

**EVALUATION OF COTTONSEED MEAL AND *ACETES INDICUS*
MEAL AS REPLACEMENTS TO FISH MEAL IN
LITOPENAEUS VANNAMEI DIETS REARED UNDER VARYING
WATER SALINITIES**

BY

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CERTIFICATE

This is to certify that the thesis entitled “**EVALUATION OF COTTONSEED MEAL AND ACETES INDICUS MEAL AS REPLACEMENTS TO FISH MEAL IN *LITOPENAEUS VANNAMEI* DIETS 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.PRAMODA MALLI. G.J.**, I.D.No.MFM/2012-005 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. G.J.PRAMODA MALLI**, hereby declare that the thesis entitled **“EVALUATION OF COTTONSEED MEAL AND ACETES INDICUS MEAL AS REPLACEMENTS TO FISH MEAL IN *LITOPENAEUS VANNAMEI* DIETS 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: 01-12-2014

(**G.J.PRAMODA MALLI**)

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Dedicated to my Mom & Dad

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CONTENTS

Chapter No.	Title	Page No.
1	INTRODUCTION	1-10
2	REVIEW OF LITERATURE	11-41
3	MATERIALS AND METHODS	42-52
4	RESULTS	53-136
5	DISCUSSION	137-155
6	SUMMARY	156-159
7	REFERENCES	160-185

LIST OF TABLES

Table No.	Title	Page No.
1	Feed formulation of the diets used in the experiment	44
2	Proximate composition of the various ingredients used in formulated feeds	45
3	Proximate composition of the formulated feeds used in the experiment	45
4	Dissolved Oxygen (ppm) in the water of aquaria with <i>L. vannamei</i> (white leg shrimp) reared at 5ppt Salinity	73
5	Temperature (°C) in water of aquaria with <i>L. vannamei</i> (white leg shrimp) reared at 5ppt Salinity	74
6	p ^H in water of aquaria with <i>L. vannamei</i> (white leg shrimp) reared at 5ppt Salinity	75
7	Total alkalinity (mg/l) in water of aquaria with <i>L. vannamei</i> (white leg shrimp) reared at 5ppt Salinity	76
8	Total Hardness (mg/l) in water of aquaria with <i>L. vannamei</i> (white leg shrimp) reared at 5ppt Salinity	77
9	Dissolved Oxygen (ppm) in water of aquaria with <i>L. vannamei</i> (white leg shrimp) reared at 15ppt Salinity	78
10	Temperature (°C) in water of aquaria with <i>L. vannamei</i> (white leg shrimp) reared at 15ppt Salinity	79
11	p ^H in water of aquaria with <i>L. vannamei</i> (white leg shrimp) reared at 15ppt Salinity	80
12	Total alkalinity (mg/l) in water of aquaria with <i>L. vannamei</i> (white leg shrimp) reared at 15ppt Salinity	81
13	Total Hardness (mg/l) in water of aquaria with <i>L. vannamei</i> (white leg shrimp) reared at 15ppt Salinity	82
14	Dissolved Oxygen (ppm) in water of aquaria with <i>L. vannamei</i> (white leg shrimp) reared at 30ppt Salinity	83

15	Temperature (°C) in water of aquaria with <i>L. vannamei</i> (white leg shrimp) reared at 30ppt Salinity	84
16	p ^H in water of aquaria with <i>L. vannamei</i> (white leg shrimp) reared at 30ppt Salinity	85
17	Total alkalinity (mg/l) in water of aquaria with <i>L. vannamei</i> (white leg shrimp) reared at 30ppt Salinity	86
18	Total Hardness (mg/l) in water of aquaria with <i>L. vannamei</i> (white leg shrimp) reared at 30ppt Salinity	87
19	The percentage of survival of <i>L. vannamei</i> (white leg shrimp) fed with different experimental diets reared at 5ppt Salinity	88
20	Statistical Analysis for percentage of survival of <i>L. vannamei</i> (white leg shrimp) fed with different experimental diets reared at 5ppt Salinity	89
21	Growth performance in water of aquaria with <i>L. vannamei</i> (white leg shrimp) reared at 5ppt Salinity	90
22	Statistical Analysis for growth performance of <i>L. vannamei</i> (white leg shrimp) fed with different experimental diets reared at 5ppt Salinity	91
23	Weight gain in <i>L. vannamei</i> (white leg shrimp) fed on different experimental diets reared at 5 Salinity	92
24	Statistical Analysis of Weight gain <i>L. vannamei</i> (white leg shrimp) fed with different experimental diets reared in 5 Salinity	93
25	Specific growth rates (%) in <i>L. vannamei</i> (white leg shrimp) fed on different experimental diets reared at 5 Salinity	94
26	The percentage of survival of <i>L. vannamei</i> (white leg shrimp) fed with different experimental diets reared at 15 Salinity	95
27	Statistical Analysis of survival percentage of <i>L. vannamei</i> (white leg shrimp) fed with different experimental diets reared at 15 Salinity	96
28	Growth performance of <i>L. vannamei</i> (White leg shrimp) fed with different experimental diets reared in 15 Salinity	97
29	Statistical Analysis of growth performance of <i>L. vannamei</i> (white leg shrimp) fed with different experimental diets reared in 15 Salinity	98
30	Weight gain in <i>L. vannamei</i> (white leg shrimp) fed on different experimental diets reared at 15 Salinity	99

31	Statistical Analysis of Weight gain in <i>L. vannamei</i> (white leg shrimp) fed with different experimental diets reared in 15 Salinity	100
32	Specific growth rates (%) in <i>L. vannamei</i> (white leg shrimp) fed on different experimental diets reared at 15 Salinity	101
33	The percentage of survival of <i>L. vannamei</i> (white leg shrimp) fed with different experimental diets reared at 30 Salinity	102
34	Statistical Analysis of survival percentage of <i>L. vannamei</i> (white leg shrimp) fed with different experimental diets reared at 30 Salinity	103
35	Growth performance of <i>L. vannamei</i> (white leg shrimp) fed with different experimental diets reared in 30 Salinity	104
36	Statistical Analysis of growth performance <i>L. vannamei</i> (white leg shrimp) fed with different experimental diets reared in 30 Salinity	105
37	Weight gain in <i>L. vannamei</i> (white leg shrimp) fed on different experimental diets reared at 30 Salinity	106
38	Statistical Analysis of Weight gain <i>L. vannamei</i> (white leg shrimp) fed with different experimental diets reared in 30 Salinity	107
39	Specific growth rates (%) in <i>L. vannamei</i> (white leg shrimp) fed on different experimental diets reared at 30 Salinity	108
40	Feed Conversion Ratio's of <i>L. vannamei</i> (white leg shrimp) fed on different experimental diets reared in 5, 15, and 30 Salinities	109

LIST OF ILLUSTRATIONS

Figure No.	Title	Page No.
1	Dissolved Oxygen (ppm) in the water of aquaria with <i>L. vannamei</i> (White leg shrimp) reared at 5 Salinity	110
2	Temperature (°C) of water in aquaria with <i>L. vannamei</i> (White leg shrimp) reared at 5 Salinity	111
3	p ^H of water in aquaria with <i>L. vannamei</i> (White leg shrimp) reared at 5 Salinity	112
4	Total alkalinity (mg/l) in the water of aquaria with <i>L. vannamei</i> (White leg shrimp) reared at 5 Salinity	113
5	Total Hardness (mg/l) in the water of aquaria with <i>L. vannamei</i> (white leg	114

shrimp) reared at 5 Salinity

6	Dissolved Oxygen (ppm) in the water of aquaria with <i>L. vannamei</i> (White leg shrimp) reared at 15 Salinity	115
7	Temperature (°C) of water in aquaria with <i>L. vannamei</i> (White leg shrimp) reared at 15 Salinity	116
8	p ^H of water in aquaria with <i>L. vannamei</i> (White leg shrimp) reared at 15 Salinity	117
9	Total alkalinity (mg/l) in the water of aquaria with <i>L. vannamei</i> (White leg shrimp) reared at 15 Salinity	118
10	Total Hardness (mg/l) in the water of aquaria with <i>L. vannamei</i> (white leg shrimp) reared at 15 Salinity	119
11	Dissolved Oxygen (ppm) in the water of aquaria with <i>L. vannamei</i> (White leg shrimp) reared at 30 Salinity	120
12	Temperature (°C) of water in aquaria with <i>L. vannamei</i> (White leg shrimp) reared at 30 Salinity	121
13	p ^H of water in aquaria with <i>L. vannamei</i> (White leg shrimp) reared at 30 Salinity	122
14	Total alkalinity (mg/l) in the water of aquaria with <i>L. vannamei</i> (White leg shrimp) reared at 30 Salinity	123
15	Total Hardness (mg/l) in the water of aquaria with <i>L. vannamei</i> (white leg shrimp) reared at 30 Salinity	124
16	The percentage of survival of <i>L. vannamei</i> (White leg shrimp) fed with different experimental diets reared at 5 Salinity	125
17	Growth performance of <i>L. vannamei</i> (White leg shrimp) fed with different experimental diets reared in 5 Salinity	126
18	Weight gain in <i>L. vannamei</i> (White leg shrimp) fed on different experimental diets reared at 5 Salinity	127
19	Specific growth rates (%) in <i>L. vannamei</i> (White leg shrimp) fed on different experimental diets reared at 5 Salinity	128
20	The percentage of survival of <i>L. vannamei</i> (White leg shrimp) fed with different experimental diets reared at 15 Salinity	129

21	Growth performance of <i>L. vannamei</i> (White leg shrimp) fed with different experimental diets reared in 15 Salinity	130
22	Weight gain in <i>L. vannamei</i> (White leg shrimp) fed on different experimental diets reared at 15 Salinity	131
23	Specific growth rates (%) in <i>L. vannamei</i> (White leg shrimp) fed on different experimental diets reared at 15 Salinity	132
24	The percentage of survival of <i>L. vannamei</i> (White leg shrimp) fed with different experimental diets reared at 30 Salinity	133
25	Growth performance of <i>L. vannamei</i> (white leg shrimp) fed with different experimental diets reared in 30 Salinity	134
26	Weight gain in <i>L. vannamei</i> (white leg shrimp) fed on different experimental diets reared at 30 Salinity	135
27	Specific growth rates (%) in <i>L. vannamei</i> (white leg shrimp) fed on different experimental diets reared at 30 Salinity	136

LIST OF PLATES

Plate No.	Title	Page No.
1	<i>Litopenaeus vannamei</i> (White leg shrimp)	10
2	Fish meal	16
3	Cottonseed Meal	27
4	<i>Acetes indicus</i>	32
5	<i>Acetes indicus</i> - wet position	33
6	<i>Acetes indicus</i> - dry position	33
7	Experimental set-up in the wet-laboratory	
	7.1. Partial set up of experimental animals reared at 5 Salinity	50
	7.2. Partial set up of experimental animals reared at 15 Salinity	50
	7.3. Partial set up of experimental animals reared at 30 Salinity	51
8	Formulated feeds used in experiments	51
9	White leg (<i>L. vannamei</i>) shrimp length measurement	52
10	White leg (<i>L. vannamei</i>) shrimp weight measurement	52

ABBREVIATIONS

FM	Fish Meal
CSM	Cottonseed Meal
AI	<i>Acetes indicus</i> Meal
T	Treatment
°C	Degree Centigrade
cm	Centimeter
mg/l	Milligram per liter
Statistical Analysis	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

Abstract

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The present study “Evaluation of Cottonseed meal and *Acetes indicus* meal as replacements to fish meal in *Litopenaeus vannamei* diets 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) *Acetes indicus* (AI) meal, 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 and *Acetes indicus* meal at three different salinities 5ppt, 15ppt and 30ppt 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 DO was found to be in the range of 5.02ppm and 8.42ppm at 5ppt, 6.29ppm to 8.52ppm at 15ppt and 7.2ppm to 8.99ppm at 30ppt salinity. The pH ranged from 8.0 to 8.3 at 5ppt, 8.1 to 8.3 at 15ppt and 8.3 to 8.5 at 30ppt, during the period of experiment. The range of total alkalinity varied between 150 mg/l to 340 mg/l at 5ppt salinity, 612mg/l to 632 mg/l at 15ppt salinity and 660mg/l to 68mg/l at 30ppt salinity. Total hardness of water ranged between 225 mg/l to 237 mg/l at 5ppt salinity, 660mg/l to 684 mg/l at 15ppt salinity and 61mg/l to 743mg/l at 30ppt salinity.

The growth performance of *L. vannamei* was found to be best when fed with diet AI30 (11.05gm) and the least with CSM25 (9.95gm) at 5ppt salinity, AI30 (12.01gm) and CSM25 (10.01gm) at 15ppt salinity, AI30 (12.22gm) and CSM25 (10.00gm) at 15ppt salinity. *L. vannamei* fed on diet AI30 showed the highest average weight gain of 1.38gm by the 63rd while the FM25 recorded the lowest weight gain of all the treatments (1.15gm) at 5ppt salinity. AI30 (2.02gm) and CSM25 (1.12gm) recorded the highest and lowest average weight gain at 15ppt salinity. AI30 (2.21gm) and CSM35 (0.41gm) recorded highest and lowest average weight gain at 30ppt salinity. Specific growth rate was highest (3.159) in shrimps fed with diet AI30, and least (2.930) in the CSM25 at 5ppt salinity. In 15ppt salinity, Specific growth rate was highest (3.302) in shrimps fed with diet AI30, and least (2.960) in the CSM25. Similarly, Specific growth rate was highest (3.287) in shrimps fed with diet AI30, and least (2.969) in the CSM25 at 30ppt salinity. Survival rates were best in shrimps fed on FM30 and FM35, which showed 78% survival rate at 5ppt salinity, FM35 showed 88% survival rate at 15ppt salinity and FM30 and FM35 showed 85% survival rate at 30ppt salinity at the end of the experiment. The shrimps fed with CSM25 and CSM30 diets showed the least survival rate (65-75%). The shrimps fed on FM30 diet showed the best FCR of 1.66 followed by

FM30 with an FCR of 1.72. The shrimps fed with CSM25 showed the least response with an FCR of 2.95

From the above studies, it can be concluded that Cottonseed meal and *Acetes indicus* meal can be included in the diets of *L. vannamei* up to 25% protein inclusion for the total replacement of fish meal.



1. Introduction

Global consumption of shrimp and fish has been increasingly 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. 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. (Amaya *et al.*, 2007b). The world production of farmed shrimp has expanded rapidly over the past two decades and these trends are expected to continue as global population increases and demand for quality sea food continues to rise (FAO, 2003).

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, *P.chinensis*; and the white leg shrimp, *L.vannamei*).

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 *L. vannamei* has a faster growth, higher stocking rate and yield, and incurs lower production costs than *P. monodon*.

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, the United States, and the other countries (Boyd and Thunjai, 2003).

Based on the results, *L. vannamei* species can be considered as a good source of fatty acid as well as protein. *L. 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). *L. vannamei* are easier to reproduce than *P.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. (Van Wyk *et al.*, 1999; Mc Graw *et al.*, 2002; Saoud *et al.*, 2003; Sowers and Tomasso, 2006) have studied the growth of *L. vannamei* in different salinities. The Government of India (GOI) permitted pilot-scale introduction of the species in 2003 and subsequently permitted the culture of *L. 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 *L.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 *L.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). 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* 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.

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.

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 (Amit 2012).

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 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.

Under aquaculture conditions shrimp are fed manufactured balanced diets which typically contain approximately 25–35% fishmeal (Tacon and Barg, 1998). A steadily increasing proportion of global fish meal production is being utilized in aquatic animal feed production; in 2003 about 53% went to produce fish and shrimp feeds (FAO, 2006). Traditionally, fish meal is the preferred dietary protein source for many farmed fish species and is appreciated for its amino acid balance, vitamin content, palatability, and unidentified growth factors.

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 Nino in 2006 and tight food regulations from CEP nations combined with continuing strong demand, particularly from 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 Gov. 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 effect 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.

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 & Dominy 1991). The replacement of fish meal with cottonseed meal in shrimp diets has met with varying degrees of success. Quite often, poor responses to high cottonseed meal diets are either due to shifts in the nutrient profile or a reduced palatability of the diet when fish meal is removed.

Due to its 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.

However, due to potential problems associated with insufficient levels of indispensable amino acids (e.g., lysine and methionine), anti-nutritional factors and poor palatability, commercial use is often limited.

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 protein. *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* may be considered to be a suitable ingredient 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).

The culture of marine shrimp primarily occurs in near-coastal areas using waters of estuarine and oceanic origin. Due to variety of social, economic and environmental issues the

inland culture of marine shrimp has been expanding in a number of countries. Commercial production of marine shrimp in US inland low salinity waters has been occurring for about 15 years.

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 cottonseed 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, plant protein and *Acetes indicus*, animal protein on the growth performance and feed efficiency in *L.vannamei* reared under different salinities.

2. Review of Literature

2.1. Protein:

Protein requirement has been defined by, Guillaume (1997), 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.

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 (Millward 1989). 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.

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; Guillaume, 1997). 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.

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.

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).

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.

2.2. Protein and *Litopenaeus vannamei*:

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.* (1997) 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, lack of antinutrients and high palatability. 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.

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 (Chamberlain, 1993, Tacon and Akiyama, 1997).

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).

Based on reports by Tacon *et al.*, (2006) marine shrimp feed uses 22.8% of the fish meal used in aquaculture feeds. Commercially shrimp formulation historically contain between 25% to 50% fish meal, representing the primary and most expensive protein ingredient (Derjant-Li, 2002; Tacon and Barg, 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). 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.

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. Fish meals, as well as other marine meals such as krill, shrimp, squid, and scallop waste, are often included in aquatic feeds as they are considered an excellent source of high quality proteins, highly unsaturated fatty acids, vitamins, minerals and attractants (Tacon and Akiyama, 1997). Due to these properties fish meal has become one of the primary component of commercial feed formulations. The demand for fish meal in aquatic feeds has been estimated to account for 31% to 42.5 % of total world fish meal production (Tacon and Barg, 1998). Of this, marine shrimp consumed about 17.6 % of the total amount.



Plate

.2:

Fish

Meal

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. 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, Klesius, and Dominy 1998). 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), and it appears that there is a little overlap between the markets for food fish and reduction fish at present. As such, it is unlikely that fishmeal price increases would immediately result in large scale conversion of low-value food fish to fishmeal, thus robbing food fish from the diets of the world's poor.

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.

2.4. Replacement of Fish Meal:

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. 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. 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. Industry research must not only measure the effect of replacement feed ingredients on standard farming parameters like growth, survival rates and FCR, but also their impact of other factors such as immune function and disease resistance. 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. (Schippe *et al.*, 2008)

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. 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). 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).

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. Many plant and animal proteins have some potential as fish meal replacers.

Tacon (FAO, 1994) 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.

By 2006, an estimate 68% of the global fish meal production was utilized for compound aquaculture feeds (Tacon and Metian, 2008). 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.

Fish meal inclusion levels in diets have been decreasing due to a combination of factors including static or diminishing global supplies, increasing market price and rising pressure by society and retailers to improve the sustainability of fisheries resources (Tacon and Metian, 2008). At the current fish meal replacement rate, up to 3 million mt of alternate protein sources are estimated to be required to sustain aqua feed production in next five years.

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).

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 cottonseed meal are commonly utilised. Usually the use of plant proteins shows some limitation due to a variety of factors including a deficiency or imbalance of essential amino acids, presence of anti nutritional factors or toxins, and decreased palatability.

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).

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).

According to Amaya *et al.*, (2007a), 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.

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 met with different degrees of success.

Considerable 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 mung bean, rice bean and leaf meals Eusebio, 1991; Eusebio and Coloso, 1998., and papaya or camote leaf meal Penaflorida, 1995.. 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.

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. It has been demonstrated that these essential fatty acids are required in the diets of crustaceans at levels between 0.5 % and 1 % for penaeid shrimp (for reviews see Lim and Akiyama, 1995).

On account of these constraints, replacement of fish meal and other marine proteins with alternative sources of proteins such as those derived from terrestrial animals or plant protein have been encouraged (Tacon and Akimaya, 1997). These feed stuffs are characterized by higher production than fish meals and less cost, but they are of variable composition, their nutritional quality is lower than fish meals, they have possible palatability problems, and possible microbial contamination. Other sources of protein for aquatic diets are plant proteins (Li *et al.*,2000) .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, Webster, Yancey, and D Abramo, 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*.

2.6. Cottonseed Meal – *Gossypim hirstium*:

Replacement of marine protein sources in practical diets for *L. vannamei* has been less successful.

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. Higher levels of replacement with cottonseed meal resulted in a reduction of shrimp growth, presumably due to gossypol content of cottonseed meal.

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. 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. Taura-resistant line of *L. vannamei* stocked at 72 shrimp/ m³ evaluated the ability of three glandless cottonseed meals that ranged from 51.9 to 61.2% crude protein, depending on processing technique, to replace approximately 50% of the fishmeal protein in a 35%-crude protein diet. 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%).



Plate.3: Cottonseed Meal

Above all, 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 Chhorn 1996) and are available at much lower costs than animal proteins. In addition, the use of by-products is a dictate of environmental sustainability, particularly with regard to the declining water resources in the eastern Mediterranean region. 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. (Rinchard *et al.*, 2003; Yue and Zhou, 2008)

Cottonseed meal contains the toxic pigment gossypol and is low in available lysine; it has been used to partially replace soybean meal in catfish feeds. (Dorsa *et al.*, 1982; Robinson and Brent 1989). A combination of soybean meal, cottonseed meal, and supplemental lysine could be used to replace fish meal in feeds for grow-out catfish raised at high densities in earthen ponds and fed to satiation. Solvent extracted cottonseed meal can be used in catfish diets Robinson 1991 b, as well as this study show that feeds containing cottonseed meal that were thought to be deficient in available lysine did not reduce weight gain or feed efficiency.

Gossypol is a naturally occurring polyphenolic compound 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). 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.

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. Our previous 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. (Richardson *et al.*, 2013)

Lim (1996) included 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.

The shrimp body tended to show a yellow-green discoloration, probably due to accumulation of free gossypol.

Fernandez and Lawrence (1988) observed 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. 8% alternative set of final body weight, weight gain; specific growth rate was the highest, lowest feed conversion ratio, no significant difference between groups. Based on the previous findings, a maximum level of inclusion of 25 % of cottonseed meal in crustacean diets should be considered. (Wang 2010), 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) .

These results indicate that CSM may be usefully included in prawn feed only at concentrations of 10% or less. The poor growth and survival at higher levels suggest gossypol toxicity and the possibility of residual pesticide contamination of CSM.

(Rose *et al.*, 1998) the studies were conducted 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. (Wang 2010).

2.7. Animal Protein- *Acetes indicus*:

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. 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 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.

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).

