.05	5.08	5.05	6.10
28	0	5.05	5.20	5.45	4.75	5.29	5.30	5.35	6.35	6.36	5.00	5.82	5.80	5.55	6.45
	5	6.10	6.70	6.69	5.02	5.99	6.00	6.01	6.88	6.85	5.40	6.00	6.00	6.01	6.95
	15	6.78	6.90	6.91	5.88	6.13	6.10	6.80	7.15	7.10	5.95	6.19	6.10	6.15	7.20
	25	6.80	6.95	7.00	5.85	6.15	6.20	6.80	7.09	7.10	5.85	6.15	6.20	6.10	7.15
35	0	6.00	6.90	7.05	5.50	6.60	6.65	6.66	7.48	7.50	5.95	6.81	6.85	6.70	7.55
	5	8.00	8.70	8.71	6.10	6.95	6.90	7.05	8.15	8.10	6.35	7.01	7.00	7.30	8.25
	15	8.61	8.99	9.02	6.75	7.20	7.10	8.00	8.95	8.90	7.00	7.35	7.30	7.85	9.00

	25	8.55	9.02	9.05	6.75	7.20	7.22	8.05	8.91	8.90	6.95	7.25	7.30	7.80	8.95
42	0	7.10	8.15	8.25	6.20	7.80	7.82	7.50	8.88	8.90	6.99	7.92	7.95	7.50	8.99
	5	9.02	9.95	9.99	7.12	8.15	8.10	8.10	9.99	10.00	7.41	8.15	8.10	8.89	9.85
	15	9.99	10.25	10.20	7.90	8.41	8.40	9.51	10.41	10.25	8.01	8.55	8.45	9.96	10.49
	25	9.95	10.33	10.33	7.95	8.40	8.42	9.55	10.38	10.35	7.97	8.50	8.50	9.90	10.47
49	0	8.20	9.30	9.30	7.15	8.89	8.85	8.50	9.91	9.90	7.89	8.86	8.85	8.51	9.95
	5	10.50	11.05	11.05	8.81	9.50	9.50	9.75	11.05	11.00	8.39	9.25	9.20	9.95	11.10
	15	10.91	11.53	11.45	8.95	9.65	9.55	10.95	11.75	11.60	9.01	9.71	9.70	10.95	11.89
	25	10.95	11.60	11.59	9.00	9.70	9.70	11.00	11.72	11.70	8.95	9.69	9.65	10.90	11.77
56	0	9.20	10.70	10.63	8.05	9.95	9.94	9.65	11.00	11.00	9.40	9.95	9.94	9.70	11.05
	5	11.15	12.02	12.00	9.62	10.50	10.45	10.80	12.00	12.00	10.05	10.31	10.30	11.00	12.50
	15	11.85	12.29	12.20	9.98	10.78	10.60	11.65	12.50	12.15	10.00	10.97	10.95	12.00	12.77
	25	11.50	12.30	12.25	9.95	10.85	10.80	11.65	12.45	12.40	9.90	10.91	10.90	11.95	12.65
63	0	10.25	11.95	12.00	9.26	10.98	10.95	10.70	12.25	12.20	10.45	10.99	10.95	10.75	12.30
	5	11.99	12.99	12.95	10.50	11.55	11.50	12.00	13.45	13.40	11.50	11.85	11.82	12.15	13.55
	15	12.75	13.55	13.45	11.11	11.99	11.90	12.70	13.93	13.50	11.45	12.20	12.00	13.10	14.01
	25	12.70	13.52	13.50	11.70	12.05	12.03	12.69	13.90	13.90	11.45	12.15	12.00	13.05	13.95

Table. No. 16 : The percentage of Survival in Water of Aquaria with
Litopenaeus Vannamei reared in 0 PPTs Salinity

