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# Effect of herbal residues on gut pathogens in cross-bred pigs

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**Keywords:**
NSP
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Nutrient Utilization.

**Abstract**

In a completely randomized design, 5 experimental diets ($T_1$ to $T_5$) were fed to 5 groups of animals with 6 animals (35 kg ± 1.3 body wt.) in each group. The diets were supplemented with or without herbal residues (turmeric, amla, ginger) and enzyme cocktail (xylanase, β-glucanase, cellulase and phytase). Thus the five diets were a standard diet ($T_1$), economic diet with enzyme cocktail but without herbal residue ($T_2$), $T_2$ with turmeric residue ($T_3$), $T_2$ with amla residue ($T_4$) and $T_2$ with ginger residue ($T_5$). After assessing the sensitivity of the individual herbal residue to inhibit the bacterial growth, Ginger residue exhibited the maximum ($P<0.05$) inhibition of Escherichia coli, Staphylococcus aureus and Salmonella typhimurium than amla while the inhibition of turmeric was comparable with ginger and amla. It was shown that the maximum inhibition of all pathogenic bacteria was at 2 per cent level. The total viable count (CFU/gm) was significantly higher ($P<0.01$) in $T_1$ or $T_2$ than in $T_3$, $T_4$ or $T_5$ fed pigs. Feeding diets containing herbal residues ($T_3$ to $T_5$) reduced ($P<0.01$) the Coliform, Staphylococci and Salmonella count. It can be concluded that herbal residues when included in the pig diets at 2% level were able to inhibit the growth of pathogens in the gut and thereby reducing the competition by the microbes for the nutrients leading to a better utilization and performance.

## Introduction

Non-starch polysaccharides (NSP) are some important anti-nutritive components in plant based feed stuffs. Exogenous enzymes can hydrolyze these NSP into smaller units that can be utilized by pigs (Partridge and Bedford, 2000). Similarly phosphorus from plants is of low bio-availability to swine and poultry as a result of phytate, the principal form of phosphorus storage in plants, being relatively indigestible by non-ruminants (NRC, 1998). Exogenous supplementation of feeds with phytase has demonstrated the ability to increase phosphorus bio-availability and thus growth rates in pigs by cleavage of phosphorus molecule from phytase. Since four decades, concern about antibiotic resistance has increased worldwide (Cromwell, 2002). The ban on some Antibiotic Growth Promoters lead to think on phytogenic feed additives which include herbs and their residues, essential oils, botanicals, extracts etc. The mode of action of plant active substances include improvement of endogenous enzyme secretions, stimulation of the appetite, improvement of the digestibility and absorption of nutrients, promote proliferation of beneficial bacteria like Lactobacillus spp. in the gut. Hence the present experiment was planned with the objective of studying the effect of exogenous enzymes on pig performance either with or without herbal residues and their role in gut pathogen inhibition in finishers.

## Materials and methods

Five experimental diets (table 1) were formulated as per NRC (1998) requirements and were evaluated

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during finisher (35-70 kg) phases. The dietary treatments were as shown below

$T_1$ = Standard diet without enzyme cocktail or herbal residues

$T_2$ = Economic diet with enzyme cocktail but without herbal residues

$T_3 = T_2$ + *turmeric* residue

$T_4 = T_2$ + *amla* residue

$T_5 = T_2$ + *ginger* residue

All the herbal residues were diluted in Diethyl ether. Dilutions were made from 0.2% to 2.0%. All concentrations of the herbal residues were prepared a day prior to use and stored at $4^0$C.

**Table 1:** Ingredient and chemical composition (%) of treatment diets

| Ingredient | $T_1$ | $T_2$ | $T_3$ | $T_4$ | $T_5$ |
|---|---|---|---|---|---|
| Maize | 42.00 | 20.00 | 20.00 | 20.00 | 20.00 |
| Soybean meal | 14.00 | 8.00 | 8.00 | 8.00 | 8.00 |
| Sunflower Cake | - | 12.00 | 12.00 | 12.00 | 12.00 |
| Deioled Rice bran | 41.50 | 57.50 | 55.50 | 55.50 | 55.50 |
| Mineral mixture # | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 |
| Salt | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 |
| Turmeric residue | 0.00 | 0.00 | 2.00 | 0.00 | 0.00 |
| Amla residue | 0.00 | 0.00 | 0.00 | 2.00 | 0.00 |
| Ginger residue | 0.00 | 0.00 | 0.00 | 0.00 | 2.00 |
| | **100** | **100** | **100** | **100** | **100** |
| Lysine (%) | 0.41 | 0.44 | 0.44 | 0.44 | 0.44 |
| Methionine (%) | 0.01 | 0.60 | 0.60 | 0.60 | 0.60 |
| $AB_2D_3$ | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 |
| Biovital | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 |
| Enzyme cocktail (xylanase 3500, β-glucanase 2500, cellulase 1250 and phytase 3000 Units / Kg) | - | + | + | + | + |
| Cost per 100 Kg (Rs.) | 1320 | 1266 | 1266 | 1266 | 1266 |