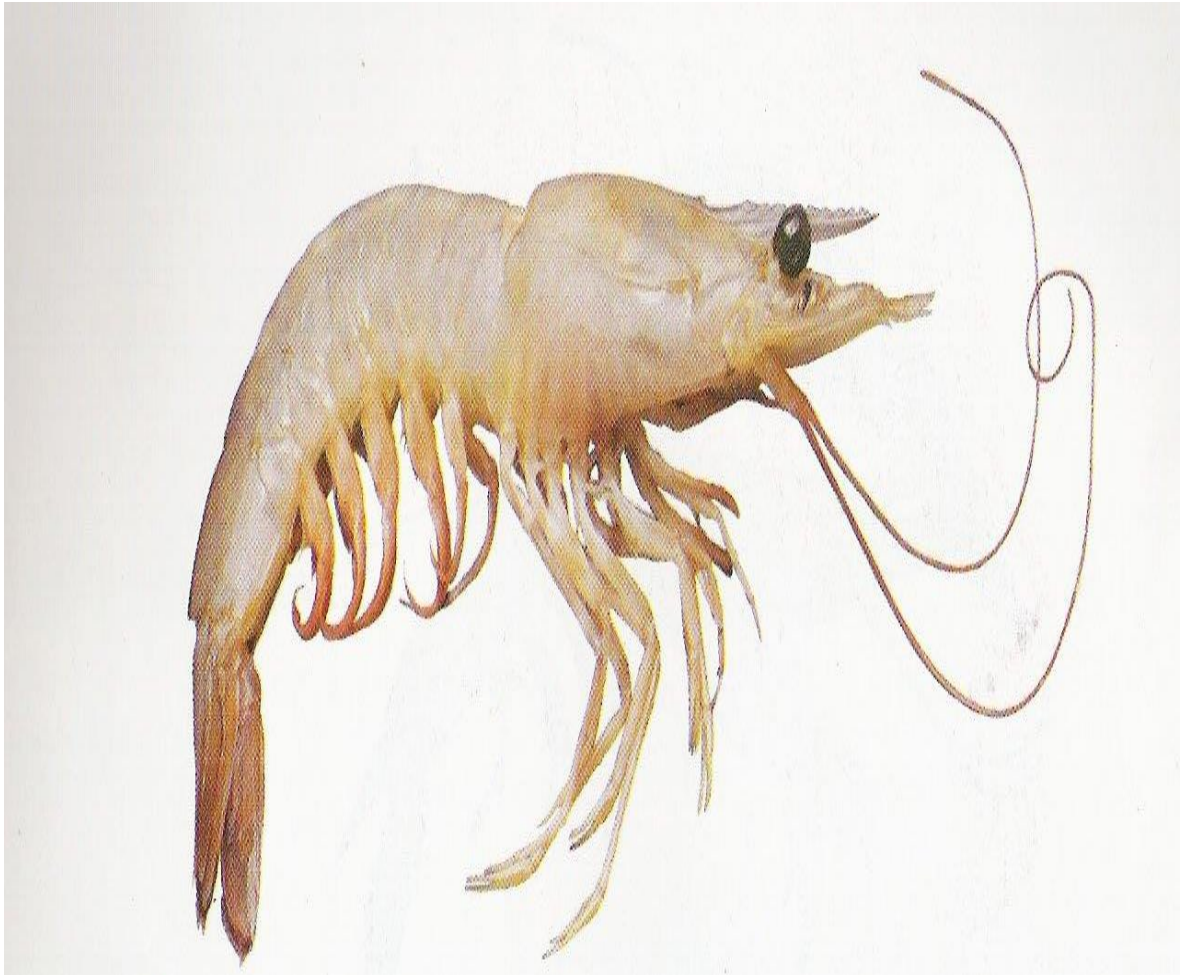


Plate.4:*Acetes indicus.*



Plate.5: *Acetes indicus* -wet position



Plate.6: *Acetes indicus*-dry position

Acetes 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).

Acetes 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).

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 one of the original feed sources used by Hameed Ali and co-workers for the preparation of a crustacean wet tissue suspension Jawa paste shrimp - *Acetes indicus*; Hameed Ali, *et al.*, 1982).

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 (CMFRI 1975). The males are smaller in size reach 27 mm in total length while the females grow up to 38 mm. Deshmukh (1988) 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)

A. indicus is a common food item of the prawns, cephalopods and juveniles of almost all the pelagic and demersal fishes occurring in shallow coastal waters around Bombay. *A.indicus* is a low-priced prawn, generally consumed after sun drying by the poor people. Due to small size and delicate nature the catch gets easily decomposed resulting in paste form which has very poor consumer acceptance.

2.8. Salinities:

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) .

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).

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 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 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 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 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. (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 an 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.

It was apparent from the experiment; however, that salinity had significant effects on both growth and food efficiency.

Previous studies had shown that holding penaeid shrimps at salinities near isosmotic 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. Similarly, in the present study, 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*

3.1. Site of the Experiment:

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 18.09.2013 to 20.11.2013)

3.2 .Experimental animals and their acclimatization:

Litopenaeus vannamei (500 numbers) seed were brought from the CP Hatchery and reared in farmer's pond till it reaches approximately weight of 1.5g for 25 days at 30 ppt salinity. Then the seed were acclimatized to the required salinity, i.e., 5 ppt and 15 ppt 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 1.5-1.8gm and 3.5-4.0 cm average lengths 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% (AI25) , *Acetes indicus* 30% (AI30) , *Acetes indicus* 35% (AI35) at three different salinities of 5ppt, 15ppt and 30ppt. 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 and *Acetes indicus* at 25%, 30%, 35% protein inclusion levels. Other ingredients used in the experimental diets were deoiled rice bran and maize, 1% of vitamin and mineral mixture added to experimental diets. All the ingredients that are Fish meal, Cottonseed meal, *Acetes indicus* meal, deoiled 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)

Name of the ingredient	FM25	FM30	FM35	CSM25	CSM30	CSM35	AI25	AI30	AI35
Fish meal	31.50	43.00	54.5	-	-	-	-	-	-
Cottonseed meal	-	-	-	30.5	48.2	83.20	-	-	-
<i>Acetes indicus</i> meal	-	-	-	-	-	-	28.68	40.00	50.5
De-oiled rice bran	33.25	27.5	21.75	33.75	24.90	7.4	34.66	29.00	23.75
Maize	33.25	27.5	21.75	33.75	24.90	7.4	34.66	29.00	23.75
Vitamins and mineral mixture	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0

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 protei

AI35 : *Acetes indicus* meal containing 35% crude protein

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

Ingredients	<i>Acetes indicus</i>	Fish meal	Cottonseed meal	De-oiled rice bran
Moisture	12.4	7.03	7.69	7.70
Crude Protein	58.4	55.00	40.00	12.50
Ether extract	6.03	4.02	16.65	22.50
Total ash	11.7	3.47	3.53	3.90
Acid insoluble ash	5.78	5.60	4.24	15.80

Table.3: Proximate Composition of formulated feeds used in the experiment (% on dry matter basis):

Feeds	FM25	FM30	FM35	CSM25	CSM30	CSM35	AI25	AI30	AI35
Moisture	8.75	8.65	8.61	9.02	8.95	9.01	9.21	9.3	9.2
Crude protein	25	30	35	25	30	35	25	30	35
Crude fiber	3.95	3.97	3.95	16.20	16.23	16.25	8.1	8.9	8.7
Ether extract	3.47	3.44	3.41	3.53	3.51	3.53	3.5	3.4	3.7

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:

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

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.

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

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.

$$\text{Weight of ash}$$

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

Weight of sample

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.

Weight of crude fiber

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

Original weight of sample

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 length and weight at 7 days interval.

3.5.1. Weight increment:

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

$$\text{Weight increment (gm)} = \text{Final body weight (gm)} - \text{Initial body weight (gm)}.$$

3.5.2. Specific growth rate:

Specific growth rate was calculated by the formula

$$[(L_n \text{ FBW} - L_n \text{ IBW}) / \text{day}] \times 100$$

FBW -- Final body weight

IBW -- Initial body weight

Ln -- Logarithm

Day -- duration of experiment (63 days)

3.6. Survival Rate:

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

Total number of fish survived

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

Total number of fish stocked

3.7. Food Conversion Ratio (FCR):

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

Feed given (dry 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.7.1: Partial set up of experimental animals reared at
5ppt Salinity**



**Plate.7.2: Partial set up of experimental animals reared at
15ppt Salinity**

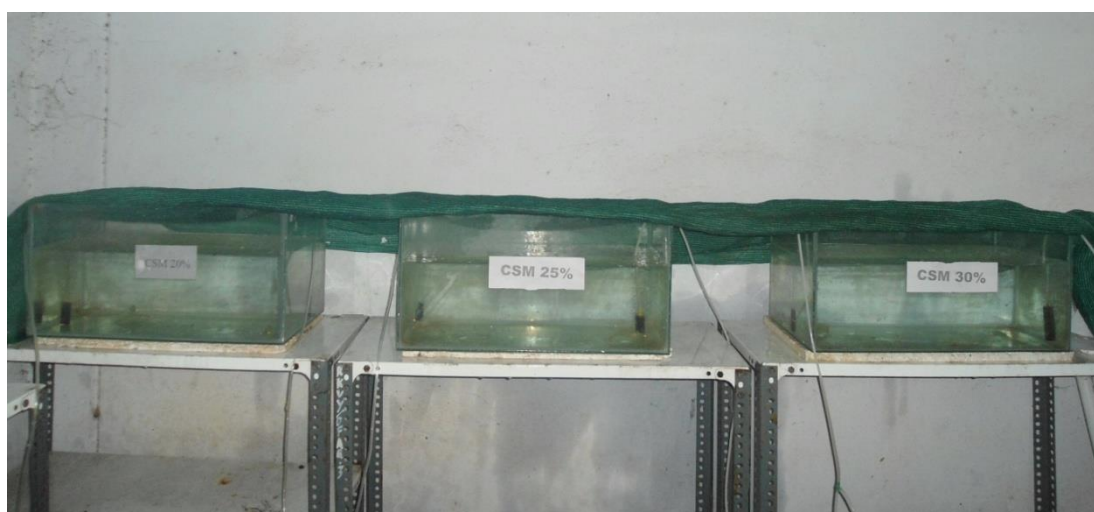


**Plate.7.3: Partial set up of experimental animals
reared at 30ppt Salinity**



Plate.8: Formulated Feeds prepared for experiment



Plate.9: Length measurement of *L.vannamei*



Plate.10: Weight Measurement of *L.vannamei*.

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 three different salinities 5ppt Salinity, 15ppt Salinity and 30ppt Salinity, and presented in tables 4 -18 And in figures from 1 -15.

4.1.1. DISSOLVED OXYGEN (PPM):

During the experimental period at 5ppt Salinity, the dissolved oxygen values varied between 5.02-8.42ppm. The highest value of 8.42ppm was recorded in CSM25 (initial day) and the lowest value of 5.22ppm was recorded in FM35 (07th day) during the entire experiment. Observations during the first sampling at the beginning of the experiment ranged between 7.00 and 8.42ppm. The highest DO was observed in CSM25 and the lowest was observed in FM35. Observations of dissolved oxygen during the 07th day recorded between 5.02 and 7.74ppm. During the sampling on the 14th day Dissolved oxygen values ranged between 5.61-7.62ppm. The highest and lowest dissolved oxygen levels were observed in AI25 and CSM25 respectively. The dissolved oxygen values observed during the sampling on the 21st day were between 5.22ppm and 7.24ppm. The highest values were recorded for CSM25 and the lowest in FM30. During the sampling on the 28th day the Dissolved oxygen values observed were in the range of 5.28ppm and 6.46ppm. AI30 recorded the highest and the lowest values were observed in FM30. The Dissolved oxygen levels observed during the sampling on the 35th day were between 5.27 and 6.01ppm, the lowest in FM30, and highest in CSM30. During the sampling on the 42nd day Dissolved oxygen values ranged between 5.23-6.46ppm. The highest and lowest dissolved oxygen levels were observed in CSM25

and FM30 respectively. The dissolved oxygen values observed during the sampling on the 49th day ranged between 5.28ppm and 6.02ppm. The highest values were recorded for AI25 and the lowest in FM30. During the sampling on the 56th day Dissolved oxygen values ranged between 5.62-6.02ppm. The highest and lowest dissolved oxygen levels were observed in AI25 and (CSM25, CSM30 and CSM35) respectively. At the final sampling done on the 63rd day the Dissolved oxygen observations recorded were between 5.42 (FM35) and 6.25ppm (CSM35). The observations are given in table 4 and presented in figure 1.

During the experimental period at 15ppt Salinity, the dissolved oxygen values varied between 6.29-8.52ppm. The highest value of 8.52ppm was recorded in FM35 (42nd day) and the lowest value of 6.29ppm was recorded in CSM35 (7th day) during the entire experiment. Observations during the first sampling at the beginning of the experiment ranged between 7.1 and 7.5ppm. The highest DO was observed in AI25 and the lowest was observed in FM35, CSM35. Observations of dissolved oxygen during the 07th day recorded between 6.29 and 7.81ppm. During the sampling on the 14th day Dissolved oxygen values ranged between 8.00-8.4ppm. The highest and lowest dissolved oxygen levels were observed in FM 30 and FM25, AI35 respectively. The dissolved oxygen values observed during the sampling on the 21st day were between 7.95ppm and 8.42ppm. The highest values were recorded for AI25 and the lowest in CSM25. During the sampling on the 28th day the Dissolved oxygen values observed were in the range of 7.24ppm and 8.24ppm. FM35 recorded the highest and the lowest values were observed in AI35. The Dissolved oxygen levels observed during the sampling on the 35th day were between 7.25 and 8.31ppm, the lowest in CSM25, and highest in FM35. During the sampling on the 42nd day Dissolved oxygen values ranged between 8.01-8.52ppm. The highest and lowest dissolved oxygen levels were observed in FM35 and CSM25 respectively. The dissolved oxygen values observed during the sampling on the 49th day ranged between 7.82ppm and 8.25ppm. The highest values were recorded for AI35 and the lowest in CSM30. During the sampling on the 56th day Dissolved oxygen values ranged between 7.94-8.32ppm. The highest and lowest dissolved oxygen levels were observed in AI35 and FM30

respectively. At the final sampling done on the 63rd day the Dissolved oxygen observations recorded were between 7.63 (FM35) and 8.42ppm (AI35). The observations are given in table 5 and presented in figure 2.

During the experimental period at 30ppt Salinity, the dissolved oxygen values varied between 7.23-8.99ppm. The highest value of 8.99ppm was recorded in FM25 (28th day) and the lowest value of 7.23ppm was recorded in FM35 (initial day) during the entire experiment. Observations during the first sampling at the beginning of the experiment ranged between 7.23 and 7.86ppm. The highest DO was observed in AI25 and the lowest was observed in FM35. Observations of dissolved oxygen during the 07th day recorded between 7.59 and 7.90ppm. During the sampling on the 14th day Dissolved oxygen values ranged between 7.79-7.99ppm. The highest and lowest dissolved oxygen levels were observed in AI35 and CSM35 respectively. The dissolved oxygen values observed during the sampling on the 21st day were between 8.10ppm and 8.98ppm. The highest values were recorded for CSM35 and the lowest in FM25. During the sampling on the 28th day the Dissolved oxygen values observed were in the range of 8.34ppm and 8.99ppm. FM25 recorded the highest and the lowest values were observed in CSM35. The Dissolved oxygen levels observed during the sampling on the 35th day were between 8.32 and 8.95ppm, the lowest in CSM35, and highest in AI35. During the sampling on the 42nd day Dissolved oxygen values ranged between 8.00-8.14ppm. The highest and lowest dissolved oxygen levels were observed in CSM25 and FM25 respectively. The dissolved oxygen values observed during the sampling on the 49th day ranged between 8.05ppm and 8.19ppm. The highest values were recorded for CSM25 and the lowest in (FM25 and FM35). During the sampling on the 56th day Dissolved oxygen values ranged between 7.92-8.32ppm. The highest and lowest dissolved oxygen levels were observed in AI35 and CSM25 respectively. At the final sampling done on the 63rd day the Dissolved oxygen observations recorded were between 7.98 (FM30, CSM25) and 8.35ppm (AI35). The observations are given in table 6 and presented in figure 3.

Table.4: Dissolved Oxygen (ppm) in the water of aquaria with *L.vannamei*

(White leg shrimp) reared at 5ppt Salinity:

TREATMENTS DAYS	TREATMENT FM			TREATMENT CSM			TREATMENT AI		
	T ₁ FM 25	T ₂ FM 30	T ₃ FM 35	T ₄ CSM 25	T ₅ CSM 30	T ₆ CSM 35	T ₇ AI 25	T ₈ AI 30	T ₉ AI 35
Initial	7.51	7.23	7.00	8.42	8.02	8.05	7.61	7.54	7.45
07	5.92	5.62	5.02	6.00	5.93	5.61	7.62	7.74	7.62
14	6.23	6.21	6.01	5.61	5.93	6.00	7.62	7.52	7.41
21	5.42	5.22	5.75	7.24	6.41	5.24	5.60	5.61	5.60
28	5.64	5.28	5.48	5.65	5.62	5.62	6.45	6.46	5.64
35	5.64	5.27	5.51	5.61	6.01	5.64	5.64	5.63	5.63
42	5.66	5.23	5.42	6.46	5.24	5.64	5.62	6.44	6.44
49	5.63	5.28	5.31	5.63	5.63	5.62	6.02	5.63	5.65
56	5.63	5.63	5.63	5.62	5.62	5.62	6.02	5.63	5.65
63	5.64	5.61	5.42	6.02	6.04	6.25	6.04	6.03	5.62

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

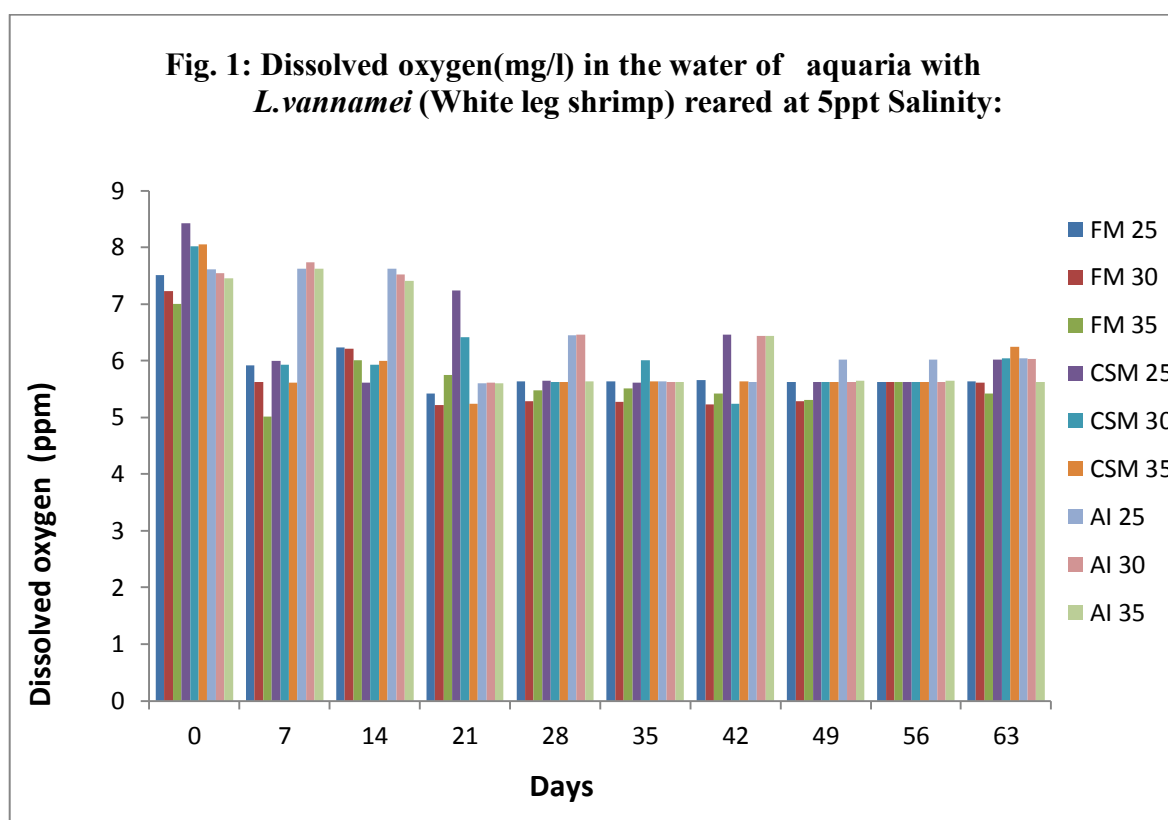
CSM30 : Cottonseed meal containing 30 % crude protein

CSM35 : Cottonseed meal containing 35% crude protein

AI25 : *Acetes indicus* meal containing 25% crude protein

AI30 : *Acetes indicus* meal containing 30% crude protei

AI35 : *Acetes indicus* meal containing 35% crude protein



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

Table.5: Dissolved Oxygen (ppm) in water of aquaria with *L.vannamei*

(White leg shrimp) reared at 15ppt Salinity

TREATMENTS / DAYS	TREATMENT FM			TREATMENT CSM			TREATMENT AI		
	T ₁₀ FM 25	T ₁₁ FM 30	T ₁₂ FM 35	T ₁₃ CSM 25	T ₁₄ CSM 30	T ₁₅ CSM 35	T ₁₆ AI 25	T ₁₇ AI 30	T ₁₈ AI 35
Initial	7.4	7.2	7.1	7.3	7.4	7.1	7.5	7.3	7.3
07	7.81	7.62	7.01	7.00	6.99	6.29	6.35	6.89	6.89
14	8.00	8.4	8.31	8.34	8.23	8.02	7.92	7.86	8.00
21	8.10	8.10	8.00	7.95	8.21	8.36	8.42	8.35	8.05
28	8.20	7.92	8.24	7.24	7.38	7.41	7.98	7.89	7.24
35	8.21	8.21	8.31	7.25	7.42	7.98	8.00	8.01	8.01
42	8.16	8.05	8.52	8.01	8.23	8.12	8.11	8.23	8.13
49	8.00	8.00	7.92	7.98	7.82	8.00	8.00	8.16	8.25
56	7.98	7.94	8.00	8.01	7.99	8.15	8.12	8.20	8.32
63	7.98	7.99	7.63	7.98	7.42	7.99	7.96	8.05	8.42

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

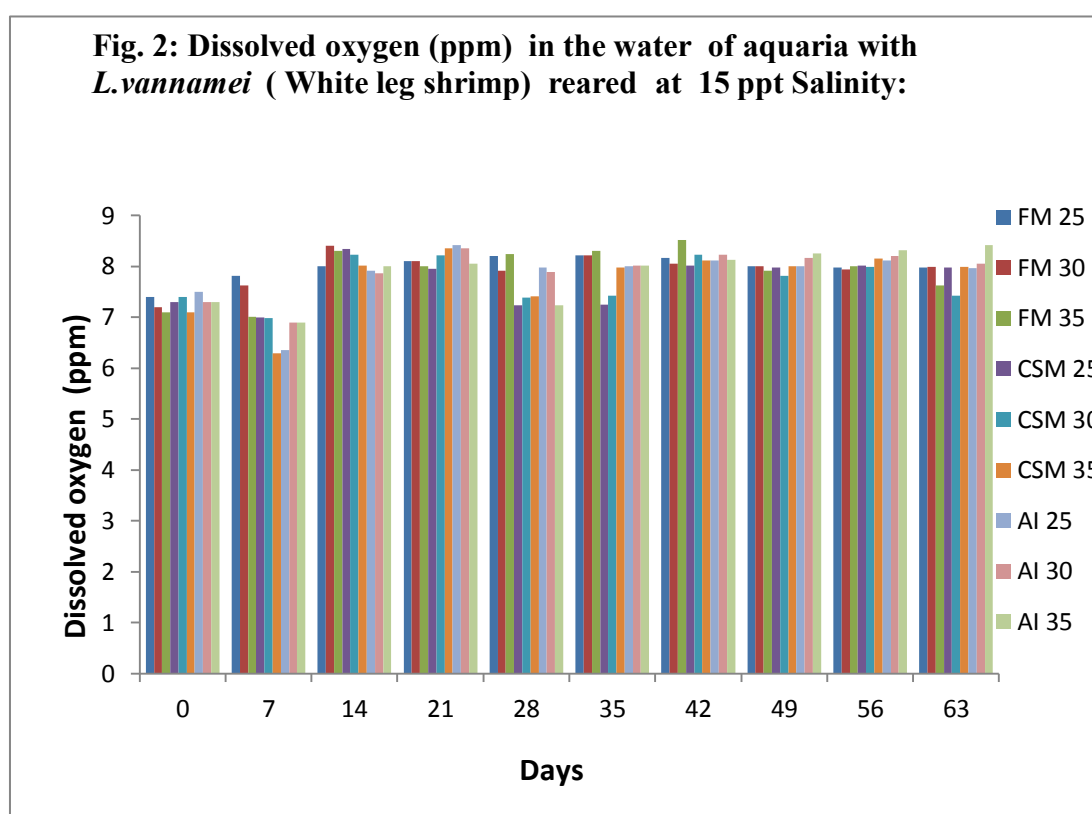
CSM30 : Cottonseed meal containing 30 % crude protein