Treatment Days		FM			CSM			AI			SF			MM		
		FM 25	FM 30	FM 35	CSM 25	CSM 30	CSM 35	AI 25	AI 30	AI 35	SF 25	SF 30	SF 35	MM 25	MM 30	MM 35
0	0	100	100	100	100	95	95	100	100	100	95	95	95	100	100	100
	5	100	100	100	95	95	95	100	100	100	95	95	95	100	100	100
	15	100	100	100	100	95	95	100	100	100	100	95	95	100	100	100
	25	100	100	100	95	95	95	100	100	100	100	100	100	100	100	100
7	0	100	100	100	95	85	90	100	95	95	95	90	95	100	100	100
	5	100	100	100	95	95	95	100	100	100	95	95	95	100	100	100
	15	100	100	100	95	95	95	100	100	100	95	95	95	100	100	100
	25	100	100	100	90	90	95	100	100	100	100	95	95	100	100	100
14	0	95	95	95	85	80	90	95	90	90	90	90	90	100	100	100
	5	100	100	100	95	90	90	100	100	100	95	90	90	100	100	100
	15	100	100	100	95	90	90	100	95	95	90	90	90	95	95	95
	25	100	100	100	90	85	90	95	95	95	100	90	95	100	100	100
21	0	95	95	95	80	75	85	90	90	90	90	85	85	100	95	95
	5	100	100	100	90	85	85	95	95	100	90	85	85	100	95	95
	15	100	100	100	90	85	85	95	95	95	85	85	90	95	95	95
	25	100	100	100	85	85	85	95	95	90	95	90	90	100	100	100
28	0	95	95	95	75	70	75	90	90	90	85	80	80	95	95	95
	5	95	95	100	85	85	85	95	95	95	85	80	85	95	95	95
	15	100	100	100	90	85	85	95	95	95	85	85	85	95	95	95
	25	100	100	100	85	80	85	90	95	90	90	85	90	95	95	100
35	0	95	90	90	70	65	70	85	85	90	80	75	80	95	90	90
	5	95	95	95	80	80	85	95	95	95	80	75	80	95	95	95
	15	100	100	100	85	80	80	95	90	90	80	80	80	95	95	95
	25	100	100	100	80	80	80	90	95	90	85	85	85	95	95	95
42	0	95	90	90	65	65	65	85	85	85	75	70	75	90	90	90
	5	95	95	95	80	75	80	95	90	90	75	70	75	95	95	95
	15	100	95	95	80	75	75	90	90	90	75	75	75	95	95	95
	25	100	100	100	75	75	75	90	95	90	80	80	80	95	95	95
49	0	90	90	90	60	60	60	85	85	80	70	65	70	90	85	85
	5	95	95	95	75	70	70	90	90	90	70	65	70	95	85	90
	15	95	95	95	75	70	70	90	90	90	70	70	70	90	90	95
	25	100	100	100	75	75	75	90	95	90	80	80	80	95	90	90

56	0	90	90	90	60	60	60	90	80	80	65	65	65	85	85	85
	5	90	90	90	60	60	60	80	85	85	65	65	65	90	85	85
	15	95	95	95	70	65	70	85	85	85	70	65	65	90	85	85
	25	100	100	100	75	70	70	90	90	90	80	75	75	90	90	90
63	0	85	85	85	60	55	55	80	80	80	65	60	60	80	80	80
	5	90	90	90	65	60	65	85	80	80	60	60	60	85	80	80
	15	95	95	95	65	60	60	85	85	85	65	65	60	85	85	85
	25	100	100	100	70	65	65	85	90	85	80	70	70	90	90	90

Table. No. 14 : Specific Growth Rate (SGR) in Water of Aquaria with *Litopenaeus Vannamei* reared in 0 PPTs Salinity