# contained, Ca 32%; P 6%; Mn 0.27%; Zn 0.26%; Cu 100 ppm; Fe 1000 ppm, Iodine 0.01%; Fluorine (max.) 0.03%

*Test to determine the antimicrobial activity*

The disc diffusion method was used to determine the antimicrobial activity of the herbal residues. The volume of 0.1ml (approximately $10^9$ cells / ml) of the tested microorganisms grown in liquid growth media at $37^0$C was inoculated on Muller – Hinton growth media and then spread on the entire surface of the Petri dish using a sterile swab. Then sterile paper discs (Whatman 1.6 mm) with 30 µl absorbed extracts of herbal residues were placed on to the Muller – Hinton agar by pressing gently. The plates were incubated at 35 $\pm$ $1^0$C for 48 hours. After the incubation period the inhibition zones around the paper discs were measured in millimeters. The sensitivity of the individual herbal residue was classified by the diameter of the inhibition zone as per the procedure of Moreira *et al.,* (2005).

Thirty entire male finisher pigs (35.41±0.65 kg) were made into five groups of 6 animals each and were fed with the diets $T_1$ to $T_5$, respectively till they attain a body weight of about 70 kg. In the present study, $T_1$ (without herbal residues and enzymes) and $T_2$ (with enzymes) act as control. At the end of the finisher phase, all the 6 animals in each group were slaughtered to study the gut pathogenic bacteria. A portion of large intestine between caecum and colon of 25cm length was ligated on both the sides it was cut behind the ligation on both the sides with a sterilized knife. The collected intestine piece was placed in a sterilized beaker and kept at refrigeration temperature ($4 \pm 1\ ^0$C)

## Results and Discussion

The Minimum Inhibitory Concentration (MIC) for different concentrations of all residues on the growth of pathogenic bacteria was different at different levels. Turmeric residue (table 2) was effective in preventing the growth of *Escherichia coli* at 0.8, 1, 1.5 and 2 percent levels; *Staphylococcus aureus* at

0.4,0.8,1,1.5 and 2 per cent levels ; *Salmonella typhimurium* at 0.4, 0.8,1, 1.5 and 2 per cent levels ; *Bacillus cereus* at 0.8,1,1.5 and 2 % levels; *Campylobacter jejuni* at 0.2, 1.5 and 2 per cent levels; *Listeria monocytogenes* at 0.6, 1, 1.5 and 2 per cent levels; *Streptococcus pyrogenes* at 0.6, 1, 1.5 and 2 per cent levels; Methicillin resistant *Staphylococcus aureus* at 1, 1.5 and 2 per cent levels. The table shows that the maximum inhibition of all bacterial pathogens was at 2 per cent level.

**Table 2:** Inhibitory effect of turmeric residue on the pathogenic bacterial growth

| Pathogenic bacteria | Concentrations of turmeric residue | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0.20% | 0.40% | 0.60% | 0.80% | 1% | 1.50% | 2% |
| *Escherichia coli* | -- | -- | -- | + | + | + | ++ |
| *Staphylococcus aureus* | -- | + | -- | + | + | + | ++ |
| *Salmonella typhimurium* | -- | + | -- | + | + | + | + |
| *Bacillus cereus* | -- | -- | -- | + | + | + | ++ |
| *Campylobacter jejuni* | + | -- | -- | -- | - | + | ++ |
| *Listeria monocytogenes* | -- | -- | + | -- | + | ++ | ++ |
| *Streptococcus pyogenes* | -- | -- | + | -- | + | + | ++ |
| Methicillin resistant *Staphylococcus aureus* | -- | -- | -- | -- | + | + | ++ |

+ : 08-09 mm; ++ : 10-13 mm; +++ : 14-16 mm

Amla residue (Table 3) was effective in preventing the growth of *Escherichia coli* at 0.8, 1, and 2 per cent levels; *Staphylococcus aureus* at 0.8, 1, 1.5 and 2 per cent levels ; *Salmonella typhimurium* at 0.8, 1, 1.5 and 2 percent levels; *Bacillus cereus* at 1,1.5 and 2 % levels; *Campylobacter jejuni* at 1, 1.5 and 2 per cent levels; *Listeria monocytogenes* at 1, 1.5 and 2 per cent levels ; *Streptococcus pyrogenes* at 0.6, 1, 1.5 and 2 per cent levels; Methicillin resistant *Staphylococcus aureus* at 1 and 2 percent levels. The table shows that the maximum inhibition of all pathogenic bacteria was at 2 per cent level.