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



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

Table.6: Dissolved oxygen (mg/l) in water of aquaria with *L.vannamei* (White

leg shrimp) reared at 30ppt Salinity:

TREATMENTS DAYS	TREATMENT FM			TREATMENT CSM			TREATMENT AI		
	T19 FM 25	T20 FM 30	T21 FM 35	T22 CSM 25	T23 CSM 30	T24 CSM 35	T25 AI 25	T26 AI 30	T27 AI 35
Initial	7.61	7.49	7.23	7.62	7.31	7.23	7.86	7.42	7.39
07	7.82	7.64	7.59	7.62	7.66	7.61	7.84	7.89	7.90
14	7.91	7.89	7.81	7.88	7.82	7.79	7.91	7.95	7.99
21	8.10	8.23	8.42	8.66	8.78	8.98	8.49	8.55	8.89
28	8.99	8.92	8.81	8.71	8.84	8.34	8.49	8.62	8.92
35	8.90	8.86	8.85	8.87	8.79	8.32	8.56	8.65	8.95
42	8.00	8.09	8.01	8.14	8.13	8.10	8.06	8.11	8.13
49	8.05	8.10	8.05	8.19	8.17	8.14	8.11	8.13	8.16
56	7.98	7.96	7.94	7.92	7.99	7.99	8.00	8.12	8.32
63	7.99	7.98	7.99	7.98	8.00	8.01	8.12	8.15	8.35

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

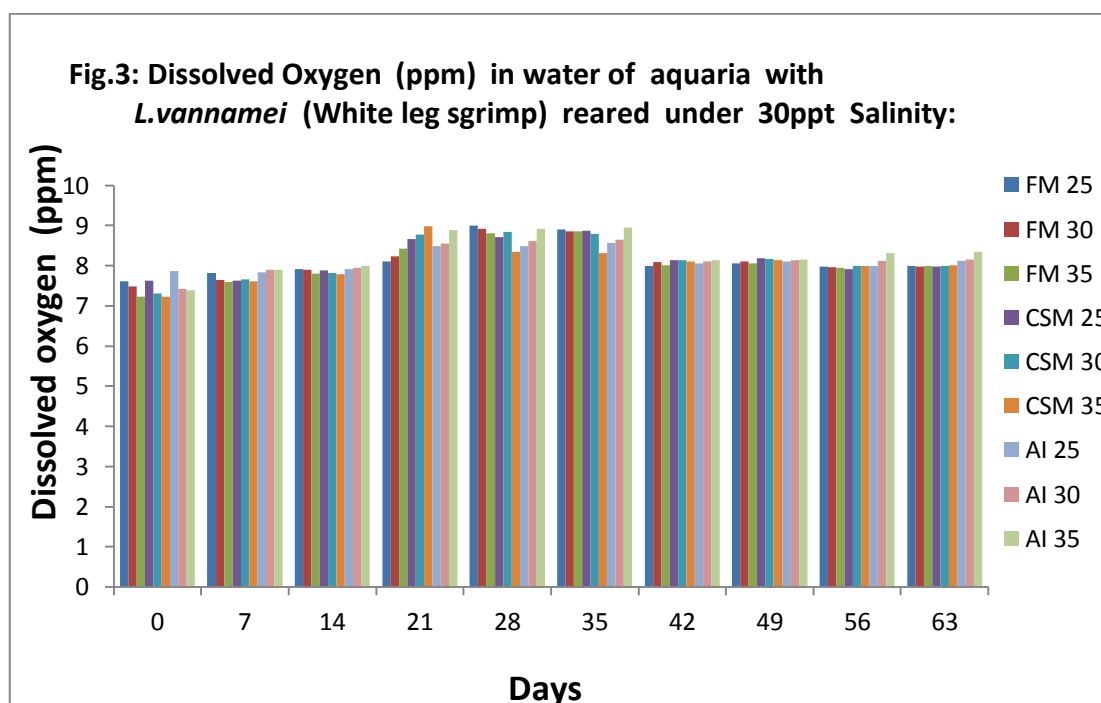
CSM30 : Cottonseed meal containing 30 % crude protein

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



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

4.1.2. TEMPERATURE (°C):

At 5ppt Salinity the temperature value recorded on the initial day was 29.5°C. The 07th day values recorded were 30.4°C. The temperature values observed on the 14th day were 29.3°C. Least variations in temperature had been noted during this period. On the 21st day temperature values were 30.2°C. Samples on 28th day recorded a temperature of 29.6°C. The temperature during the sampling on 35th day recorded were 30.8°C. The temperature values observed on the 42nd day were 31.6°C. High temperature was recorded in all treatments in this sampling. Samples on 49th day recorded a temperature of 29.3°C. On 56th day, temperature for the various treatments ranged between 30.4°C. The temperature values observed on the 63rd day were 30.4°C. The temperature value during this period was almost similar to that of 07th day. The observations are given in table 7 and presented in figure 4.

At 15ppt Salinity, the temperature value recorded on the initial day was 29.5°C. The 07th day values recorded were 30.3°C. The temperature values observed on the 14th day were 29.2°C. On the 21st day temperature values were 30.2°C. Samples on 28th day recorded a temperature of 29.6°C. The temperature during the sampling on 35th day recorded were 30.8°C. The temperature

values observed on the 42nd day were 31.6°C. High temperature was recorded in all treatments in this sampling. Samples on 49th day recorded a temperature of 29.3°C. On 56th day, temperature for the various treatments ranged between 29.1°C. Least variations in temperature had been noted during this period. The temperature values observed on the 63rd day were 30.4°C. The observations are given in table 8 and presented in figure 5.

At 30ppt Salinity the temperature value recorded on the initial day was 29.5°C. The 07th day values recorded were 30.4°C. The temperature values observed on the 14th day were 29.3°C. Least variations in temperature had been noted during this period. On the 21st day temperature values were 30.2°C. Samples on 28th day recorded a temperature of 29.5°C. The temperature during the sampling on 35th day recorded were 30.7°C. The temperature values observed on the 42nd day were 31.6°C. High temperature was recorded in all treatments in this sampling. Samples on 49th day recorded a temperature of 29.3°C. Least variations in temperature had been noted during this period also. On 56th day, temperature for the various treatments ranged between 30.3°C. The temperature values observed on the 63rd day were 30.4°C. The observations are given in table 9 and presented in figure 6.

Table.7: Temperature (°C) in water of aquaria with *L.vannamei*

(White leg shrimp) reared at 5ppt Salinity:

TREATMENTS DAYS	TREATMENT FM			TREATMENT CSM			TREATMENT AI		
	T ₁	T ₂	T ₃	T ₄	T ₅	T ₆	T ₇	T ₈	T ₉
	FM 25	FM 30	FM 35	CSM 25	CSM 30	CSM 35	AI 25	AI 30	AI 35
Initial	29.5	29.5	29.5	29.5	29.5	29.5	29.5	29.5	29.5
07	30.4	30.4	30.4	30.4	30.4	30.4	30.4	30.4	30.4

14	29.3	29.3	29.3	29.3	29.3	29.3	29.3	29.3	29.3
21	30.2	30.2	30.2	30.2	30.2	30.2	30.2	30.2	30.2
28	29.6	29.6	29.6	29.6	29.6	29.6	29.6	29.6	29.6
35	30.8	30.8	30.8	30.8	30.8	30.8	30.8	30.8	30.8
42	31.6	31.6	31.6	31.6	31.6	31.6	31.6	31.6	31.6
49	29.3	29.3	29.3	29.3	29.3	29.3	29.3	29.3	29.3
56	30.4	30.4	30.4	30.4	30.4	30.4	30.4	30.4	30.4
63	30.4	30.4	30.4	30.4	30.4	30.4	30.4	30.4	30.4

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

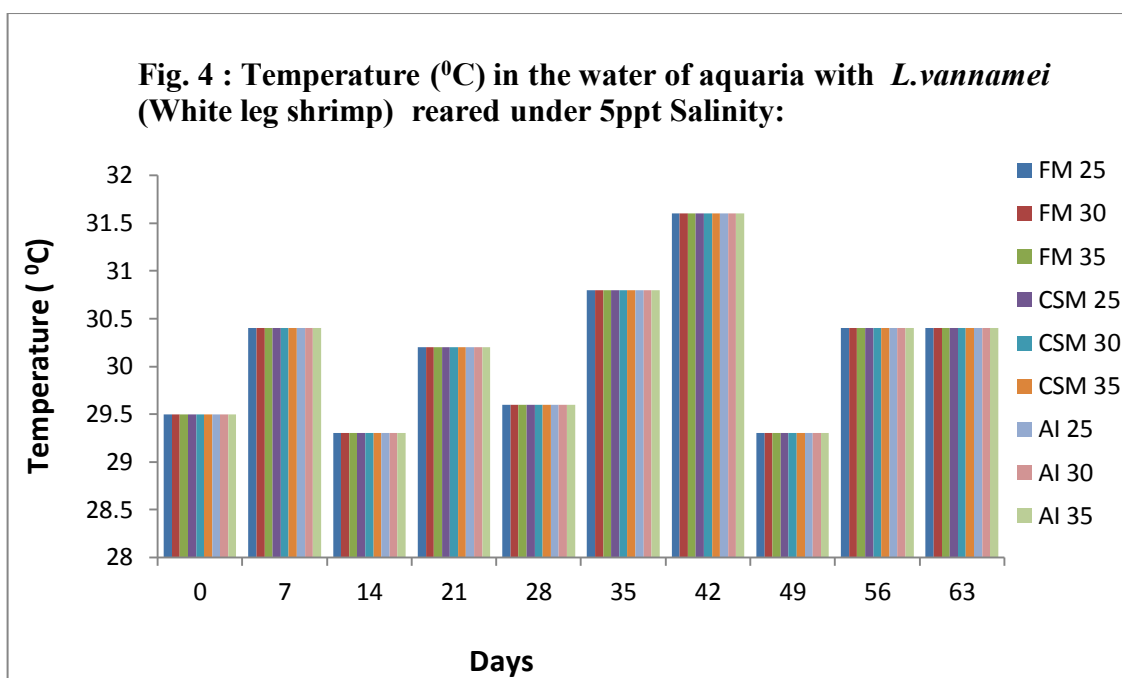
CSM30 : Cottonseed meal containing 30 % crude protein

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



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

Table.8: Temperature ($^{\circ}$ C) in water of aquaria with *L.vannamei*

(White leg shrimp) reared at 15ppt Salinity:

TREATMENTS DAYS	TREATMENT FM			TREATMENT CSM			TREATMENT AI		
	T ₁₀ FM 25	T ₁₁ FM 30	T ₁₂ FM 35	T ₁₃ CSM 25	T ₁₄ CSM 30	T ₁₅ CSM 35	T ₁₆ AI 25	T ₁₇ AI 30	T ₁₈ AI 35
Initial	29.5	29.5	29.5	29.5	29.5	29.5	29.5	29.5	29.5
07	30.3	30.3	30.3	30.3	30.3	30.3	30.3	30.3	30.3
14	29.2	29.2	29.2	29.2	29.2	29.2	29.2	29.2	29.2
21	30.2	30.2	30.2	30.2	30.2	30.2	30.2	30.2	30.2
28	29.6	29.6	29.6	29.6	29.6	29.6	29.6	29.6	29.6
35	30.8	30.8	30.8	30.8	30.8	30.8	30.8	30.8	30.8
42	31.6	31.6	31.6	31.6	31.6	31.6	31.6	31.6	31.6
49	29.3	29.3	29.3	29.3	29.3	29.3	29.3	29.3	29.3
56	29.1	29.1	29.1	29.1	29.1	29.1	29.1	29.1	29.1
63	30.4	30.4	30.4	30.4	30.4	30.4	30.4	30.4	30.4

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

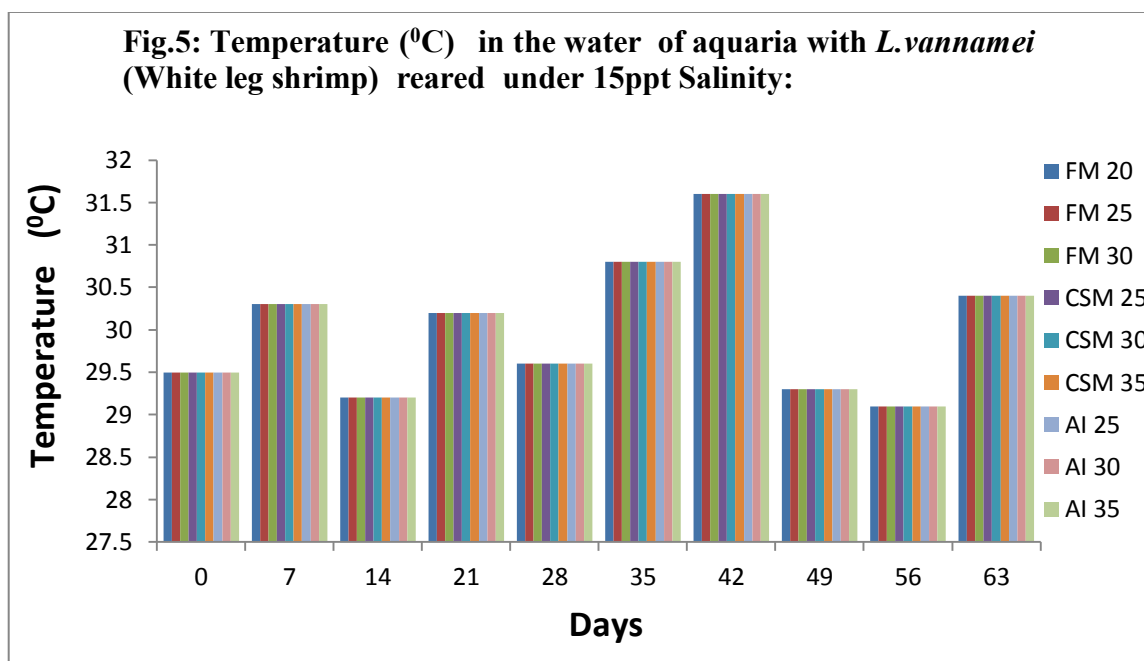
CSM30 : Cottonseed meal containing 30 % crude protein

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



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

Table.9: Temperature ($^{\circ}$ C) in water of aquaria with *L.vannamei*

(White leg shrimp) reared at 30ppt Salinity:

TREATMENTS DAYS	TREATMENT FM			TREATMENT CSM			TREATMENT AI		
	T ₁₉ FM 25	T ₂₀ FM 30	T ₂₁ FM 35	T ₂₂ CSM 25	T ₂₃ CSM 30	T ₂₄ CSM 35	T ₂₅ AI 25	T ₂₆ AI 30	T ₂₇ AI 35
Initial	29.5	29.5	29.5	29.5	29.5	29.5	29.5	29.5	29.5
07	30.4	30.4	30.4	30.4	30.4	30.4	30.4	30.4	30.4
14	29.3	29.3	29.3	29.3	29.3	29.3	29.3	29.3	29.3
21	30.2	30.2	30.2	30.2	30.2	30.2	30.2	30.2	30.2
28	29.5	29.5	29.5	29.5	29.5	29.5	29.5	29.5	29.5
35	30.7	30.7	30.7	30.7	30.7	30.7	30.7	30.7	30.7
42	31.6	31.6	31.6	31.6	31.6	31.6	31.6	31.6	31.6
49	29.3	29.3	29.3	29.3	29.3	29.3	29.3	29.3	29.3
56	30.3	30.3	30.3	30.3	30.3	30.3	30.3	30.3	30.3
63	30.4	30.4	30.4	30.4	30.4	30.4	30.4	30.4	30.4

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

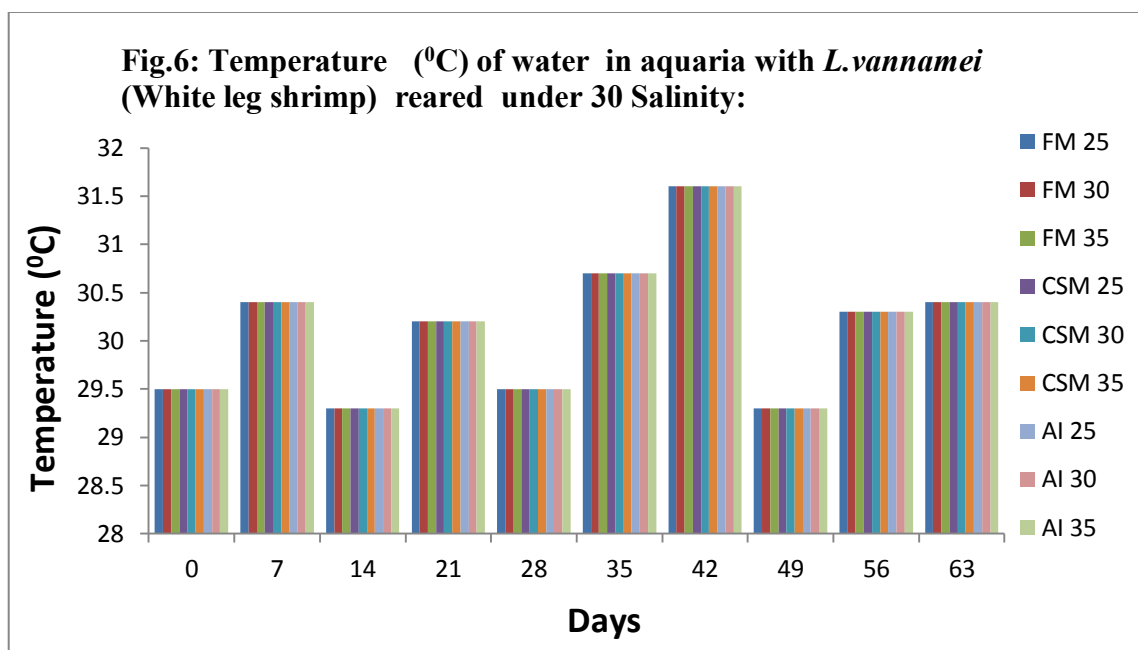
CSM30 : Cottonseed meal containing 30 % crude protein

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



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

4.1.3. pH:

The pH values recorded during the period of experiment at 5ppt Salinity ranged between 8.0 and 8.3. Initially the pH had ranged between 8.0 and 8.3 among all the treatments. The pH range recorded for 7th, 21st, 28th, 42nd and 63rd day was same between 8.1-8.2. The pH range recorded for 14th day between 8.0-8.3 as same as initial day. The pH range recorded for 35th, 49th and 56th day was same between 8.0-8.2. The observations are given in table 10 and presented in figure 7.

The pH values recorded during the period of experiment at 15ppt Salinity, ranged between 8.1 and 8.3. Initially the pH had ranged between 8.2 and 8.3 among all the treatments. The pH range recorded for the 07th day sampling was 8.2 (CSM25, CSM35, AI25, AI30) to 8.3 (CSM30). The pH range was the same for 14th and 21st, 42nd, 49th, 56th and 63rd day showed the pH had ranged between 8.2-8.3. The pH range was the same for 28th day and 35th day between 8.1-8.3. The observations are given in table 11 and presented in fig.8.

The pH values recorded during the period of experiment at 30ppt Salinity, ranged between 8.3 and 8.5. Initially the pH had ranged between 8.4 and 8.5 among all the treatments. The pH range recorded for the 07th and 63rd day was same as initial day between 8.4-8.5. The pH range was the same for 14th and 21st, 28th, 35th, 42nd, 49th and 56th day showed the pH had ranged between 8.3-8.5. The observations are given in table 12 and presented in figure 9.

Table.10: pH in water of aquaria with *L.vannamei* (white leg shrimp)

Reared at 5ppt Salinity:

TREATMENTS	TREATMENT FM			TREATMENT CSM			TREATMENT AI		
	T ₁ FM 25	T ₂ FM 30	T ₃ FM 35	T ₄ CSM 25	T ₅ CSM 30	T ₆ CSM 35	T ₇ AI 25	T ₈ AI 30	T ₉ AI 35
DAYS									
Initial	8.2	8.2	8.2	8.2	8.3	8.2	8.2	8.0	8.2
07	8.2	8.1	8.1	8.2	8.2	8.2	8.1	8.1	8.1
14	8.1	8.2	8.0	8.3	8.1	8.1	8.2	8.2	8.2
21	8.2	8.1	8.1	8.2	8.2	8.1	8.2	8.2	8.1
28	8.1	8.2	8.2	8.1	8.2	8.2	8.1	8.1	8.2
35	8.1	8.2	8.0	8.2	8.1	8.2	8.2	8.0	8.1
42	8.2	8.1	8.1	8.2	8.2	8.1	8.2	8.1	8.2
49	8.2	8.2	8.1	8.0	8.0	8.0	8.1	8.2	8.2
56	8.1	8.2	8.2	8.2	8.2	8.1	8.0	8.1	8.1
63	8.2	8.2	8.2	8.1	8.2	8.2	8.1	8.2	8.1

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

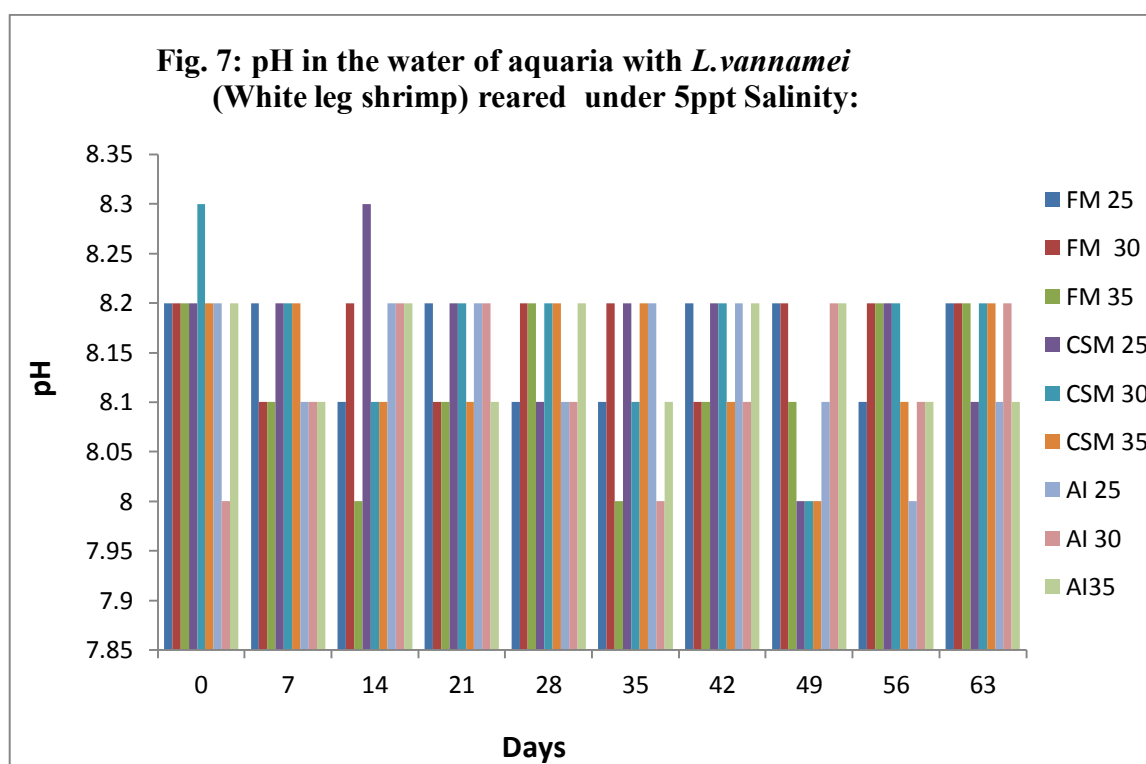
CSM30 : Cottonseed meal containing 30 % crude protein