Treatment Days		FM			CSM			AI			SF			MM		
		FM 25	FM 30	FM 35	CSM 25	CSM 30	CSM 35	AI 25	AI 30	AI 35	SF 25	SF 30	SF 35	MM 25	MM 30	MM 35
0	0	2.50	2.51	2.50	2.49	2.53	2.52	2.50	2.49	2.48	2.49	2.49	2.50	2.51	2.52	2.51
	5	2.52	2.50	2.49	2.48	2.50	2.51	2.51	2.49	2.48	2.48	2.50	2.51	2.49	2.50	2.50
	15	2.50	2.50	2.49	2.51	2.50	2.52	2.52	2.50	2.51	2.51	2.51	2.50	2.50	2.49	2.49
	25	2.53	2.53	2.52	2.52	2.49	2.53	2.51	2.52	2.50	2.52	2.50	2.51	2.51	2.48	2.49
7	0	0.49	0.89	0.92	0.06	0.42	0.47	0.38	0.66	0.69	0.28	0.46	0.50	0.39	0.05	0.66
	5	0.51	1.00	1.07	0.23	0.50	0.49	0.50	1.01	0.01	0.17	0.60	0.53	0.52	1.00	0.99
	15	1.10	1.11	1.09	0.54	0.65	0.63	0.99	1.22	1.25	0.49	0.68	0.65	0.52	1.28	1.26
	25	1.07	1.11	1.13	0.54	0.65	0.66	1.01	1.24	1.25	0.49	0.67	0.66	0.49	1.29	1.31
14	0	0.81	0.59	0.60	0.55	0.40	0.37	0.62	1.05	1.08	0.38	0.96	0.95	0.61	1.05	1.08
	5	0.98	1.14	1.17	0.73	0.95	0.85	1.01	1.05	0.99	0.99	0.95	0.95	1.47	0.95	1.08
	15	1.11	1.13	1.04	0.80	0.86	0.96	1.10	1.04	1.01	0.90	0.93	0.97	1.01	1.08	0.96
	25	0.93	1.15	1.15	0.98	0.88	0.91	1.02	1.03	1.05	0.99	0.93	0.95	1.50	1.03	1.05
21	0	0.35	0.16	0.21	0.80	0.80	0.83	0.94	1.05	1.05	0.84	0.80	0.80	0.94	1.06	1.05
	5	1.00	0.85	1.15	0.97	1.00	0.19	0.58	1.35	1.25	0.96	0.95	0.95	1.06	1.35	1.26
	15	1.04	1.13	0.85	0.45	1.01	0.99	0.87	1.22	1.91	1.06	0.98	0.95	0.99	1.25	1.37
	25	1.05	1.07	1.10	0.76	0.97	0.99	1.01	1.28	1.26	0.90	0.95	0.96	1.00	1.30	1.27
28	0	0.90	1.07	1.10	0.76	0.97	0.99	1.01	1.10	1.06	1.01	1.11	1.05	1.10	1.17	1.19
	5	1.20	1.20	1.01	1.13	1.04	1.91	1.30	0.98	1.10	1.01	1.11	1.05	1.10	1.17	1.19
	15	0.95	1.10	1.29	1.02	1.13	1.05	1.02	1.04	0.99	1.00	1.00	1.05	0.60	1.15	1.11
	25	1.22	2.07	1.10	1.05	1.05	1.15	1.25	1.82	1.04	0.95	1.10	1.12	0.60	1.80	1.06
35	0	0.95	1.7	1.6	0.75	1.31	1.35	1.31	1.13	1.14	0.95	0.99	1.05	1.15	1.10	1.11
	5	1.83	2.00	2.11	0.87	0.96	1.00	1.20	1.27	1.80	1.05	1.01	1.20	1.70	1.30	1.81
	15	1.90	2.09	2.02	1.08	1.07	0.90	1.04	1.80	1.25	0.95	1.16	1.00	1.29	1.80	1.20
	25	1.75	2.07	2.05	0.90	1.05	1.02	1.25	1.82	1.80	9.10	1.10	1.10	1.10	1.80	1.81
42	0	1.70	1.25	1.20	0.70	1.20	1.17	0.84	1.40	1.40	1.04	1.11	1.10	0.80	1.44	1.40
	5	1.38	1.20	1.18	1.15	1.20	1.30	1.51	1.84	1.35	1.01	1.14	1.15	2.11	1.60	1.44
	15	1.02	1.26	1.28	1.02	1.21	1.20	1.05	1.46	1.90	1.06	1.12	1.10	1.59	1.49	1.60
	25	1.40	1.31	1.28	1.20	1.20	1.20	1.50	1.47	1.45	1.02	1.25	1.20	2.10	1.52	1.51
49	0	1.10	1.15	1.05	0.95	1.09	1.03	1.00	1.03	1.00	0.90	0.94	0.90	1.01	0.96	0.95
	5	0.92	1.10	1.25	1.05	1.35	1.15	1.44	1.06	1.35	1.00	1.10	1.25	0.99	1.25	1.35
	15	1.48	1.28	1.06	1.69	1.24	1.90	1.68	1.34	1.00	0.98	1.16	1.10	1.06	1.40	1.28
	25	1.10	1.27	1.26	1.05	1.30	1.28	1.45	1.34	1.35	0.98	1.19	1.15	1.00	1.30	1.25
56	0	1.00	1.27	1.26	1.05	1.30	1.28	1.45	1.09	1.10	1.06	1.09	1.09	1.19	1.10	1.05

	5	0.94	0.97	0.75	1.03	1.00	1.05	0.70	0.95	0.55	1.04	1.06	1.25	1.05	0.40	0.95
	15	0.65	0.76	0.95	0.81	1.13	0.95	1.08	0.75	1.00	1.01	1.08	1.10	1.05	0.88	1.37
	25	0.55	0.70	0.66	0.95	1.15	1.10	0.65	0.73	0.70	1.05	1.22	1.25	1.05	1.25	1.25
63	0	1.05	1.25	1.37	1.21	1.03	1.01	1.05	1.25	1.20	0.95	1.04	1.01	1.05	1.25	1.25
	5	0.90	9.97	1.25	1.13	1.04	1.30	1.05	1.45	1.35	1.45	1.54	1.05	1.10	2.05	1.25
	15	0.84	1.26	0.95	0.88	1.21	1.05	1.20	1.43	1.90	1.05	1.23	1.52	1.15	1.24	1.05
	25	1.20	1.22	1.25	1.75	1.20	1.23	1.05	1.43	1.50	1.45	1.24	1.10	1.10	1.30	1.30

**Table No. 7 : Total Hardness (mg/l) in the water of aquaria with
L.vannamei (White leg Shrimp) reared under 0 ppt, 5 PPT, 15 PPT and 25 PPT Salinity :**