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



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

Table.11: pH in water of aquaria with *L.vannamei* (White leg shrimp)

reared at 15ppt Salinity:

TREATMENTS DAYS	TREATMENT FM			TREATMENT CSM			TREATMENT AI		
	T ₁₀	T ₁₁	T ₁₂	T ₁₃	T ₁₄	T ₁₅	T ₁₆	T ₁₇	T ₁₈
	FM 25	FM 30	FM 35	CSM 25	CSM 30	CSM 35	AI 25	AI 30	AI 35
Initial	8.2	8.2	8.3	8.3	8.2	8.2	8.2	8.3	8.3
07	8.3	8.3	8.3	8.2	8.3	8.2	8.2	8.2	8.3
14	8.3	8.3	8.2	8.3	8.2	8.3	8.3	8.3	8.2
21	8.2	8.3	8.3	8.2	8.3	8.3	8.2	8.2	8.3
28	8.3	8.2	8.3	8.1	8.2	8.2	8.3	8.3	8.3
35	8.3	8.3	8.3	8.3	8.1	8.3	8.2	8.3	8.2
42	8.3	8.2	8.3	8.2	8.3	8.2	8.3	8.3	8.3
49	8.2	8.3	8.3	8.3	8.2	8.2	8.3	8.2	8.2
56	8.2	8.3	8.3	8.3	8.2	8.2	8.3	8.2	8.2
63	8.3	8.3	8.3	8.3	8.2	8.3	8.3	8.3	8.3

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

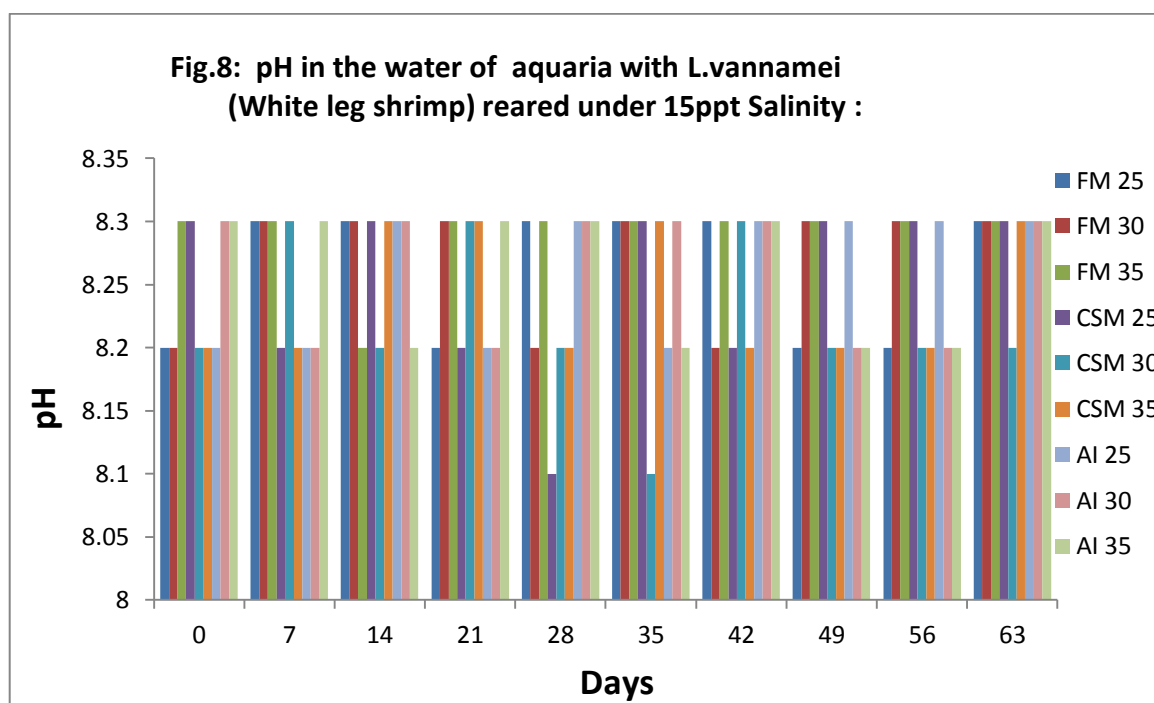
CSM30 : Cottonseed meal containing 30 % crude protein

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



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

Table.12: pH in water of aquaria with *L.vannamei* (white leg shrimp)

reared at 30ppt Salinity:

TREATMENTS DAYS	TREATMENT FM			TREATMENT CSM			TREATMENT AI		
	T ₁₉	T ₂₀	T ₂₁	T ₂₂	T ₂₃	T ₂₄	T ₂₅	T ₂₆	T ₂₇
	FM 25	FM 30	FM 35	CSM 25	CSM 30	CSM 35	AI 25	AI 30	AI 35
Initial	8.5	8.5	8.5	8.5	8.4	8.5	8.4	8.5	8.4
07	8.5	8.4	8.5	8.4	8.4	8.4	8.4	8.5	8.5
14	8.3	8.4	8.4	8.4	8.5	8.5	8.5	8.4	8.4
21	8.4	8.5	8.5	8.3	8.4	8.4	8.5	8.3	8.5
28	8.4	8.3	8.4	8.4	8.5	8.3	8.4	8.4	8.5
35	8.5	8.4	8.3	8.5	8.4	8.4	8.3	8.5	8.4

42	8.4	8.5	8.4	8.4	8.4	8.3	8.4	8.3	8.5
49	8.4	8.4	8.5	8.5	8.4	8.4	8.5	8.4	8.3
56	8.3	8.5	8.4	8.4	8.5	8.5	8.4	8.5	8.4
63	8.5	8.5	8.5	8.4	8.5	8.4	8.5	8.5	8.5

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

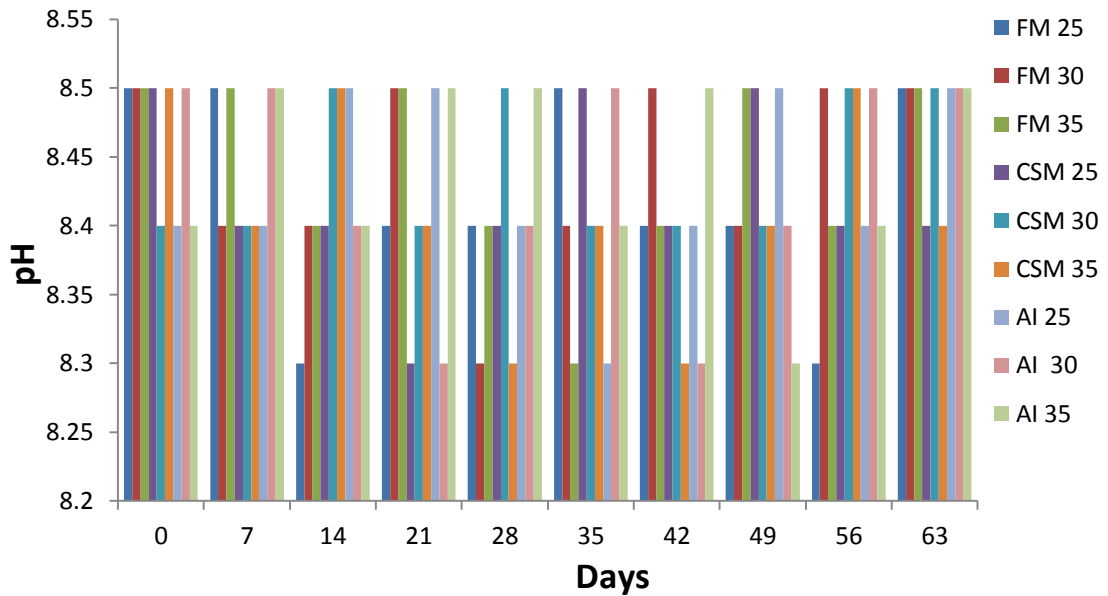
CSM35 : Cottonseed meal containing 35% crude protein

AI25 : *Acetes indicus* meal containing 25% crude protein

AI30 : *Acetes indicus* meal containing 30% crude protein

AI35 : *Acetes indicus* meal containing 35% crude protein

Fig.9. pH of water in aquaria with *L.vannamei* (White leg shrimp) reared under 30ppt 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

4.1.4. TOTAL ALKALINITY (mg/l):

During the initial observation, the total alkalinity values recorded in the experiment at 5ppt Salinity were in a range of 280 to 340mg/l. The highest and the lowest total alkalinity values were observed in AI30 and CSM25 respectively. The total alkalinity levels observed during the sampling on the 07th day were between 220 to 300mg/l. The highest and lowest total alkalinity values were observed in FM35 and CSM30. During the sampling on the 14th day the total alkalinity was 170mg/l- CSM30, 220mg/l -FM35. The 21st day results revealed that the highest was for CSM35, AI25 and AI30 and the lowest for FM35 in the range of 170-220mg/l. 28th day observations were in the range of 220-200mg/l (highest in CSM35,AI30,AI35,FM30 and lowest in FM25). On 35th day the sampling results were 200mg/l-230mg/l. FM25 recorded the lowest while the highest was in CSM30. The 42nd day results revealed that the highest was for FM30, and the lowest for CSM30, CSM35 in the range of 220 - 160 mg/l. During the sampling on the 49th day the total alkalinity was 210mg/l (AI30)- 160mg/l (CSM30, CSM35, AI25, AI35, FM25, FM30, and FM35). The 56th day the total alkalinity levels observed between 210mg/l-150mg/l. The 63rd day the total alkalinity levels observed between 220mg/l-210mg/l. The highest and lowest values observed were in CSM35, FM35 and CSM25, CSM30, AI25, AI30, AI35, FM25, FM30 respectively. The observations are given in table 13 and presented in figure 10.

During the initial observation, the total alkalinity values recorded in the experiment at 15ppt Salinity were in a range of 612 to 629mg/l. The highest and the lowest total alkalinity values were observed in AI35 and CSM30 respectively. The total alkalinity levels observed during the sampling on the 07th day were between 614 to 629mg/l. The highest and lowest total alkalinity values were observed in AI35 and CSM30. During the sampling on the 14th day the total alkalinity was 612mg/l- CSM30 624mg/l - FM25. The 21st day results revealed that the highest was for FM25, FM35 and the lowest for CSM30 in the range of 626-614mg/l. 28th day observations were in the range of 628-616mg/l, highest in FM25 and lowest in CSM30. On 35th day the sampling results were

614mg/l-630mg/l. CSM30 recorded the lowest while the highest was in AI35. The 42nd day results revealed that the highest was for AI35 and the lowest for CSM30 in the range of 632 - 616 mg/l. During the sampling on the 49th day the total alkalinity was 628 mg/l CSM25, FM25 - 618mg/l CSM30. The 56th day the total alkalinity levels observed between 626mg/l-616mg/l. The 63rd day the total alkalinity levels observed between 630mg/l-620mg/l. The highest and lowest values observed were in AI35 and CSM30 respectively. The observations are given in table 14 and presented in figure 11.

During the initial observation, the total alkalinity values recorded in the experiment at 30ppt Salinity were in a range of 660 to 682mg/l. The highest and the lowest total alkalinity values were observed in FM30 and CSM30 respectively. The total alkalinity levels observed during the sampling on the 07th day were between 664 to 682mg/l. The highest and lowest total alkalinity values were observed in CSM25 and FM25. During the sampling on the 14th day the total alkalinity was 664mg/l- CSM35 681mg/l – CSM30. The 21st day results revealed that the highest was for CSM30 and the lowest for CSM35 in the range of 684-670mg/l. 28th day observations were in the range of 683-668mg/l, highest in FM25 and lowest in CSM25, CSM35, and AI35. On 35th day the sampling results were 672mg/l-683mg/l. FM35, CSM25 recorded the lowest while the highest was in FM30. The 42nd day results revealed that the highest was for CSM35 and the lowest for FM25 in the range of 689 - 681 mg/l. During the sampling on the 49th day the total alkalinity was 680 mg/l (CSM25, CSM30) 664mg/l (CSM35). The 56th day the total alkalinity levels observed between 680mg/l-676mg/l. The 63rd day the total alkalinity levels observed between 685mg/l-680mg/l. The highest and lowest values observed were in FM35, CSM25, CSM30 and FM25, FM30, CSM35, AI25, AI30, AI35 respectively. The observations are given in table 15 and presented in figure 12.

Table.13: Total Alkalinity (mg/l) in water of aquaria with *L.vannamei*

(White leg shrimp) reared at 5ppt Salinity:

TREATMENTS DAYS	TREATMENT FM			TREATMENT CSM			TREATMENT AI		
	T ₁ FM 25	T ₂ FM 30	T ₃ FM 35	T ₄ CSM 25	T ₅ CSM 30	T ₆ CSM 35	T ₇ AI 25	T ₈ AI 30	T ₉ AI 35
Initial	300	340	320	280	300	290	300	340	320
07	250	250	300	250	220	230	230	250	260
14	210	210	220	210	170	190	200	210	210
21	200	210	170	200	210	220	220	220	210
28	200	220	210	210	210	220	210	220	220
35	230	210	210	210	200	220	220	220	220
42	210	220	220	170	160	160	170	210	210
49	160	160	160	170	160	160	160	210	160
56	150	210	170	210	160	170	170	160	160
63	210	210	220	210	210	220	210	210	210

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

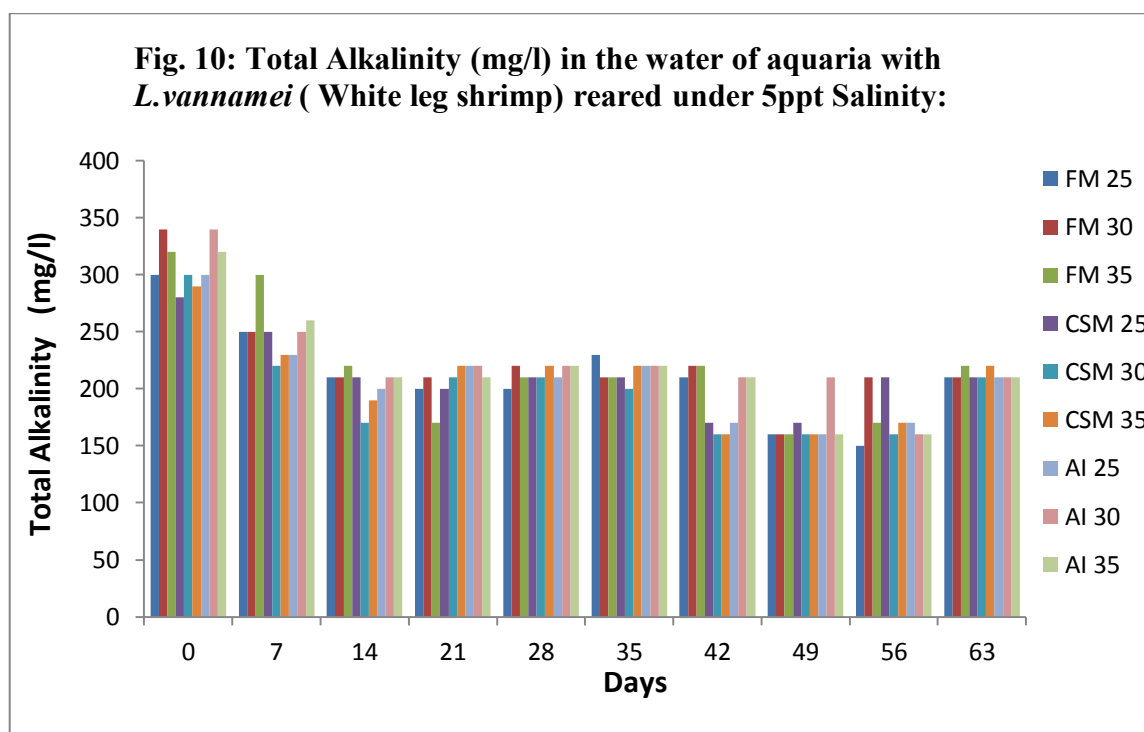
CSM30 : Cottonseed meal containing 30 % crude protein

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



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

**Table.14: Total Alkalinity (mg/l) in water of aquaria with *L.vannamei*
(White leg shrimp) reared at 15ppt Salinity:**

TREATMENTS DAYS	TREATMENT FM			TREATMENT CSM			TREATMENT AI		
	T ₁₀ FM 25	T ₁₁ FM 30	T ₁₂ FM 35	T ₁₃ CSM 25	T ₁₄ CSM 30	T ₁₅ CSM 35	T ₁₆ AI 25	T ₁₇ AI 30	T ₁₈ AI 35
Initial	622	615	619	620	612	618	618	624	629
07	622	618	621	622	614	620	620	626	629
14	624	620	623	622	612	622	622	622	622
21	626	621	626	624	614	620	618	624	621
28	628	623	622	626	616	624	626	626	625
35	622	625	628	624	614	626	622	622	630
42	626	622	632	626	616	628	624	620	632
49	628	624	622	628	618	626	622	624	626
56	624	626	624	626	616	620	620	622	624
63	628	628	626	628	620	624	628	626	630

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

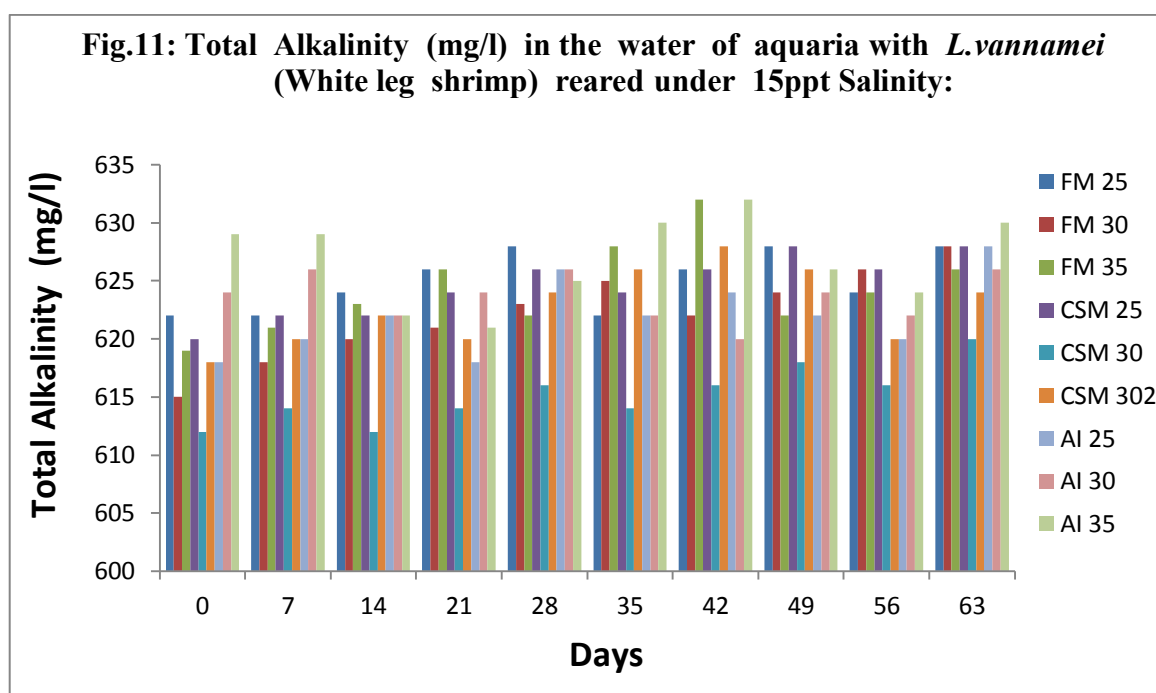
CSM30 : Cottonseed meal containing 30 % crude protein

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



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

Table.15: Total Alkalinity (mg/l) in water of aquaria with *L.vannamei* (White

leg shrimp) reared at 30ppt Salinity:

TREATMENTS / DAYS	TREATMENT FM			TREATMENT CSM			TREATMENT AI		
	T ₁₉	T ₂₀	T ₂₁	T ₂₂	T ₂₃	T ₂₄	T ₂₅	T ₂₆	T ₂₇
	FM 25	FM 30	FM 35	CSM 25	CSM 30	CSM 35	AI 25	AI 30	AI 35
Initial	680	682	672	674	660	675	670	672	674
07	664	678	665	682	672	666	676	671	668
14	677	678	665	674	681	664	670	672	669
21	681	680	676	681	684	670	672	675	673
28	683	681	669	668	673	668	671	671	668
35	676	683	672	672	676	679	680	682	681
42	681	683	686	683	687	689	684	683	680
49	676	672	676	680	680	664	666	676	672
56	680	678	680	680	680	678	678	676	676
63	680	680	685	685	685	680	680	680	680

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

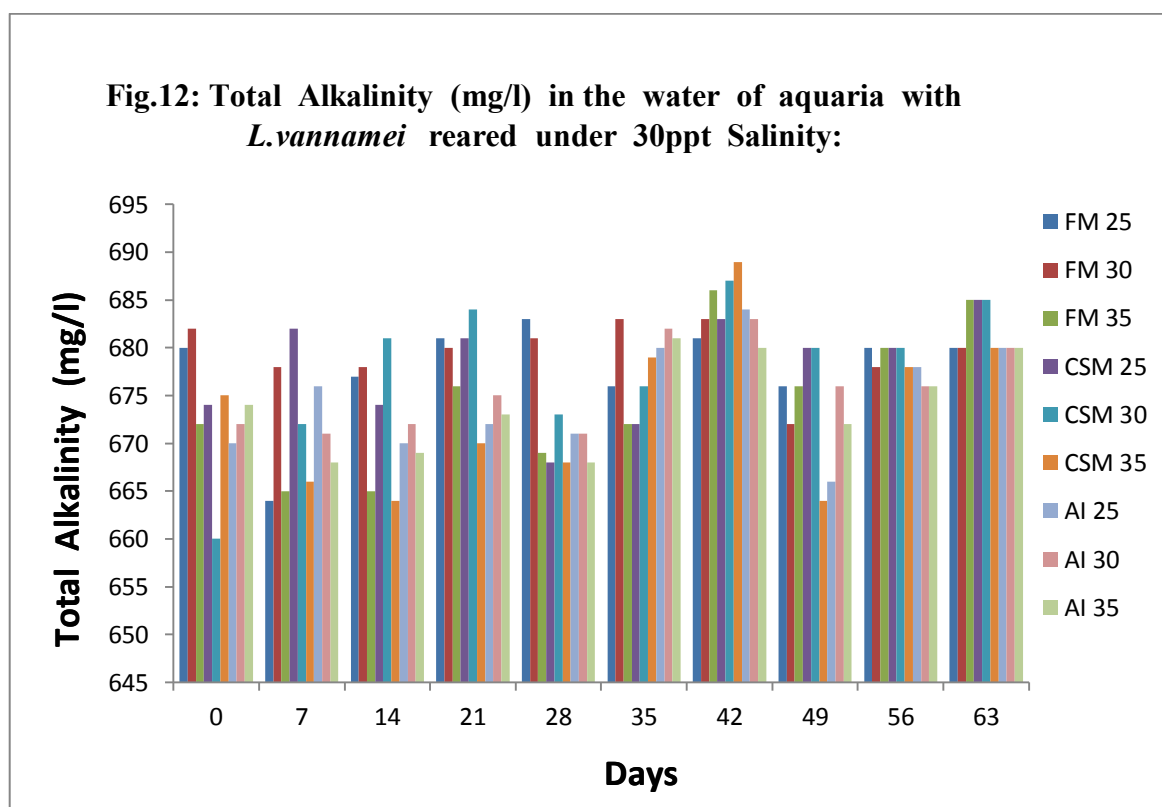
CSM30 : Cottonseed meal containing 30 % crude protein

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



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

4.1.5. TOTAL HARDNESS (mg/l):

At 5ppt Salinity, the total hardness values observed during the initial sampling were between 225mg/l and 230mg/l. The highest and the lowest hardness values were observed in AI25, AI30 and AI35 respectively. During the 07th day sampling the total hardness values ranged between 226mg/l and 230mg/l. The highest and lowest hardness values were observed in the treatments AI25 and FM25, FM30 respectively. Results of 14th day sampling recorded highest value for FM25, FM30, while the lowest being for CSM30, CSM35 and the values were 231mg/l and 228mg/l respectively. 21st day observation ranged between 228mg/l-232mg/l, highest in FM35 and lowest in CSM30. On the 28th day's highest and lowest values recorded were for AI25, FM35 and AI30 respectively. The 35th day observations ranged between 229-235mg/l. The sampling results of the treatments on the 42nd day were 226-234mg/l. Among the values observed for 42nd day's sampling the highest was for AI30, FM35 and the lowest being for FM30. The 49th day observations ranged between 229-234mg/l. The 56th day observations ranged between 226-235mg/l. The final days' (63rd

day) sampling results were 231mg/l(lowest) in AI35 and 237mg/l (highest) in AI25. During this sampling, highest Total hardness value was recorded. The observations are given in table 16 and presented in figure 13.

At 15ppt Salinity, the total hardness values observed during the initial sampling were between 666mg/l and 684mg/l. The highest and the lowest hardness values were observed in FM25 and CSM30 respectively. During the 07th day sampling the total hardness values ranged between 660mg/l and 676mg/l. The highest and lowest hardness values were observed in the treatments CSM30 and FM25, respectively. Results of 14th day sampling recorded highest value for FM30 while the lowest being for FM35 and the values were 680mg/l and 662mg/l respectively. 21st day observation ranged between 662mg/l-678mg/l, highest in FM25 and lowest in CSM30,AI25,AI35. On the 28th day's highest and lowest values recorded were for FM25 and CSM25 respectively. The 35th day observations ranged between 670-680mg/l. The sampling results of the treatments on the 42nd day were 674-680mg/l. Among the values observed for 42nd day's sampling the highest was for CSM35, AI35, FM25 and the lowest being for CSM25, AI25, AI30. The 49th day observations ranged between 666-680mg/l. The 56th day observations ranged between 672-678mg/l. The final days' (63rd day) sampling results were 678mg/l-(lowest) in AI35, FM30 and 680mg/l (highest) in CSM25,CSM30,CSM35,AI25,AI30,FM25,FM35. During this sampling, highest Total hardness value was recorded. The observations are given in table 17 and presented in figure 14.

At 30ppt Salinity, the total hardness values observed during the initial sampling were between 691mg/l and 699mg/l. The highest and the lowest hardness values were observed in AI30 and CSM25 respectively. During the 07th day sampling the total hardness values ranged between 700mg/l and 710mg/l. The highest and lowest hardness values were observed in the treatments CSM35 and FM35 respectively. Results of 14th day sampling recorded highest value for FM35, CSM30 while the lowest being for FM25 and the values were 715mg/l and 709mg/l respectively. 21st day observation ranged between 709mg/l-719mg/l, highest in FM30, CSM35 and lowest in

AI35. On the 28th day's highest and lowest values recorded were for FM35 and AI25 respectively. The 35th day observations ranged between 720-729mg/l. The sampling results of the treatments on the 42nd day were 709-719mg/l. Among the values observed for 42nd day's sampling the highest was for CSM35 and the lowest being for AI35. The 49th day observations ranged between 720-732mg/l. The 56th day observations ranged between 730-738mg/l. The final day's (63rd day) sampling results were 740mg/l (lowest) in CSM25,CSM35,AI30 and 743mg/l (highest) in CSM30. During this sampling, highest Total hardness value was recorded. The observations are given in table 18 and presented in figure 15.

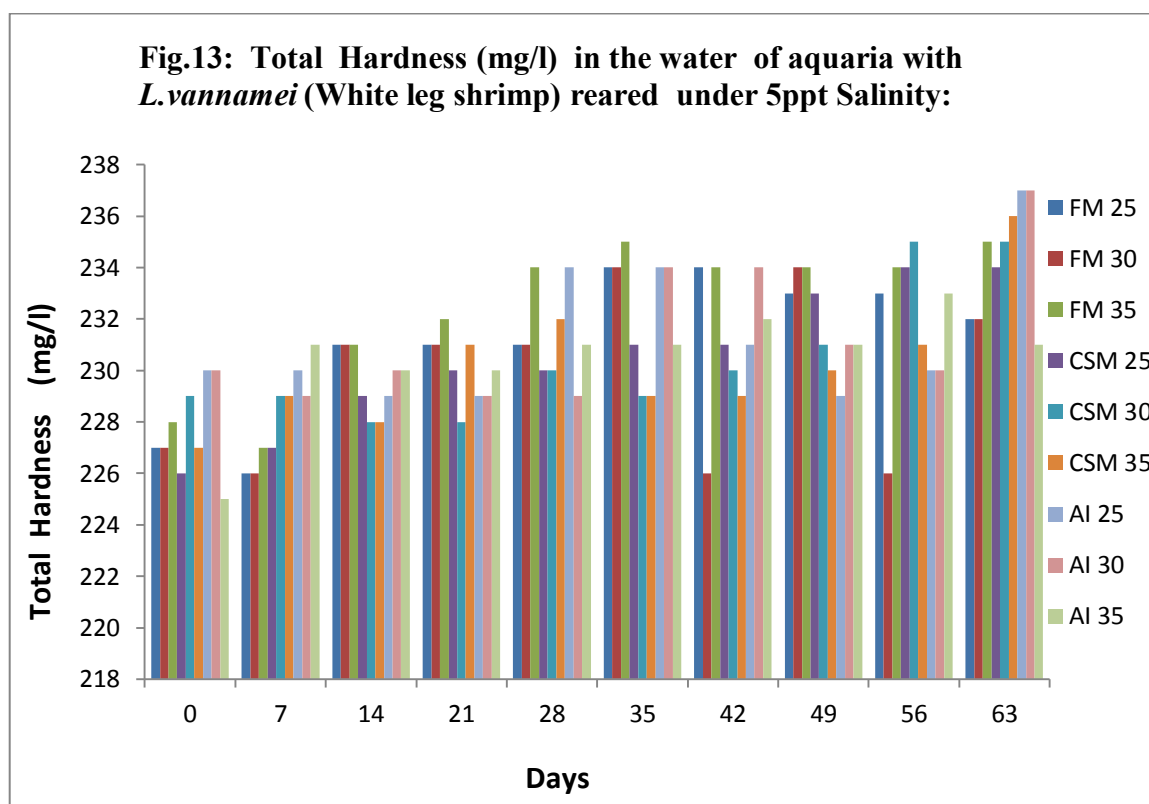
Table.16: Total Hardness (mg/l) in water of aquaria with *L.vannamei*

(White leg shrimp) reared at 5ppt Salinity:

TREATMENT DAYS	TREATMENT FM			TREATMENT CSM			TREATMENT AI		
	T ₁	T ₂	T ₃	T ₄	T ₅	T ₆	T ₇	T ₈	T ₉
	FM 25	FM 30	FM 35	CSM 25	CSM 30	CSM 35	AI 25	AI 30	AI 35
Initial	227	227	228	226	229	227	230	230	225
07	226	226	227	227	229	229	230	229	231
14	231	231	231	229	228	228	229	230	230
21	231	231	232	230	228	231	229	229	230
28	231	231	234	230	230	232	234	229	231
35	234	234	235	231	229	229	234	234	231
42	234	226	234	231	230	229	231	234	232
49	233	234	234	233	231	230	229	231	231
56	233	226	234	234	235	231	230	230	233
63	232	232	235	234	235	236	237	237	231

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



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

Table.17: Total Hardness (mg/l) in water of aquaria with *L.vannamei*

(White leg shrimp) reared at 15ppt Salinity:

TREATMENTS DAYS	TREATMENT FM			TREATMENT CSM			TREATMENT AI		
	T10	T11	T12	T13	T14	T15	T16	T17	T18
	FM 25	FM 30	FM 35	CSM 25	CSM 30	CSM 35	AI 25	AI 30	AI 35
Initial	684	680	674	673	666	671	675	668	676
07	660	676	667	664	676	670	664	672	666
14	674	680	662	671	678	666	668	674	668

21	678	676	668	665	662	668	662	670	662
28	680	678	662	662	670	672	668	676	668
35	678	680	670	670	676	678	670	680	674
42	680	678	675	674	678	680	674	674	680
49	678	672	672	680	680	666	670	672	676
56	676	678	678	672	678	676	676	678	678
63	680	678	680	680	680	680	680	680	678

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

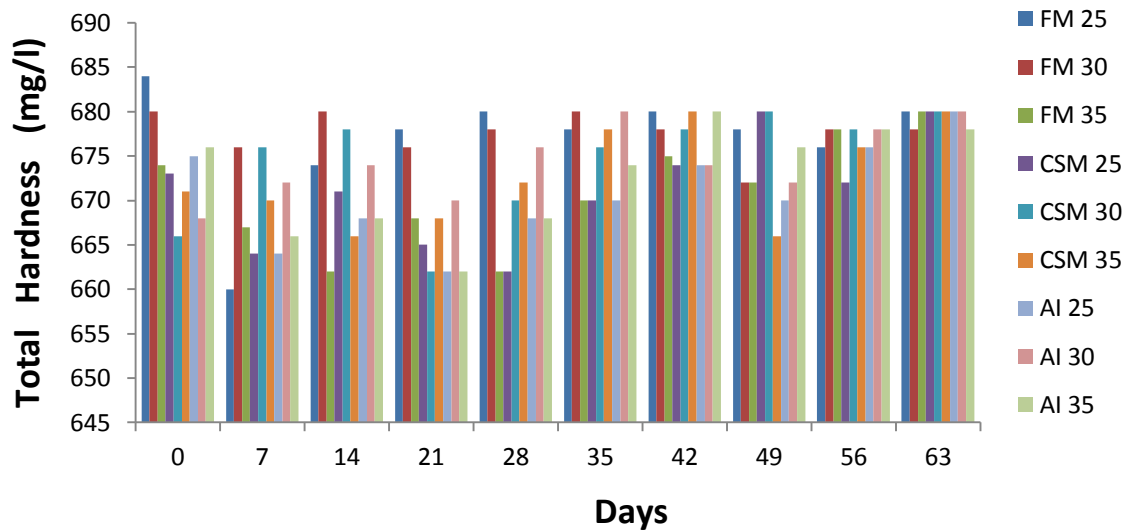
CSM35 : Cottonseed meal containing 35% crude protein

AI25 : *Acetes indicus* meal containing 25% crude protein

AI30 : *Acetes indicus* meal containing 30% crude protein

AI35 : *Acetes indicus* meal containing 35% crude protein

Fig.14: Total Hardness (mg/l) in the water of aquaria with *L.vannamei* (White leg shrimp) reared under 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 : *Acetes indicus* meal containing 25% crude protein

AI30 : *Acetes indicus* meal containing 30% crude protein

AI35 : *Acetes indicus* meal containing 35% crude protein

Table.18: Total Hardness (mg/l) in water of aquaria with *L.vannamei* (White leg shrimp) reared at 30ppt Salinity:

TREATMENTS DAYS	TREATMENT FM			TREATMENT CSM			TREATMENT AI		
	T ₁₉ FM 25	T ₂₀ FM 30	T ₂₁ FM 35	T ₂₂ CSM 25	T ₂₃ CSM 30	T ₂₄ CSM 35	T ₂₅ AI 25	T ₂₆ AI 30	T ₂₇ AI 35
Initial	692	695	697	691	692	693	697	699	698
07	705	706	700	701	702	710	708	706	707
14	709	710	715	713	715	711	714	711	713
21	712	719	712	719	711	719	716	710	709
28	702	705	709	701	708	702	700	701	706
35	720	723	721	729	726	727	724	728	727
42	710	714	715	711	716	719	713	711	709
49	725	728	720	722	723	721	727	732	730
56	733	731	737	732	738	730	735	734	736
63	742	741	741	740	743	740	742	740	741

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

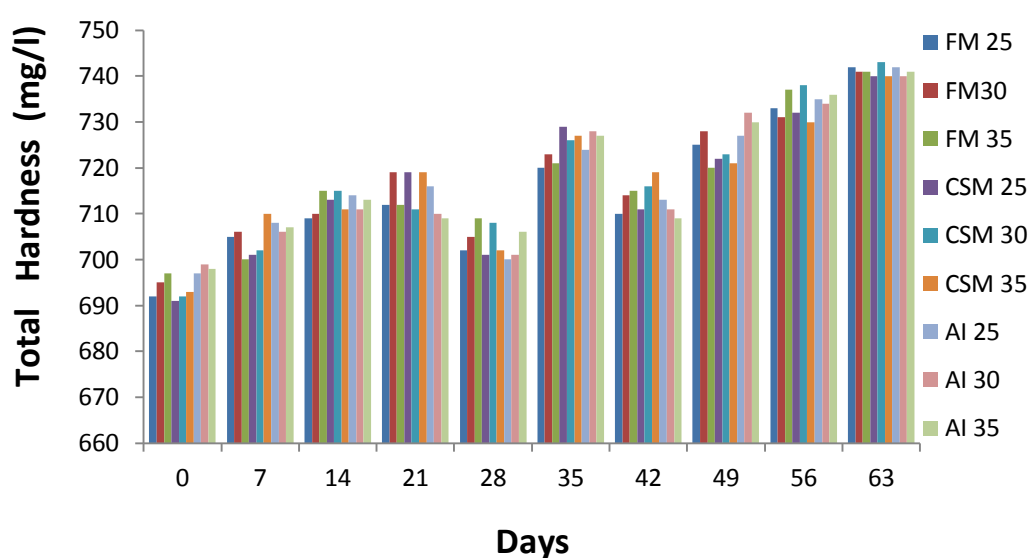
CSM35 : Cottonseed meal containing 35% crude protein

AI25 : *Acetes indicus* meal containing 25% crude protein

AI30 : *Acetes indicus* meal containing 30% crude protein

AI35 : *Acetes indicus* meal containing 35% crude protein

Fig. 15: Total Hardness (mg/l) in the water of aquaria with *L.vannamei* (White leg shrimp) reared under 30ppt 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

4.2. GROWTH PERFORMANCE OF *LITOPENAEUS VANNAMEI*

(WHITE LEG SHRIMP) FED ON DIFFERENT DIETS:

Weight of shrimp in grams and weight increment data observed weekly for different treatments at different salinities were presented below:

Observations on the growth of *L.vannamei* reared at 5ppt Salinity during the first week (07th day) revealed that weight increment varied between 1.33 and 1.69gm for treatment CSM35 and both AI35, FM 35 respectively. Highest and lowest average weight values were observed in the treatments AI35 (3.25 ± 0.12) and CSM35 (2.91 ± 0.09). On the 14th day highest increment of 0.89gms and lowest increment of 0.62 gms were recorded for the FM25 and FM35 respectively. Highest average weight values (4.01 ± 0.23) and lowest average weight values (3.65 ± 0.41) were recorded for AI30 and CSM25 respectively during the second sample (14th day). On the 21st day, The highest and lowest weight growth of increment observed were 0.80 and 0.56gms for FM35 and CSM25 respectively, while the highest and lowest average weight values observed were (4.80 ± 0.31) and (4.21 ± 0.07) for AI30 and CSM25 respectively. Treatments AI30 and AI 25 stood in second and third positions with growth in weight of 0.79 and 0.77gms respectively. During the 28th day, the highest and lowest weight increments observed were 0.82 and 0.66gms for CSM30, AI35 and FM35 respectively. The highest and lowest average weight values observed were (5.55 ± 0.06) and (5.01 ± 0.14) for AI30 and CSM25 respectively. Treatments CSM35, CSM25 stood in second, AI30 in third positions with weight growth of 0.80 and 0.75gms respectively. During the 35th day of the experiment, highest and lowest weight growth increments observed were 0.99 and 0.66 gms for FM35 and FM25 respectively. The highest and lowest average weight values observed were (6.45 ± 0.30) and (5.83 ± 0.07) for AI30 and CSM25 respectively. AI35 stood in second and both AI30,

CSM30 in third positions with growth weight gain of 0.91 and 0.90gms respectively. On the 42nd day highest increment of 1.21gms and lowest increment of 0.73gms were recorded for the AI30 and FM35 respectively. Highest average weight values 7.66 ± 0.23 and lowest average weight values 6.81 ± 0.10 were recorded for AI30 and CSM25 respectively. On the 49th day highest increment of 1.14gms and lowest increment of 0.89gms were recorded for the AI25 and CSM25 respectively. Highest average weight values 8.59 ± 0.13 and lowest average weight values 7.70 ± 0.15 were recorded for AI30 and CSM25 respectively. On the 56th day, highest increment of 1.09 and lowest increment of 0.83 were recorded for the AI35 and AI25 respectively. Highest average weight values of 9.67 ± 0.13 from AI30 and lowest average weight values of 8.65 ± 0.16 from CSM25. At the end of the experiment, i.e., on the 63rd day highest average weight values of 11.05 and lowest average weight values of 9.95 from AI30 and CSM25 respectively. An overall study indicated that the AI30 recorded total weight increment of 9.54 gm in the 63 days experimental period. This was followed by the AI35 (9.37gm), FM30 (8.70) and AI25 (8.67gm), they stood in second, third and fourth positions respectively.

The growth data was subjected to analysis of variance (ANOVA) at 1% and 5% level of significance and the observations are given in table 20. The statistical analysis has shown that F-value is found to be significant among treatments. Since F-value is found to be significant, the pair wise comparison of any two Treatments could be done by computing RBD two way classifications. The Treatment AI30 is found to be significantly superior when compare to other Treatments. The second and third positions were occupied by AI35 and FM30, FM35 respectively. There was a significant difference between the culture periods also.

Observations on the growth of *L.vannamei* reared at 15ppt Salinity during the first week (07th day) revealed that weight increment varied between 1.6 and 1.94 for treatment FM25, CSM25 and AI35 respectively. Highest and lowest average weight values were observed in the treatments AI35 (3.49 ± 0.13) and FM25 (3.15 ± 0.11), CSM25 (3.15 ± 0.06). On the 14th day highest increment of

0.86gms and lowest increment of 0.54 gms were recorded for the FM25 and AI25 respectively. Highest average weight values (4.15 ± 0.06) and lowest average weight values (3.88 ± 0.10) were recorded for FM35 and CSM25 respectively during the second sample (14th day). On the 21st day, The highest and lowest weight growth of increment observed were 1.04 and 0.56 gms for AI30 and CSM25 respectively, while the highest and lowest average weight values observed were (5.05 ± 0.21) and (4.44 ± 0.08) for AI35 and CSM25 respectively. Treatments AI35 and FM35 stood in second and third positions with growth in weight of 1.00 and 0.86gms respectively. During the 28th day, the highest and lowest weight increments observed were 0.95 and 0.55 gms for both AI35 and AI25 respectively. The highest and lowest average weight values observed were (6.0 ± 0.22) and (5.05 ± 0.01) for AI35 and CSM25 respectively. Treatments FM25 stood in second, FM35 in third positions with weight growth of 0.75 and 0.74gms respectively. During the 35th day of the experiment, highest and lowest weight growth increments observed were 1.09 and 0.49gms for CSM30 and AI35 respectively. The highest and lowest average weight values observed were (6.50 ± 0.32) and (6.0 ± 0.08) for AI30 and CSM25 respectively. CSM 35 stood in second and CSM25 in third positions with growth weight gain of 1.0 and 0.95gms respectively. On the 42nd day highest increment of 1.4gms and lowest increment of 0.73gms were recorded for the AI35 and FM30 respectively. Highest average weight values 7.89 ± 0.24 and lowest average weight values 7.0 ± 0.14 were recorded for AI30, AI35 and FM25, CSM25 respectively. On the 49th day highest increment of 1.16gms and lowest increment of 0.83 gms were recorded for the FM35 and CSM25 respectively. Highest average weight values 8.75 ± 0.14 and lowest average weight values 7.83 ± 0.14 were recorded for AI30 and CSM25 respectively. On the 56th day, highest weight increment values of 1.24gms and lowest weight increment values of 0.93gms were recorded for AI30 and AI25 respectively. Highest average weight values of 9.99 ± 0.15 from AI30 and lowest average weight values of 8.89 ± 0.17 from CSM25. At the end of the experiment, i.e., on the 63rd day highest average weight values of 12.01gms and lowest average weight values of 10.01 from AI30 and CSM25 respectively. An overall study indicated that the AI30 recorded total weight increment of 10.51gm

in the 63 days experimental period. This was followed by the AI35 (10.35gm), AI25 (9.05gms) and CSM35 (8.88gm), they stood in second, third and fourth positions respectively.

The growth data was subjected to analysis of variance (ANOVA) at 1% and 5% level of significance and the observations are given in table 24. The statistical analysis has shown that F-value is found to be significant among treatments. Since F-value is found to be significant, the pair wise comparison of any two Treatments could be done by computing RBD two way classifications. The Treatment AI35 is found to be significantly superior when compare to other Treatments. The second and third positions were occupied by AI30 and FM35 respectively. There was a significant difference between the culture periods also.

Observations on the growth of *L.vannamei* reared at 30ppt Salinity during the first week (07th day) revealed that weight increment varied between 2.0 and 1.60gms for treatment AI35 and CSM25 respectively. Highest and lowest average weight values were observed in the treatments AI35 (3.55 ± 0.14) and CSM25 (3.14 ± 0.11). On the 14th day highest increment of 0.98gms and lowest increment of 0.61gms were recorded for the FM25 and AI25 respectively. Highest average weight values (4.25 ± 0.57) and lowest average weight values (4.0 ± 0.19) were recorded for AI35 and AI25 respectively during the second sample (14th day). On the 21st day, The highest and lowest weight growth of increment observed were 0.96 and 0.55gms for AI30 and CSM25 respectively, while the highest and lowest average weight values observed were (5.55 ± 0.22) and (4.56 ± 0.11) for AI35 and CSM25 respectively. Treatments AI35, FM35 and AI25 stood in second and third positions with growth in weight of 0.90 and 0.85gms respectively. During the 28th day, the highest and lowest weight increments observed were 0.95 and 0.32gms for both AI35 and CSM35 respectively. The highest and lowest average weight values observed were (6.10 ± 0.23) and (5.15 ± 0.03) for AI35 and CSM25 respectively. Treatments AI30 stood in second, FM35 in third positions with weight growth of 0.90 and 0.80gms respectively. During the 35th day of the experiment, highest and lowest weight growth increments observed were 1.03 and 0.44gms for CSM35 and FM25 respectively. The highest

and lowest average weight values observed were (6.66 ± 0.25) and (6.05 ± 0.13) for AI35 and FM25 respectively. CSM 30 stood in second and CSM25 in third positions with growth weight gain of 1.02 and 0.95 g0ms respectively. On the 42nd day highest increment of 1.39gms and lowest increment of 0.65gms were recorded for the AI30 and FM35 respectively. Highest average weight values 7.99 ± 0.25 and lowest average weight values 7.0 ± 0.17 were recorded for AI30, AI35 and FM25, CSM25 respectively. On the 49th day highest increment of 1.31gms and lowest increment of 0.90gms were recorded for the AI30 and FM25, CSM25, CSM30 respectively. Highest average weight values 9.30 ± 0.15 and lowest average weight values 7.90 ± 0.17 were recorded for AI30 and CSM25, FM25 respectively. On the 56th day, highest weight increment values of 1.35gms and lowest weight increment values of 0.71gms were recorded for CSM30 and AI30 respectively. Highest average weight values of 10.02 ± 0.15 from AI35 and lowest average weight values of 8.99 ± 0.21 from FM25. At the end of the experiment, i.e., on the 63rd day highest average weight values of 12.22gms and lowest average weight values of 10.0 from AI30 and CSM25 respectively. An overall study indicated that the AI30 recorded total weight increment of 10.68 gm in the 63 days experimental period. This was followed by the AI35 (10.45gm), AI25 (9.22gms) both FM30 and FM35 (8.85gm), they stood in second, third and fourth positions respectively.