Treatment Days	FM			CSM			AI			SF			MM			
	FM 25	FM 30	FM 35	CSM 25	CSM 30	CSM 35	AI 25	AI 30	AI 35	SF 25	SF 30	SF 35	MM 25	MM 30	MM 35	
0	0	191	194	194	194	195	196	195	195	195	199	195	198	199	197	199
	5	241	240	241	245	242	242	251	250	250	252	250	250	251	251	252
	15	601	606	605	606	609	610	612	611	611	606	610	607	611	610	612
	25	700	701	700	705	707	707	702	702	702	700	699	710	710	707	708
7	0	202	199	200	199	202	200	201	202	202	205	207	207	208	210	211
	5	249	245	249	241	240	241	257	259	258	249	250	248	252	252	252
	15	609	610	617	617	610	616	615	615	616	616	616	620	619	620	620
	25	705	710	709	710	713	713	713	715	719	719	715	718	719	719	720
14	0	212	202	210	202	203	205	210	210	209	209	210	212	213	215	215
	5	279	264	284	282	280	288	288	289	289	288	285	288	283	280	288
	15	620	619	620	621	622	623	629	630	635	631	629	619	629	631	635
	25	721	718	719	721	722	725	727	725	725	725	722	722	731	735	739
21	0	202	205	205	206	210	207	208	211	210	210	211	210	220	214	220
	5	281	280	281	284	282	281	287	282	288	289	285	291	298	289	300
	15	621	622	625	624	625	626	623	631	631	635	635	636	639	636	635
	25	721	725	722	725	725	725	730	729	732	735	733	735	740	736	739
28	0	209	208	208	210	210	210	211	210	209	215	215	214	215	216	217
	5	279	268	281	285	281	288	290	285	288	293	209	299	297	289	303
	15	631	630	631	632	631	632	635	635	631	639	639	640	651	641	651
	25	735	731	729	729	733	731	735	731	733	739	741	745	747	745	750
35	0	213	215	215	216	214	214	317	213	218	215	215	216	217	218	217
	5	290	285	291	292	259	299	291	281	293	285	285	286	2897	289	287
	15	634	631	635	635	634	639	640	640	642	641	641	641	649	644	645
	25	735	731	731	735	736	739	740	740	741	749	744	745	751	749	751
42	0	215	214	215	217	214	217	218	218	218	218	219	222	222	220	220

	5	288	289	289	291	281	290	281	283	281	288	241	288	291	242	300	
	15	639	635	635	637	637	635	641	645	642	645	645	647	649	650	649	
	25	735	733	737	739	739	741	745	744	745	749	745	751	753	750	755	
49	0	216	215	216	220	219	222	220	222	221	225	225	226	227	226	229	
	5	288	269	288	291	263	280	281	285	288	288	284	287	291	245	299	
	15	641	639	642	642	641	643	647	649	647	651	651	652	656	655	655	
	25	741	741	739	744	745	747	751	751	755	749	749	751	751	755	753	
56	0	220	219	220	222	222	220	221	225	221	225	221	221	221	228	229	229
	5	292	291	298	290	285	289	291	289	297	288	285	287	299	299	305	
	15	641	639	644	645	645	647	649	650	651	653	655	655	662	660	666	
	25	749	745	745	748	749	751	753	755	751	751	751	753	761	760	763	
63	0	225	222	220	221	233	230	235	230	235	230	231	235	238	233	237	
	5	300	305	310	307	305	310	311	301	315	305	300	309	317	351	322	
	15	641	639	644	644	650	645	661	666	666	669	670	671	673	671	675	
	25	751	749	751	752	750	748	760	756	761	769	769	770	771	776	777	

Table No. 15: Feed Conversion Ratio of L.vannamei (White leg shrimp) fed with different diets in water of aquaria 0 ppt, 5 ppt, 15 ppt and 25 ppt salinities

Treatment	FM			CSM			AI			SF			MM			
	FM 25	FM 30	FM 35	CSM 25	CSM 30	CSM 35	AI 25	AI 30	AI 35	SF 25	SF 30	SF 35	MM 25	MM 30	MM 35	
Days																
	0	1.98	1.85	1.91	2.50	2.45	2.50	2.10	1.95	2.05	2.50	2.23	2.30	2.03	1.91	2.00
	5	1.79	1.75	1.77	2.44	2.30	2.35	2.00	1.90	1.92	2.45	2.15	2.25	1.95	1.88	1.90
	15	1.66	1.59	1.62	2.40	2.22	2.25	1.93	1.80	1.85	2.38	2.10	2.15	1.83	1.78	1.85
	25	1.58	1.45	1.60	2.31	2.10	2.22	1.81	1.70	1.75	2.30	1.98	2.01	1.69	1.60	1.62