The growth data was subjected to analysis of variance (ANOVA) at 1% and 5% level of significance and the observations are given in table 28. The statistical analysis has shown that F-value is found to be significant among treatments. Since F-value is found to be significant, the pair wise comparison of any two Treatments could be done by computing RBD two way classification. The Treatment AI35 is found to be significantly superior when compare to other Treatments. The second and third positions were occupied by AI30 and FM35 respectively. There was a significant difference between the culture periods also.

Table.19: Growth Performance in water of aquaria with *L.vannamei*
(White leg shrimp) reared at 5ppt Salinity:

TREATMENTS DAYS	TREATMENT FM			TREATMENT CSM			TREATMENT AI		
	T ₁	T ₂	T ₃	T ₄	T ₅	T ₆	T ₇	T ₈	T ₉
	FM 25	FM 30	FM 35	CSM 25	CSM 30	CSM 35	AI 25	AI 30	AI 35
Initial	1.53 ±0.19	1.55 ±0.50	1.54 ±0.40	1.57 ±0.17	1.54 ±0.22	1.58 ±0.18	1.58 ±0.30	1.51 ±0.12	1.56 ±0.10
07	3.01 ±0.10	3.21 ±0.20	3.23 ±0.25	2.98 ±0.08	2.99 ±0.14	2.91 ±0.09	3.04 ±0.06	3.15 ±0.11	3.25 ±0.12
14	3.90 ±0.42	3.99 ±0.16	3.85 ±0.05	3.65 ±0.41	3.69 ±0.19	3.71 ±0.42	3.88 ±0.30	4.01 ±0.23	3.99 ±0.55
21	4.65 ±0.10	4.69 ±0.02	4.65 ±0.10	4.21 ±0.07	4.27 ±0.08	4.31 ±0.08	4.65 ±0.03	4.80 ±0.31	4.66 ±0.20
28	5.35 ±0.01	5.39 ±0.18	5.31 ±0.14	5.01 ±0.01	5.09 ±0.06	5.11 ±0.01	5.19 ±0.10	5.55 ±0.21	5.48 ±0.20
35	6.01 ±0.10	6.22 ±0.18	6.30 ±0.10	5.83 ±0.07	5.99 ±0.21	5.90 ±0.08	6.05 ±0.15	6.45 ±0.30	6.39 ±0.22
42	6.91 ±0.13	6.97 ±0.40	7.03 ±0.23	6.81 ±0.10	6.85 ±0.11	6.99 ±0.12	7.01 ±0.12	7.66 ±0.23	7.59 ±0.16
49	7.85 ±0.17	7.99 ±0.10	8.11 ±0.41	7.70 ±0.15	7.89 ±0.22	7.88 ±0.16	8.15 ±0.10	8.59 ±0.13	8.50 ±0.14
56	8.90 ±0.18	8.99 ±0.09	9.00 ±0.40	8.65 ±0.16	8.80 ±0.21	8.79 ±0.17	8.98 ±0.10	9.67 ±0.13	9.59 ±0.14
63	10.05 ±0.10	10.25 ±0.21	10.20 ±0.22	9.95 ±0.08	10.15 ±0.20	10.03 ±0.09	10.25 ±0.11	11.05 ±0.65	10.93 ±0.11

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

Table.20: Statistical Analysis for Growth performance of *L. vannamei*
(White leg shrimp) fed with different experimental diets
reared in 5ppt Salinity

Treatment means	
S.No	Average
Treatment 1	5.816
Treatment 2	5.925
Treatment 3	5.922
Treatment 4	5.636
Treatment 5	5.726

Treatment 6	5.721
Treatment 7	5.878
Treatment 8	6.244
Treatment 9	6.194

Anova Table					
Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F cal	F prob
Replications	9	619.511	68.834	3048.600	3.244
Treatments	8	3.457	0.435	19.117	4.630
Error	72	1.626	0.025	-	-
Total	89	-	-	-	-

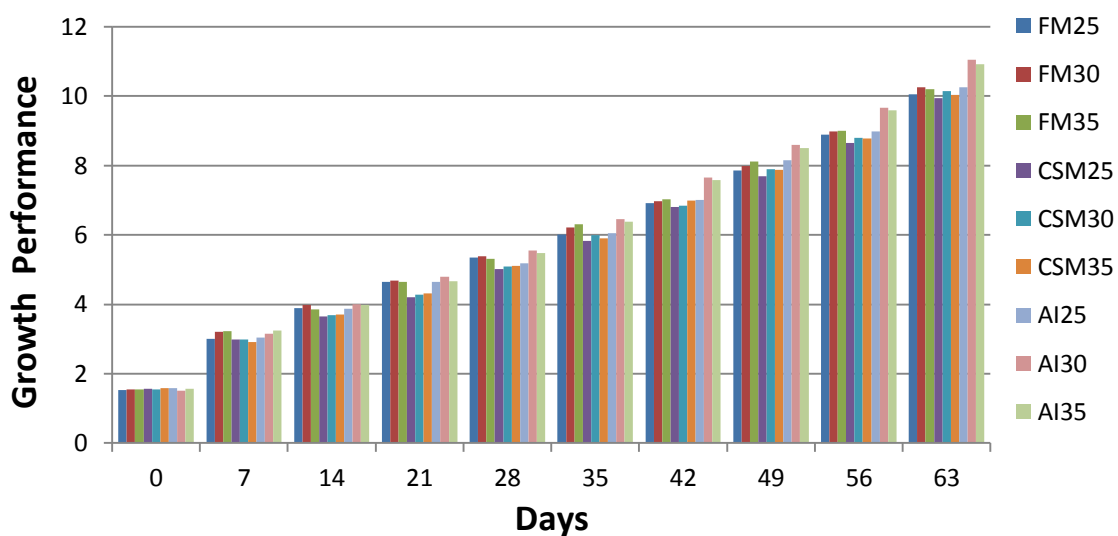
Coefficient of Variation = 2.546

Treatments found Significant at 1% and 5% level of significance CD(0.01) = 0.178 CD(0.05) = 0.139

Comparison of Treatment Means with Critical Difference (0.05)

Treatment No.	T 8	T 9	T 2	T 3	T 7	T 1	T 5	T 6	T 4
Treatment Average	6.244	6.194	5.925	5.922	5.878	5.816	5.726	5.721	5.636
Critical Difference (CD) Compared	a	a	b	b	b	bc	cd	cd	d

Fig.16: Growth Performance of *L.vannamei* (White leg shrimp) fed with different experimental diets 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 : *Acetes indicus* meal containing 25% crude protein

AI30 : *Acetes indicus* meal containing 30% crude protein

AI35 : *Acetes indicus* meal containing 35% crude protein

Table.21: Weight Gain in water of aquaria with *L.vannamei* (White leg shrimp) reared at 5ppt Salinity:

TREATMENTS DAYS	TREATMENT FM			TREATMENT CSM			TREATMENT AI		
	T ₁	T ₂	T ₃	T ₄	T ₅	T ₆	T ₇	T ₈	T ₉
	FM 25	FM 30	FM 35	CSM 25	CSM 30	CSM 35	AI 25	AI 30	AI 35
07	1.48	1.66	1.69	1.41	1.45	1.33	1.46	1.64	1.69
14	0.89	0.78	0.62	0.67	0.70	0.80	0.84	0.86	0.74
21	0.75	0.70	0.8	0.56	0.58	0.60	0.77	0.79	0.67
28	0.70	0.70	0.66	0.80	0.82	0.80	0.54	0.75	0.82
35	0.66	0.83	0.99	0.82	0.90	0.79	0.86	0.90	0.91
42	0.90	0.75	0.73	0.98	0.86	1.09	0.96	1.21	1.20
49	0.94	1.02	1.08	0.89	1.04	0.89	1.14	0.93	0.91
56	1.05	1.00	0.89	0.95	0.91	0.91	0.83	1.08	1.09
63	1.15	1.26	1.20	1.30	1.35	1.24	1.27	1.38	1.34

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

Table.22: Statistical Analysis for Weight gain in *L. vannamei* (White leg shrimp) fed on different experimental diets reared at 5ppt Salinity

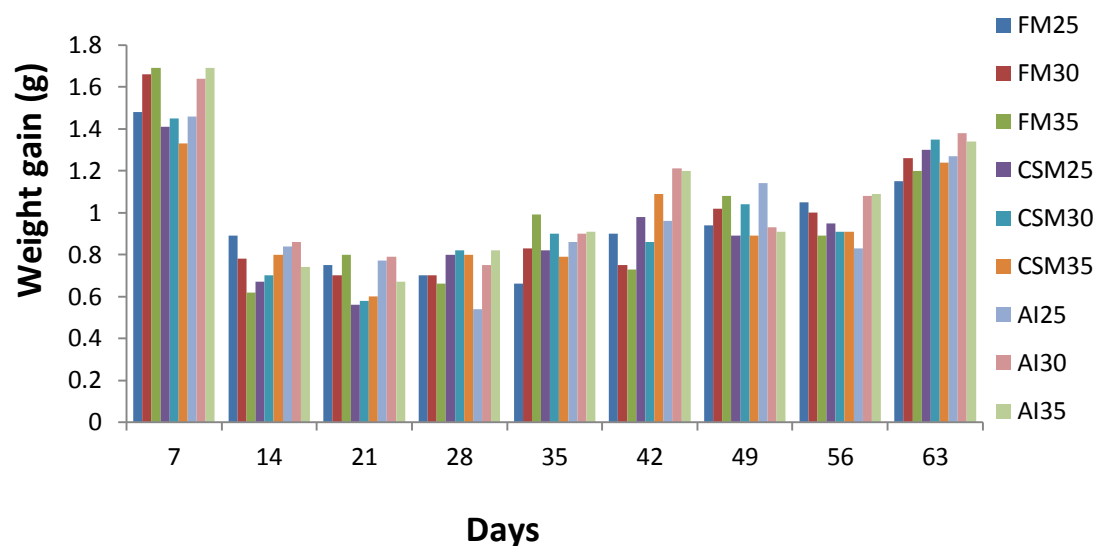
Treatment means	
S.No	Average
Treatment 1	0.946
Treatment 2	0.966
Treatment 3	0.962
Treatment 4	0.931
Treatment 5	0.956
Treatment 6	0.938
Treatment 7	0.963
Treatment 8	1.060
Treatment 9	1.046

Anova Table					
Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F cal	F prob
Replications	8	5.407	0.672	62.278	2.921
Treatments	8	0.158	0.012	1.779	0.097
Error	64	0.699	0.018	-	-
Total	80	-	-	-	-

Coefficient of Variation = 10.684

Treatments found to be Non Significant.

Fig.17: Weight gain in *L. vannamei* (White leg shrimp) fed with different experimental diets 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 : *Acetes indicus* meal containing 25% crude protein

AI30 : *Acetes indicus* meal containing 30% crude protein

AI35 : *Acetes indicus* meal containing 35% crude protein

Table.23: Growth Performance in water of aquaria with *L.vannamei*

(White leg shrimp) reared at 15 ppt Salinity:

TREATMENTS DAYS	TREATMENT FM			TREATMENT CSM			TREATMENT AI		
	T ₁₀	T ₁₁	T ₁₂	T ₁₃	T ₁₄	T ₁₅	T ₁₆	T ₁₇	T ₁₈
	FM 25	FM 30	FM 35	CSM 25	CSM 30	CSM 35	AI 25	AI 30	AI 35
Initial	1.55 ±0.20	1.53 ±0.52	1.55 ±0.41	1.55 ±0.15	1.55 ±0.21	1.57 ±0.18	1.55 ±0.31	1.50 ±0.14	1.55 ±0.11
07	3.15 ±0.11	3.27 ±0.22	3.31 ±0.26	3.15 ±0.06	3.19 ±0.15	3.20 ±0.09	3.35 ±0.07	3.33 ±0.13	3.49 ±0.13
14	4.01 ±0.43	4.11 ±0.18	4.15 ±0.06	3.88 ±0.10	3.95 ±0.20	3.99 ±0.12	3.89 ±0.31	3.95 ±0.24	4.05 ±0.56
21	4.80 ±0.11	4.95 ±0.04	5.01 ±0.11	4.44 ±0.08	4.53 ±0.09	4.61 ±0.09	4.70 ±0.04	4.99 ±0.33	5.05 ±0.21
28	5.55 ±0.02	5.61 ±0.20	5.75 ±0.15	5.05 ±0.01	5.10 ±0.07	5.19 ±0.01	5.25 ±0.11	5.61 ±0.22	6.00 ±0.22
35	6.05 ±0.11	6.29 ±0.20	6.31 ±0.11	6.00 ±0.08	6.19 ±0.22	6.19 ±0.10	6.07 ±0.16	6.50 ±0.32	6.49 ±0.24
42	7.00	7.02 ±0.42	7.09 ±0.24	7.00 ±0.11	7.09 ±0.13	7.10 ±0.13	7.10 ±0.14	7.89 ±0.24	7.89 ±0.18

	±0.14								
49	7.89 ±0.18	8.05 ±0.11	8.25 ±0.42	7.83 ±0.14	7.99 ±0.23	8.01 ±0.15	8.17 ±0.11	8.75 ±0.14	8.74 ±0.16
56	9.00 ±0.19	9.15 ±0.12	9.20 ±0.41	8.89 ±0.17	9.00 ±0.23	9.05 ±0.18	9.10 ±0.12	9.99 ±0.15	9.96 ±0.14
63	10.25 ±0.11	10.39 ±0.22	10.41 ±0.23	10.01 ±0.09	10.20 ±0.20	10.45 ±0.10	10.60 ±0.13	12.01 ±0.66	11.90 ±0.13

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 protei

AI35 : *Acetes indicus* meal containing 35% crude protein

Table.24: Statistical Analysis for Growth performance of *L.vannamei*
(Whiteleg shrimp) fed with different experimental diets
reared in 15ppt Salinity

Treatment means	
S.No	Average
Treatment 10	5.925
Treatment 11	6.037
Treatment 12	6.103
Treatment 13	5.780
Treatment 14	5.879
Treatment 15	5.936
Treatment 16	5.978
Treatment 17	6.452
Treatment 18	6.512

Anova Table					
Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F cal	F prob
Replications	9	648.382	72.040	1200.879	1.022
Treatments	8	5.110	0.632	10.657	9.556
Error	72	4.314	0.059	-	-
Total	89	-	-	-	-

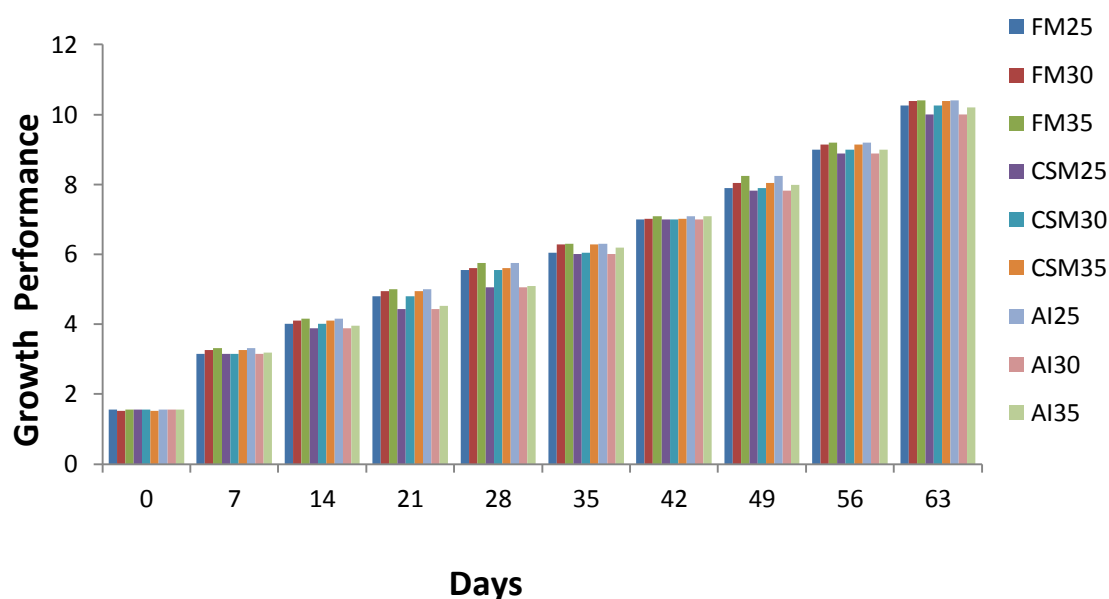
Coefficient of Variation = 4.032

Treatments found Significant at 1% and 5% level of significance CD(0.01) = 0.288 CD(0.05) = 0.213

Comparison of Treatment Means with Critical Difference (0.05)

Treatment No.	T 18	T 17	T 12	T 11	T 16	T 15	T 10	T 14	T 13
Treatment Average	6.512	6.452	6.103	6.037	5.978	5.936	5.925	5.879	5.780
Critical Difference (CD) Compared	a	a	b	bc	bcd	bcd	bcd	cd	d

Fig.18: Growth Performance of *L.vannamei* (White leg shrimp) fed with different experimental diets 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 : *Acetes indicus* meal containing 25% crude protein

AI30 : *Acetes indicus* meal containing 30% crude protein

AI35 : *Acetes indicus* meal containing 35% crude protein

Table.25: Weight Gain in water of aquaria with *L.vannamei* (white leg shrimp) reared at 15ppt Salinity:

TREATMENTS DAYS	TREATMENT FM			TREATMENT CSM			TREATMENT AI		
	T ₁₀	T ₁₁	T ₁₂	T ₁₃	T ₁₄	T ₁₅	T ₁₆	T ₁₇	T ₁₈
	FM 25	FM 30	FM 35	CSM 25	CSM 30	CSM 35	AI 25	AI 30	AI 35
07	1.60	1.74	1.76	1.60	1.64	1.63	1.80	1.83	1.94
14	0.86	0.84	0.84	0.73	0.76	0.79	0.54	0.62	0.56
21	0.79	0.84	0.86	0.56	0.58	0.62	0.81	1.04	1.00
28	0.75	0.66	0.74	0.61	0.57	0.58	0.55	0.62	0.95
35	0.5	0.68	0.56	0.95	1.09	1.00	0.82	0.89	0.49
42	0.95	0.73	0.78	1.00	0.9	0.91	1.03	1.39	1.4
49	0.89	1.03	1.16	0.83	0.9	0.91	1.07	0.86	0.85
56	1.11	1.1	0.95	1.06	1.01	1.04	0.93	1.24	1.22
63	1.25	1.24	1.21	1.12	1.2	1.4	1.5	2.02	1.94

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

Table.26: Statistical Analysis for Weight gain in *L. vannamei* (White leg shrimp) fed with different experimental diets reared at 15pptSalinity

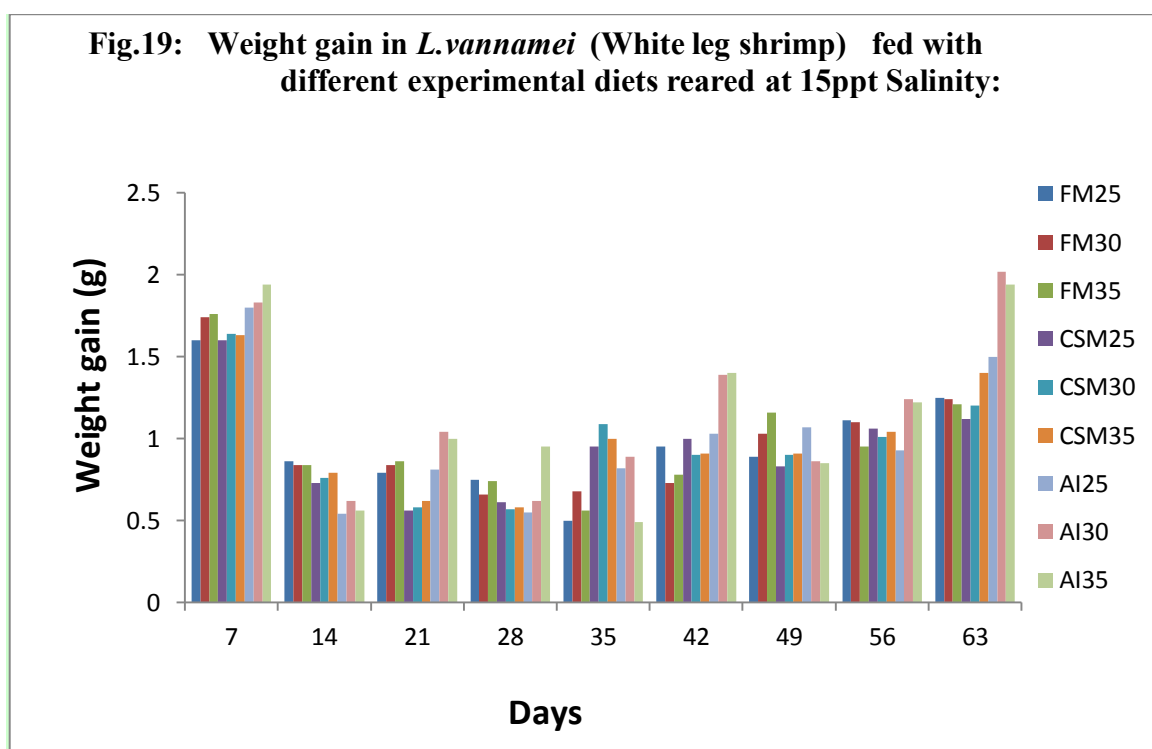
Treatment means	
S.No	Average
Treatment 10	0.966
Treatment 11	0.984
Treatment 12	0.984
Treatment 13	0.940
Treatment 14	0.961
Treatment 15	0.986
Treatment 16	1.005
Treatment 17	1.167

Treatment 18	1.150
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Anova Table					
Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F cal	F prob
Replications	8	8.986	1.124	35.159	1.367
Treatments	8	0.495	0.060	1.941	0.066
Error	64	2.044	0.039	-	-
Total	80	-	-	-	-

Coefficient of Variation = 17.597

Treatments found to be Non Significant



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

Table.27: Growth Performance in water of aquaria with *L.vannamei*

(White leg shrimp) reared at 30ppt Salinity:

TREATMENTS DAYS	TREATMENT FM			TREATMENT CSM			TREATMENT AI		
	T ₁₉ FM 25	T ₂₀ FM 30	T ₂₁ FM 35	T ₂₂ CSM 25	T ₂₃ CSM 30	T ₂₄ CSM 35	T ₂₅ AI 25	T ₂₆ AI 30	T ₂₇ AI 35
Initial	1.55 ±0.21	1.56 ±0.32	1.57 ±0.33	1.54 ±0.20	1.53 ±0.22	1.56 ±0.42	1.53 ±0.53	1.54 ±0.16	1.55 ±0.12
07	3.17 ±0.12	3.25 ±0.07	3.35 ±0.07	3.14 ±0.11	3.20 ±0.16	3.25 ±0.27	3.39 ±0.23	3.40 ±0.15	3.55 ±0.14
14	4.15 ±0.44	4.15 ±0.32	4.20 ±0.31	4.01 ±0.13	4.10 ±0.21	4.10 ±0.08	4.00 ±0.19	4.09 ±0.25	4.25 ±0.57
21	4.90	4.99	5.10	4.56	4.71	4.90	4.85	5.05	5.15

	±0.13	±0.04	±0.04	±0.11	±0.10	±0.13	±0.06	±0.35	±0.22
28	5.61	5.65	5.90	5.15	5.20	5.22	5.50	5.95	6.10
	±0.05	±0.11	±0.10	±0.03	±0.08	±0.17	±0.22	±0.23	±0.23
35	6.05	6.39	6.50	6.10	6.22	6.25	6.10	6.60	6.66
	0.13	±0.15	±0.16	±0.13	±0.23	±0.13	±0.22	±0.33	±0.25
42	7.00	7.05	7.15	7.00	7.10	7.15	7.20	7.99	7.99
	±0.17	±0.15	±0.16	±0.15	±0.14	±0.26	±0.44	±0.25	±0.19
49	7.90	8.10	8.35	7.90	8.00	8.10	8.50	9.30	9.20
	±0.19	±0.12	±0.13	±0.17	±0.25	±0.44	±0.12	±0.15	±0.17
56	8.99	9.25	9.45	9.00	9.35	9.90	9.70	10.01	10.02
	±0.12	±0.13	±0.14	±0.19	±0.25	±0.43	±0.14	±0.16	±0.15
63	10.25	10.41	10.42	10.00	10.24	10.31	10.75	12.22	12.00
	±0.13	±0.14	±0.14	±0.11	±0.22	±0.25	±0.24	±0.68	±0.17

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

**Table.28: Statistical Analysis for Growth Performance of *L. Vannamei* (White leg shrimp) fed with different experimental diets
reared in 30ppt Salinity**

Treatment means	
S.No	Average
Treatment 19	5.858
Treatment 20	6.080
Treatment 21	6.199
Treatment 22	5.840
Treatment 23	5.965
Treatment 24	6.074
Treatment 25	6.152
Treatment 26	6.615
Treatment 27	6.647

Anova Table					
Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F cal	F prob
Replications	9	658.662	73.188	752.196	1.807
Treatments	8	6.919	0.869	8.880	2.250
Error	72	7.002	0.092	-	-
Total	89	-	-	-	-

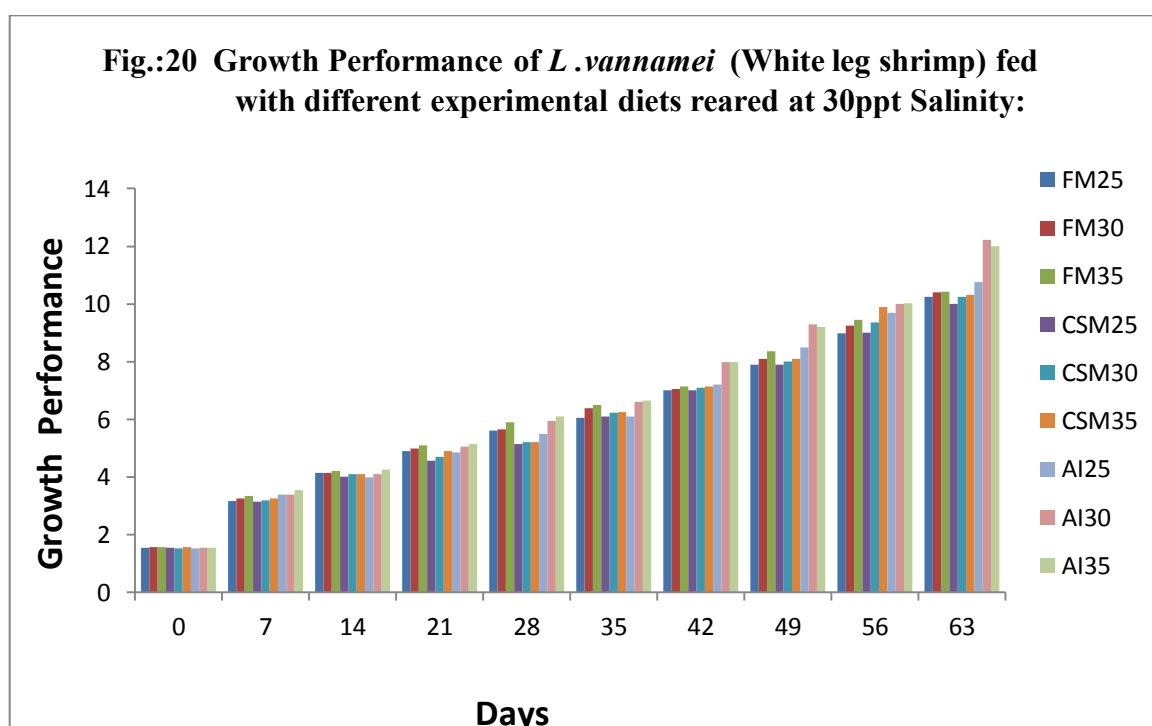
Coefficient of Variation = 5.065

Treatments found Significant at 1% and 5% level of significance

CD (0.01) = 0.361 CD (0.05) = 0.270

Comparison of Treatment Means with Critical Difference (0.05)

Treatment No.	T 27	T 26	T 21	T 25	T 20	T 24	T 23	T 19	T 22
Treatment Average	6.647	6.615	6.199	6.152	6.080	6.074	5.965	5.858	5.840
Critical Difference (CD) Compared	a	a	b	b	bc	bc	bc	c	c



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

Table.29: Weight Gain in water of aquaria with *L.vannamei* (White leg shrimp) reared at 30ppt Salinity:

TREATMENTS DAYS	TREATMENT FM			TREATMENT CSM			TREATMENT AI		
	T ₁₉	T ₂₀	T ₂₁	T ₂₂	T ₂₃	T ₂₄	T ₂₅	T ₂₆	T ₂₇
	FM 25	FM 30	FM 35	CSM 25	CSM 30	CSM 35	AI 25	AI 30	AI 35
07	1.62	1.69	1.78	1.60	1.67	1.69	1.86	1.86	2.00
14	0.98	0.90	0.85	0.87	0.90	0.85	0.61	0.69	0.70
21	0.75	0.84	0.90	0.55	0.61	0.80	0.85	0.96	0.90
28	0.71	0.66	0.80	0.59	0.49	0.32	0.65	0.90	0.95
35	0.44	0.74	0.60	0.95	1.02	1.03	0.60	0.65	0.56
42	0.95	0.66	0.65	0.90	0.88	0.90	1.10	1.39	1.33
49	0.90	1.05	1.20	0.90	0.90	0.95	1.30	1.31	1.21
56	1.09	1.15	1.10	1.10	1.35	1.80	1.20	0.71	0.82
63	1.26	1.16	0.97	1.00	0.89	0.41	1.05	2.21	1.98

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

Table.30: Statistical Analysis for Weight gain in *L. vannamei* (White leg shrimp) fed on different experimental diets reared at 30ppt Salinity

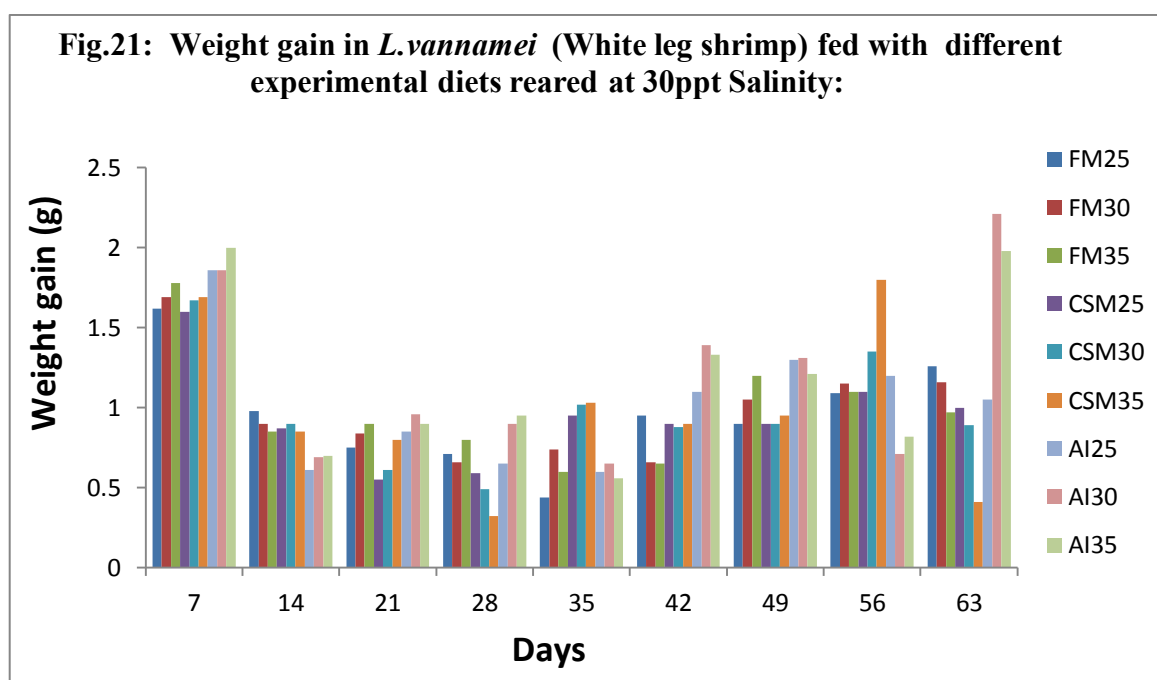
Treatment means	
S.No	Average
Treatment 19	0.966
Treatment 20	1.034
Treatment 21	0.983
Treatment 22	0.940
Treatment 23	0.967
Treatment 24	0.972

Treatment 25	1.024
Treatment 26	1.186
Treatment 27	1.161

Anova Table					
Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F cal	F prob
Replications	8	8.207	1.028	13.971	1.590
Treatments	8	0.564	0.070	0.962	0.466
Error	64	4.695	0.073	-	-
Total	80	-	-	-	-

Coefficient of Variation = 26.392

Treatments found to be Non Significant.



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

4.3. SURVIVAL RATE:

Survival rate of *L. vannamei* (White leg shrimp) fed on experimental diets at 5ppt 15ppt and 30ppt Salinities:

Survival percentages of *L. vannamei* shrimp reared at 5ppt Salinity in various experimental treatments are presented in table 31 and figure 22. The survival percentage throughout the period of experiment was lowest for the CSM25 and highest for FM35, FM30. By the final sampling (63rd day) the survival percentage was (highest) 78.0 for FM30, FM35 and (lowest) 65.0 for CSM25.

The survival data was subjected to analysis of variance (ANOVA) presented in the table 32. Statistical analysis has shown that F- value is found to be significant among treatments. Since F- value is found to be significant, the pair- wise comparison of any two treatments could be done by computing RBD two way classification. The treatment FM30 had shown highest survival rate when compared to the other treatments. The subsequent positions were occupied by Treatments FM35,

FM25, AI35, AI30, AI25 CSM35 followed by CSM30, CSM25. Treatment FM30 have shown significant difference from all other treatments. There was significant difference in between experimental period also.

Survival percentages of *L. vannamei* shrimp reared at 15ppt Salinity in various experimental treatments are presented in table 33 and figure 23. The survival percentage throughout the period of experiment was lowest for the CSM25 and highest for AI30. By the final sampling (63rd day) the survival percentage was (highest) 88.0 for FM35 - and (lowest) 75.0 for CSM25.

The survival data was subjected to analysis of variance (ANOVA) presented in the table 34. Statistical analysis has shown that F- value is found to be significant among treatments. Since F- value is found to be significant, the pair- wise comparison of any two treatments could be done by computing RBD two way classifications. The treatment FM25 had shown highest survival rate when compared to the other treatments. The subsequent positions were occupied by Treatments FM35, AI30, and FM30, AI35 followed by AI25, CSM35, CSM30, and CSM25. Treatment CE30 have shown significant difference from all other treatments. There was significant difference in between experimental period also.

Survival percentages of *L. vannamei* shrimp reared at 30ppt Salinity in various experimental treatments are presented in table 35 and figure 24. The survival percentage throughout the period of experiment was lowest for the CSM25, CSM35 and highest for FM25, FM30, FM35. By the final sampling (63rd day) the survival percentage was (highest) 85.0 for FM30, FM35 - and (lowest) 75.0 for CSM25 and CSM35.

The survival data was subjected to analysis of variance (ANOVA) presented in the table 36. Statistical analysis has shown that F- value is found to be significant among treatments. Since F- value is found to be significant, the pair- wise comparison of any two treatments could be done by computing RBD two way classification. The treatment FM35 had shown highest survival rate when

compared to the other treatments. The subsequent positions were occupied by Treatments FM25, FM30, AI35, AI30 followed by AI25, CSM30, CSM25, and CSM35. There was significant difference in between experimental period also.

Table.31: The percentage of survival in water of aquaria with *L.vannamei* (White leg shrimp) fed with different experimental diets reared at 5ppt Salinity:

TREATMENTS DAYS	TREATMENT FM			TREATMENT CSM			TREATMENT AI		
	T ₁	T ₂	T ₃	T ₄	T ₅	T ₆	T ₇	T ₈	T ₉
	FM 25	FM 30	FM 35	CSM 25	CSM 30	CSM 35	AI 25	AI 30	AI 35
07	95	97	97	90	90	85	92	90	95
14	95	97	90	90	85	85	92	90	95
21	92	97	90	88	85	85	87	90	90
28	92	90	90	83	85	80	80	83	85
35	92	90	88	79	77	80	80	83	85
42	80	90	88	70	77	80	77	75	77
49	80	85	80	70	77	75	77	75	74
56	80	78	80	70	68	74	73	75	74
63	75	78	78	65	68	70	73	73	74

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

**Table.32: Statistical Analysis for percentage of survival of *L. vannamei*
(White leg shrimp) fed with different experimental diets
reared at 5ppt Salinity**

Treatment means	
S.No	Average
Treatment 1	86.777
Treatment 2	89.111
Treatment 3	86.777
Treatment 4	78.333
Treatment 5	79.111
Treatment 6	79.333
Treatment 7	81.222
Treatment 8	81.555
Treatment 9	83.222

Anova Table					
Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F cal	F prob
Replications	8	3804.026	475.500	72.084	4.667
Treatments	8	1091.350	136.417	20.675	4.580
Error	64	422.195	6.598	-	-
Total	80	-	-	-	-

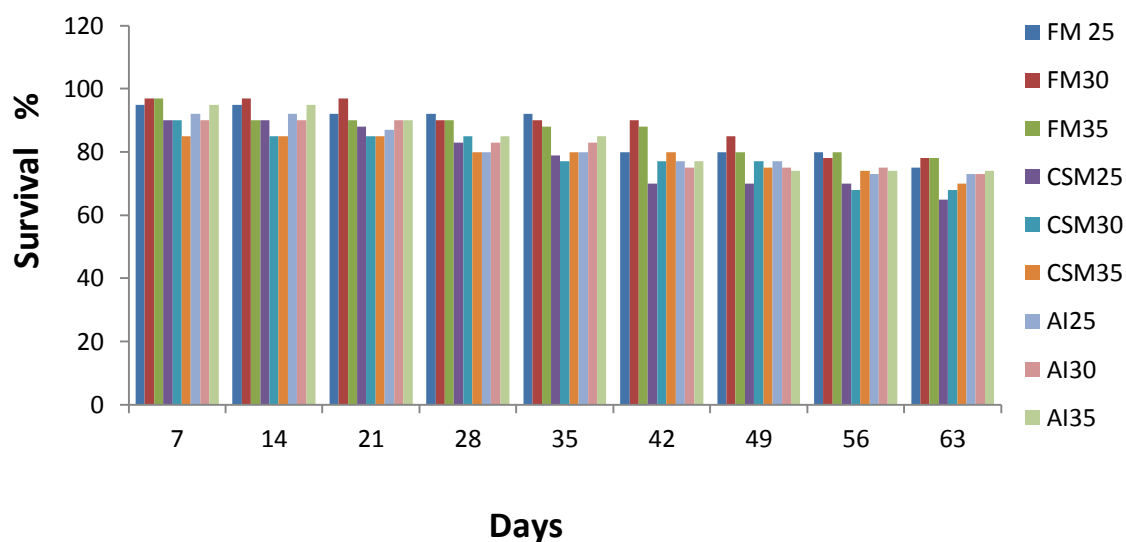
Coefficient of Variation = 3.109

Treatments found Significant at 1% and 5% level of significance CD (0.01) = 3.215 CD (0.05) = 2.411

Comparison of Treatment Means with Critical Difference (0.05)

Treatment No.	T 2	T 3	T 1	T 9	T 8	T 7	T 6	T 5	T 4
Treatment Average	89.111	86.777	86.777	83.222	81.555	81.222	79.333	79.111	78.333
Critical Difference (CD) Compared	a	a	a	b	bc	bcd	cde	de	e

Fig:22: The percentage of Survival of *L.vannamei* (White leg shrimp) 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

Table.33: The percentage of survival in water of aquaria with *L.vannamei*

(White leg shrimp) fed with different experimental diet reared at 15ppt Salinity:

TREATMENTS DAYS	TREATMENT FM			TREATMENT CSM			TREATMENT AI		
	T ₁₀	T ₁₁	T ₁₂	T ₁₃	T ₁₄	T ₁₅	T ₁₆	T ₁₇	T ₁₈
	FM 25	FM 30	FM 35	CSM 25	CSM 30	CSM 35	AI 25	AI 30	AI 35
07	97	97	96	95	95	95	98	98	96
14	93	94	93	91	92	94	95	95	95
21	93	93	90	85	90	90	90	92	92
28	90	89	90	85	90	90	88	92	90
35	90	89	90	81	90	88	88	90	88
42	90	88	90	81	81	85	88	90	85
49	88	88	88	77	81	80	84	88	85
56	88	88	88	77	79	77	82	84	85
63	85	86	88	75	77	77	82	84	84

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

Table.34: Statistical Analysis for Percentage of survival of *L.vannamei* (White leg shrimp) fed with different experimental diets reared at 15ppt Salinity:

Treatment means	
S.No	Average
Treatment 10	90.444
Treatment 11	90.222
Treatment 12	90.333
Treatment 13	83.000
Treatment 14	86.111
Treatment 15	86.222
Treatment 16	88.333
Treatment 17	90.333
Treatment 18	88.888

Anova Table					
Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F cal	F prob
Replications	8	1656.329	207.041	49.666	1.501
Treatments	8	486.329	60.791	14.588	7.012
Error	64	266.791	4.165	-	-
Total	80	-	-	-	-

Coefficient of Variation = 2.316

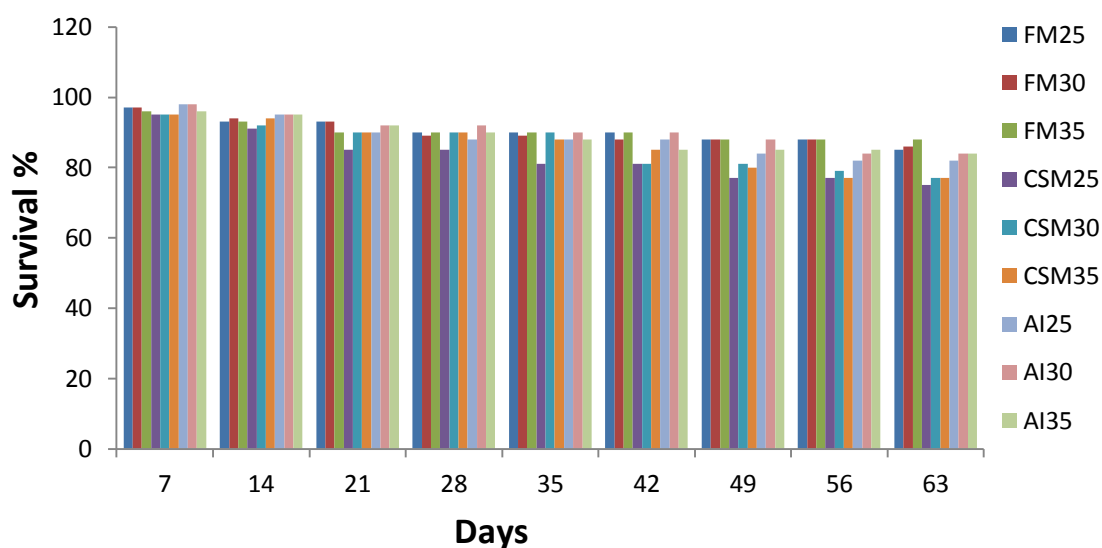
Treatments found Significant at 1% and 5% level of significance

Treatment No.	T 10	T 12	T 17	T 11	T 18	T 16	T 15	T 14	T 13
Treatment Average	90.444	90.333	90.333	90.222	88.888	88.333	86.222	86.111	83.000
Critical Difference (CD) Compared	a	a	a	ab	ab	b	c	c	d

CD (0.01) = 2.553 CD (0.05) = 1.920

Comparison of Treatment Means with Critical Difference (0.05)

Fig.23: The percentage of Survival of *L.vannamei* (White leg shrimp) 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 : *Acetes indicus* meal containing 25% crude protein

AI30 : *Acetes indicus* meal containing 30% crude protein

AI35 : *Acetes indicus* 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

**Table.36: Statistical Analysis for Percentage of survival of
L. vannamei (White leg shrimp) fed with different
experimental diets reared at 30ppt Salinity**

Treatment means	
S.No	Average
Treatment 19	90.111
Treatment 20	90.111
Treatment 21	90.333
Treatment 22	84.000
Treatment 23	84.333
Treatment 24	82.222
Treatment 25	84.666
Treatment 26	85.555
Treatment 27	87.222

Anova Table					
Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F cal	F prob
Replications	8	1496.461	187.056	57.374	2.854
Treatments	8	673.138	84.149	25.811	2.791
Error	64	208.649	3.260	-	-
Total	80	-	-	-	-

Coefficient of Variation = 2.081

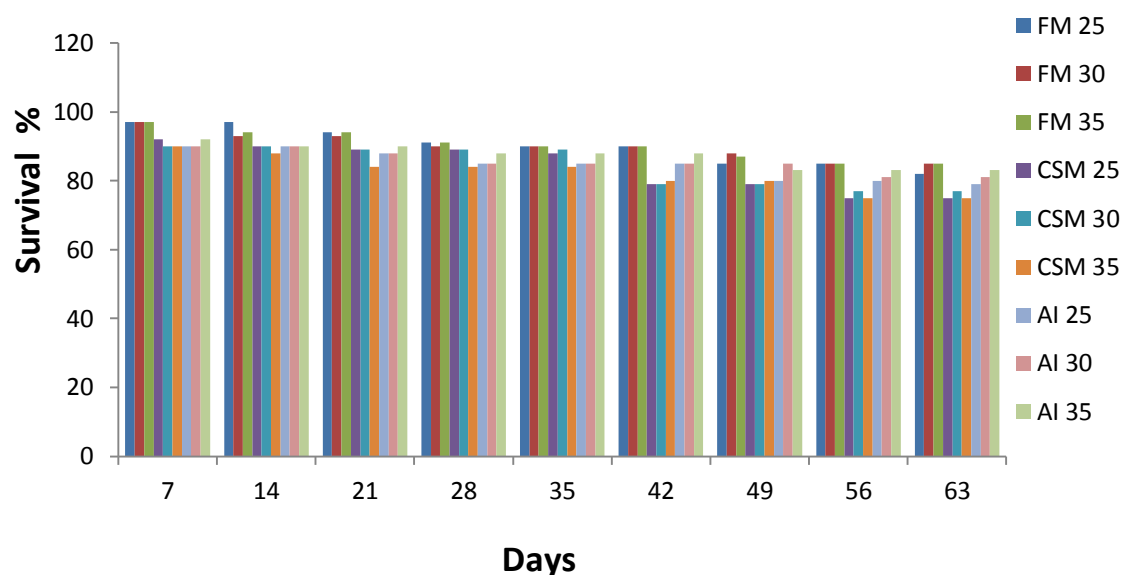
Treatments found Significant at 1% and 5% level of significance

CD (0.01) = 2.257 CD(0.05) = 1.705

Comparison of Treatment Means with Critical Difference (0.05)

Treatment No.	T 21	T 19	T 20	T 27	T 26	T 25	T 23	T 22	T 24
Treatment Average	90.333	90.111	90.111	87.222	85.555	84.666	84.333	84.000	82.222
Critical Difference (CD) Compared	a	a	a	b	bc	c	c	c	d

Fig.24: Percentage of Survival in water of aquaria with *L.vannamei* (white leg shrimp) reared at 30 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

4.4. Specific Growth Rates:

Specific growth rates for *L. vannamei* (White leg shrimp) treated with different diets at three different salinities were calculated and presented in tables 25, 32, 39 and figure 19, 23, 27 respectively at 5ppt 15ppt and 30ppt Salinities. The specific growth rates by end of the experimental period (63 days) were calculated for all treatments reared at 5, 15 and 30 salinities.

Specific growth rates for *L. vannamei* treated with different diets reared at 5 Salinity are as follows: CSM25 up has the lowest Specific Growth Rate of 2.930%. The highest value was in AI30 with 3.159%. The treatments that stood second and third positions was AI35 (3.141) and FM35 (3.0). These were followed by FM30 (2.998), CSM30 (2.993), FM25 (2.987), AI25 (2.968), CSM35 (2.933) respectively.

Specific growth rates for *L. vannamei* treated with different diets reared at 15 Salinity are as follows: CSM25 has the lowest Specific Growth Rate of 2.960%. The highest value was in AI30 with 3.302%. The treatments that stood second and third positions was AI35 (3.235) and AI25 (3.051). These were followed by FM30 (3.040), FM35 (3.023), CSM35 (3.008), FM25 (2.998), CSM30 (2.990) respectively.

Specific growth rates for *L. vannamei* treated with different diets reared at 30 Salinity are as follows: CSM25 has the lowest Specific Growth Rate of 2.969%. The highest value was in AI30 with 3.287%. The treatments that stood second and third positions was AI35 (3.248) and AI25 (3.094). These were followed By CSM30 (3.017), FM30 (3.012), FM35 (3.004), FM25 (2.998) and CSM35 (2.997) respectively.

Table .37: Specific Growth Rate (SGR) in water of aquaria with *L.vannamei* (white leg shrimp) reared at 5ppt Salinity

(white

Treatments	TREATMENT FM			TREATMENT CSM			TREATMENT AI		
	T ₁ FM 25	T ₂ FM 30	T ₃ FM 35	T ₄ CSM 25	T ₅ CSM 30	T ₆ CSM 35	T ₇ AI 25	T ₈ AI 30	T ₉ AI 35
Days									
Initial	1.53	1.55	1.54	1.57	1.54	1.58	1.58	1.51	1.56
Final	10.05	10.25	10.20	9.95	10.15	10.03	10.25	11.05	10.93
SGR	2.987	2.998	3.000	2.930	2.993	2.933	2.968	3.159	3.141

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

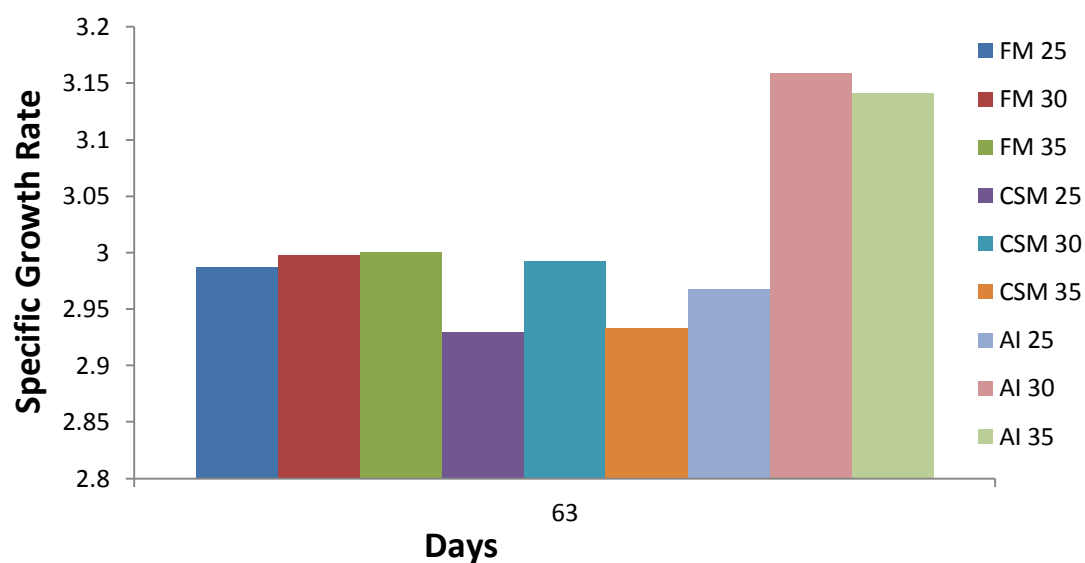
CSM35 : Cottonseed meal containing 35% crude protein

AI25 : *Acetes indicus* meal containing 25% crude protein

AI30 : *Acetes indicus* meal containing 30% crude protein

AI35 : *Acetes indicus* meal containing 35% crude protein

Fig:25. Specific Growth Rates in *L.vannamei* (White leg shrimp) 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

Table .38: Specific Growth Rate (SGR) in water of aquaria with *L.vannamei*

(white leg shrimp) reared at 15ppt Salinity:

Treatment Days	TREATMENT FM			TREATMENT CSM			TREATMENT AI		
	T ₁₀ FM25	T ₁₁ FM30	T ₁₂ FM35	T ₁₃ CSM25	T ₁₄ CSM30	T ₁₅ CSM35	T ₁₆ AI25	T ₁₇ AI30	T ₁₈ AI35
Initial	1.55	1.53	1.55	1.55	1.55	1.57	1.55	1.50	1.55
Final	10.25	10.39	10.41	10.01	10.20	10.45	10.60	12.01	11.90
SGR	2.998	3.040	3.023	2.960	2.990	3.008	3.051	3.302	3.235

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

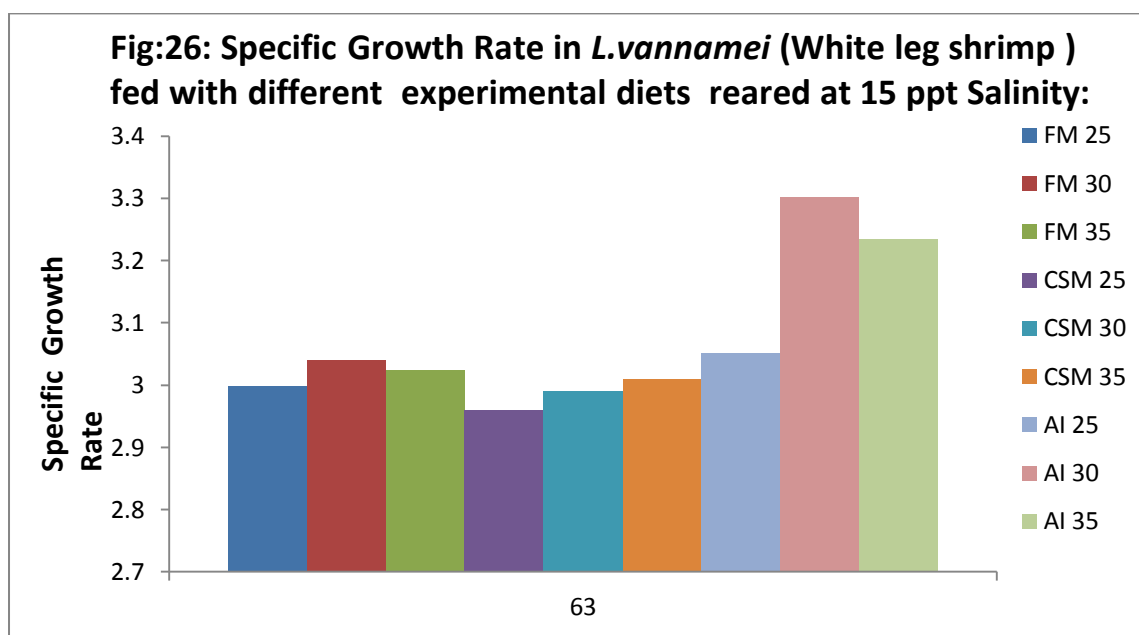
CSM30 : Cottonseed meal containing 30 % crude protein

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



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

**Table 39: Specific Growth Rate (SGR) in water of aquaria with
(white leg shrimp) reared at 30ppt Salinity:**

L.vannamei

Treatments Days	TREATMENT FM			TREATMENT CSM			TREATMENT AI		
	T ₁₉ FM 25	T ₂₀ FM 30	T ₂₁ FM 35	T ₂₂ CSM 25	T ₂₃ CSM 30	T ₂₄ CSM 35	T ₂₅ AI 25	T ₂₆ AI 30	T ₂₇ AI 35
Initial	1.55	1.56	1.57	1.54	1.53	1.56	1.53	1.54	1.55
Final	10.25	10.41	10.42	10.00	10.24	10.31	10.75	12.22	12.00
SGR	2.998	3.012	3.004	2.969	3.017	2.997	3.094	3.287	3.248

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

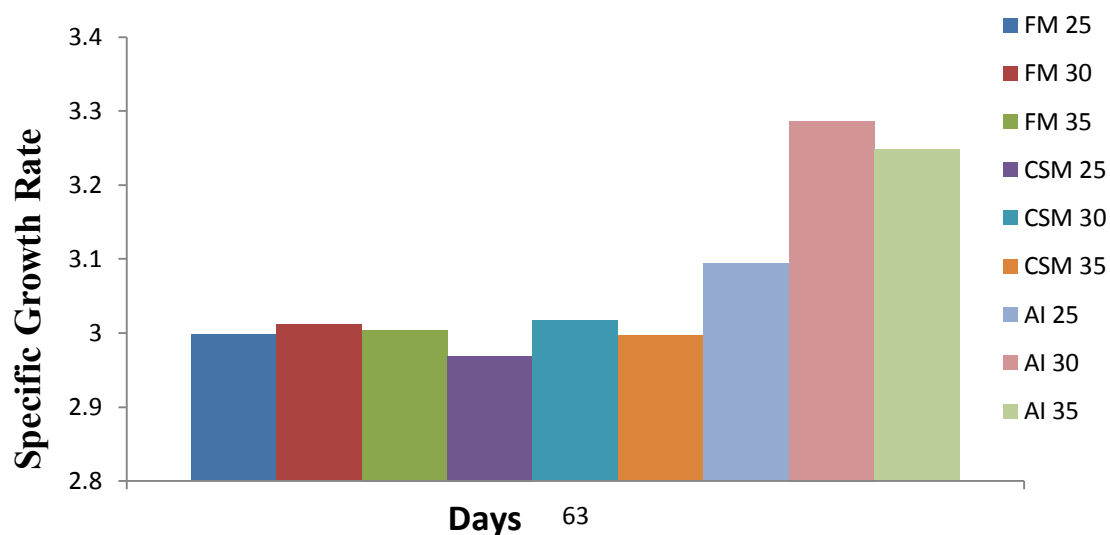
CSM35 : Cottonseed meal containing 35% crude protein

AI25 : *Acetes indicus* meal containing 25% crude protein

AI30 : *Acetes indicus* meal containing 30% crude protein

AI35 : *Acetes indicus* meal containing 35% crude protein

Fig:27:Specific Growth Rates in *L.vannamei* (White leg shrimp) fed with different experimental diets reared at 30 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

4.5. FEED CONVERSION RATIO:

Feed Conversion Ratio in *L. vannamei* (White leg shrimp) fed different experimental diets:

The Feed Conversion Ratio in different experiments of *L. vannamei* groups reared at three different salinities: 5ppt, 15ppt and 30ppt were calculated and presented in the table 40. During the final day of the sampling, the highest Food Conversion Ratio value 1.66 (FM30) at 5ppt and lowest value of 3.30 for treatment of (AI30) at 30ppt. The treatments that stood second and third positions were CSM25 in 30 ppt (2.95) and CSM25 in 5ppt (2.92). These were followed by CSM25 (2.90) in 15ppt, CSM35 (2.69) in 5ppt, CSM35 (2.60) in both 15 & 30ppt, CSM 30 (2.55) at 30ppt.

Table 40: Feed Conversion Ratios of *L.vannamei* (White leg shrimp) fed with

SALINITIES TREATMENTS	5 % Salinity	15 % Salinity	30 % Salinity
FM 25	1.89	1.81	1.91
FM 30	1.66	1.70	2.00
FM 35	1.75	1.72	1.95
CSM 25	2.92	2.90	2.95
CSM 30	2.52	2.48	2.55
CSM 35	2.69	2.60	2.60
AI 25	2.10	2.20	2.39
AI 30	2.12	2.27	2.30
AI 35	2.19	2.33	2.35

different diets in water of aquaria 5 ppt, 15 ppt and 30 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 : *Acetes indicus* meal containing 25% crude protein

AI30 : *Acetes indicus* meal containing 30% crude protein

AI35 : *Acetes indicus* meal containing 35% crude protein

5. Discussion

The fastest growing forms of aquaculture, now accounts for one-third of the shrimp produced globally. It is a rich source of animal protein and its culture is an efficient protein food production system from aquatic environment. The main role of shrimp culture is its contribution in improving the nutritional standards of the people. 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, 2004; Liao, 2011). 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 (Thomas, 1980; Varadarajan, 2013).

5.1. Protein:

Although a 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) fed post larval and juvenile *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. The dietary protein requirement by post larval shrimp was reported as 30-35%. Also, it was determined that juveniles have a dietary protein requirement of less than 30%. It has also been shown with shrimp that size (weight) affects growth response relative to the protein content of the diet.

Smith et al. (1984) studied 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. Dietary protein content only affected growth for 4.0g shrimp, with a significant increase in weight gain corresponding to the increase in dietary protein content. However, the range of the protein levels of

diets used in this study was too narrow to estimate an optimum protein level. 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.

Another study conducted by Khureshy and Davis, 2000, juveniles shrimp exhibited significantly higher weight gain, percent weight gain, FE, and PCE when fed the 32% protein diet as compared with the 16% protein diet. Juveniles fed the 16% protein diet did not reach a point at which growth did not increase as protein ration increased, and a polynomial regression best described the growth curve. 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. Sub-adult shrimp fed the 32% protein diet had significantly higher weight gain, FE, and PCE on an isoproteic basis than those fed the 16% protein diet.

Results of other research conducted in Honduras indicate that 30% protein feed did not result in increased shrimp production compared to 20% protein feed (Teichert-Coddington *et al.*, 1997). Shrimp yield and growth were similar when shrimp were stocked in ponds at 5 to 11 *L.vannamei* m⁻² and offered a 20 or 40% protein formulated ration (Teichert-Coddington and Rodriguez, 1995).

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 ahypocholesterolemic 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) *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.

5.2. Water:

In the present study optimum range of temperature for *L. vannamei* was between 29.1 to 31.6°C as observed in earlier studies. 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 26 °C and 33 °C (Nunes 2000) and 22 °C and 32 °C.

During their experimental period, water temperatures remained between 27.5-58.5° C and 26.5-28.5° C for groups 1-4 & group 5 respectively. During the experimental period, ambient water temperature ranged between 18 and 22° 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).

The optimal temperature for growth 27-30°C (Ponce-Palafox *et al.*, 1997). Other authors have suggested that temperature is a key factor affecting growth and production of shrimp

(Teichert-Coddington *et al.*, 1994; Jackson and Wang, 1998). *L. vannamei* grew more slowly when the temperature was 22°C or below, with an optimal growth temperature of 23-34°C.

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. In the present study pH of 7.7 to 8.5 was recorded for all salinities.

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). According to (Boyd 2000), 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), (Boyd, 2003) 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. 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 between 5.02 and 8.42ppm at 5ppt, 6.2 and 8.52ppm at 15ppt, 7.23 to 8.99ppm at 30 ppt.

For best performance, 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.

Seidman and Lawrence (1985) reported the critical DO point for shrimp as being below 2 mg/l. Aragon and Garcia (1996) recommend that the minimum oxygen concentration for optimal shrimp growth be approximately 5 mg/l. Brock and Main, 1994 stated that average oxygen concentration for each treatment was above the range considered stress inducing (approximately 3.0 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 salinity values were well within the appropriate ranges for *L. vannamei* culture as it correlates with the Treece 2000 and Audelo-Naranjo *et al.*, 2012. The values of water quality parameters reveal that all these are in the acceptable range for survival and growth for *L.vannamei*.

5.3. Replacement of Fish meal:

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). 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.

Results from the study of 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.

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)

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 and animal protein source like *Acetes indicus* meal, both are locally available and cheaper than fish meal.

5.4. Plant Protein Source- Cottonseed Meal:

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. (Venero *et al.*, 2008)

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.

Currently, tests are being conducted using a diet without animal proteins or fish meal (Davis *et al.*, 2004b). Inclusion of cottonseed meal in an all-plant protein feed may be desirable not only for economic reasons but it may also improve palatability. (Robinson *et al.*, 1994)

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. (Watanabe *et al.*, 1995; Regost *et al.*, 1999; Soltan *et al.*, 2008)

Cottonseed meal usually contains 0.4 to 1.7% gossypol. Free gossypol, when present in large quantity in the diet, has been shown to be toxic to mono –gastric animals including fish. 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.

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).

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). Higher levels of replacement with cottonseed meal resulted in reduced performance of the shrimp, presumably due to the gossypol content of cottonseed meal.

Lim (1996) demonstrated that 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.

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.

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.

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)

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 ($P < 0.05$). It 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. (Wang 2010).

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. In the present study Iron supplementation was done.

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 wk. These adverse effects were probably due to the toxicity of free gossypol.

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.

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. Animal Protein-*Acetes indicus*:

In a study of the nutritional quality of rendered meat and bone meals (MBM) as a feed ingredient in diets for juvenile *L.vannamei* under experimental intensive rearing conditions, Forster *et al.*, (2003) concluded that MBM could effectively replace between 25 and 75% of fish meal in 35% protein diets, depending on MBM source; a general decrease in growth above 25% replacement was observed for all MBM sources. In theory, 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 this 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 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, Teshima, Koshio & Ishikawa 1997; D Abramo 1998; Roustaiian, Kamarudin, Omar, Saad & Ahmad 1999; Mitra *et al.*, 2005. (Anh *et al.*, 2009).

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. (Anh *et al.*, 2009)

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.

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 CSM diet, the growth response was also better.

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). FCR values obtained in the present investigation (2.10-2.39) 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.

5.6. 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.

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.

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

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) However, there are disadvantages to the culture of *L.vannamei* in that they do not normally grow as large as *P.monodon* and *P.stylostris* and cannot access the lucrative market for larger sized shrimp which have a much higher price per kilo.

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 our 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, % C) 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, % C) 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 PL. The results showed 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).

The results of the present study are coincided with the studies of (Sowers and Tomasso 2006) and (Atwood *et al.* 2003). The result of (Bray *et al.* 1994), (Samocha *et al.* 1998a) indicated that shrimp grow better at lower salinities 2-10ppt. 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.

5.7. Conclusion:

Results from this study demonstrate that in commercially manufactured shrimp feeds, fish meal can be completely removed from the formulation using alternative vegetable protein sources like cottonseed meal and marine animal protein sources like *Acetes indicus* without negatively compromising the production performance and economic returns of *L.vannamei*. The most important implication from these findings, are that fish meal inclusion in commercial shrimp diets can be considerably minimized, up to a 0%, without affecting animal production, while using alternative ingredients , such as cottonseed meal and *Acetes indicus* as protein sources. Due to limited published studies, more research is needed to determine the upper limit of cottonseed meal/protein concentrate that can be included in aqua feeds.

Replacing fish meal with cottonseed meal can decrease shrimp body cholesterol, and yet not affect growth performance of shrimp. Because cottonseed meal widely available and produced through sustainable practices, their use in shrimp feed should be encouraged. As human living standards improve, the demand for seafood, such as shrimp, will increase.

However, although plant protein sources may be a cheaper and more sustainable source of dietary protein for use by the aquaculture industry, their success or not will rest upon the shoulder and skills of the aqua feed formulator, the aqua feed manufacturer, and last but not least, the farmer.

The results indicate that among the animal and plant protein sources evaluated, *Acetes indicus* shows the best potential for substituting the FM completely. The objectives of this study were to ascertain the maintenance for protein and the protein requirement for maximum growth of juveniles and sub-adult *L.vannamei*. This knowledge may eventually lead to reduce the fish meal content in feeds and to contribute to achieve sustainable farming.

Low-salinity shrimp culture is a viable and profitable alternative for areas with low water salinity or saline soil features unsuitable for agriculture or other purposes. Economic analysis indicated that fish meal replacement by vegetable protein ingredients and low cost marine animal protein ingredients was more advantageous. The decreased growth performance of shrimp in Cottonseed meal dietary protein may be because of inadequate amino acid or fatty acid content that may be overcome by supplementation with additional ingredients.

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 “Evaluation of cottonseed meal and *Acetes indicus* meal as replacements to fish meal in *Litopenaeus vannamei* diets 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 1.5g for 25 days at 30 ppt salinity. Then the seed were acclimatized to the required salinity, i.e., 5 ppt and 15 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 and *Acetes indicus* meal at 25%, 30%, 35% protein inclusion levels maintained at three different salinities such as 5 ppt, 15 ppt and 30 ppt salinities. Shrimp were fed with Cottonseed meal and *Acetes indicus* meal diets and compared with shrimp fed on fish meal diets for growth and survival rates at three different salinities 5, 15 and 30 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.

- During the experimental period the dissolved oxygen values varied between 5.02- 8.42 ppm at 5 ppt, 6.29-8.52 ppm at 15 ppt and 7.23-8.99 ppm at 30 ppt salinities.
- The temperature ranged from 29.3°C to 31.6°C at 5 ppt, 29.1°C to 31.6°C at 15 ppt and 29.3°C to 31.6°C at 30 ppt over the 63 days period.
- pH levels during the course of the experiment ranged from 8.0 to 8.3 at 5 ppt, 8.1 to 8.3 at 15 ppt and 8.3 to 8.5 at 30 ppt salinities.
- The total alkalinity recorded was in the range of 150 to 340 mg/l at 5 ppt, 612 to 632 mg/l at 15 ppt and 660 to 689 at 30 ppt during the experimental period.
- The total hardness was recorded was in the range of 226 to 237 mg/l at 5 ppt, 660 to 680 mg/l at 15 ppt and 691 to 743 mg/l at 30 ppt during the experimental period.
- Weekly sampling for 63 days was done to study Growth, Survival, and Feed Conversion Ratio.
- The highest growth performance of 9.54g was recorded for treatment AI30 and lowest 8.38g recorded for the CSM25 at 5 ppt salinity.
- Similar trend continues in 15 ppt and 30 ppt salinities, highest growth performance of 12.01g was recorded for AI30 and lowest 10.01g for the CSM25 at 15 ppt.
- In 30 ppt highest growth performance of 12.22g was recorded for AI30 and lowest 10.00g for the CSM25.
- The Analysis of variance for growth performance (at both levels) showed significant difference among the treatments at all salinities.
- The specific growth rate recorded was in the range of (lowest) 2.930 for CSM25 and (highest) 3.159 for AI30 at 5 ppt salinity, whereas at 15 ppt specific growth rate recorded

was in the range of (lowest) 2.960 for CSM25 and (highest) 3.302 for AI30. Similar trend continues at 30 ppt with lowest specific growth rate of 2.969 for CSM25 and highest specific growth rate of 3.287 for AI30.

- The highest survival of 78% (FM30 and FM35) and lowest survival of 65% (CSM25) were recorded respectively at 5 ppt salinity.
- The highest survival of 88% (FM35) and lowest survival of 75% (CSM25) were recorded respectively at 15 ppt salinity.
- The highest survival of 85% (FM30 and FM35) and lowest survival of 75% (CSM25 and CSM35) were recorded respectively at 30 ppt salinity.
- Analysis of variance for survival revealed significant differences among the treatments for survival at all salinities.
- Shrimp fed on CSM25 had showed highest Feed Conversion Ratio in all salinities while FM30 has shown the least at 5 ppt and 15 ppt only, whereas FM25 has shown the least at 30 ppt.
- Shrimps fed on all the test diets in the present study performed better and equal to the shrimp fed on fish meal diets.
- It can be concluded that Cottonseed meal and *Acetes indicus* meal can be included in the diets of *L. vannamei* up to 25% protein inclusion for the total replacement of fish meal.

7. References

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