**Table 3:** Inhibitory effect of amla residue on the pathogenic bacterial growth

| Pathogenic bacteria | Concentrations of amla residue | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0.20% | 0.40% | 0.60% | 0.80% | 1% | 1.50% | 2% |
| *Escherichia coli* | -- | -- | -- | + | + | -- | + |
| *Staphylococcus aureus* | -- | -- | -- | + | + | + | + |
| *Salmonella typhimurium* | -- | -- | -- | + | + | + | + |
| *Bacillus cereus* | -- | -- | -- | -- | + | + | ++ |
| *Campylobacter jejuni* | -- | -- | -- | -- | + | + | + |
| *Listeria monocytogenes* | -- | -- | -- | -- | + | ++ | + |
| *Streptococcus pyogenes* | -- | -- | + | -- | + | + | ++ |
| Methicillin resistant *Staphylococcus aureus* | -- | -- | -- | -- | + | -- | ++ |

+ : 08-09 mm; ++ : 10-13 mm; +++ : 14-16 mm

The ginger residue (table 4) has shown the maximum inhibition at 2 per cent level for all pathogens. It was effective in preventing the growth of *Escherichia coli* at 0.8, 1, and 2 per cent levels; *Staphylococcus aureus* at 0.4, 0.8, 1, 1.5 and 2 per cent levels ; *Salmonella typhimurium* at 0.4, 0.8, 1, 1.5 and 2 per cent levels; *Bacillus cereus* at 0.8, 1,1.5 and 2 % levels; *Campylobacter jejuni* at 0.2, 1.5 and 2 per cent levels; *Listeria monocytogenes* at 0.6, 1, 1.5 and 2 per cent levels ; *Streptococcus pyrogenes* at 0.6, 1, 1.5 and 2 per cent levels; Methicillin resistant *Staphylococcus aureus* at 1, 1.5 and 2 per cent levels. The table shows that the maximum inhibition of all pathogenic bacteria was at 2 percent level.

The maximum inhibition for all the pathogenic bacteria was observed at 2% with all the herbal residues. Hence the herbal residues were included at 2% in the experimental diets $T_3$ to $T_5$.

**Table 4:** Inhibitory effect of ginger residue on the pathogenic bacterial growth

| Pathogenic bacteria | Concentrations of ginger residue | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0.20% | 0.40% | 0.60% | 0.80% | 1% | 1.50% | 2% |
| *Escherichia coli* | -- | -- | -- | + | + | + | ++ |
| *Staphylococcus aureus* | -- | + | -- | + | + | + | ++ |
| *Salmonella typhimurium* | -- | + | -- | + | + | + | + |
| *Bacillus cereus* | -- | -- | -- | + | + | + | ++ |
| *Campylobacter jejuni* | + | -- | -- | -- | - | + | ++ |
| *Listeria monocytogenes* | -- | -- | + | -- | + | ++ | +++ |
| *Streptococcus pyogenes* | -- | -- | + | -- | + | + | +++ |
| Methicillin resistant *Staphylococcus aureus* | -- | -- | -- | -- | + | + | +++ |

+ : 08-09 mm; ++ : 10-13 mm; +++ : 14-16 mm

*Antibacterial activity (inhibition zones) exhibited by herbal residues*

Ginger (Table 5) exhibited the maximum (P<0.05) inhibition of *Escherichia coli*, *Staphylococcus aureus* and *Salmonella typhimurium* than amla while the inhibition of turmeric was comparable with ginger and amla. The values (mm) were 26.00, 30.67 and 24.33 (ginger); 21.00, 25.00 and 22.00 (turmeric) and 18.00, 19.33 and 13.33 (amla) for *Escherichia coli*, *Staphylococcus aureus* and *Salmonella typhimurium* respectively. The inhibition (mm) of *Bacillus cereus* was maximum (P<0.05) with ginger residue (18.00) than with amla (14.00) or turmeric (12.00). The inhibition of *Campylobacter jejuni* was comparable among three residues and the values (mm) were 13.00 for all the three residues. Ginger exhibited maximum (P<0.05) inhibition of *Listeria monocytogenes* and *Streptococcus pyogenes* than amla while the effect of turmeric was comparable to amla and ginger and the values (mm) were 16.67, 12.00 (amla); 21.00, 18.00 (turmeric) and 25.00, 20.33 (ginger) for *Listeria monocytogenes* and S*treptococcus pyogenes* respectively. The inhibition zone (mm) for *Methicillin resistant Staphylococcus aureus* was maximum (P<0.01) with ginger (22.67) than with turmeric (18.66) or amla (13.00).

**Table 5:** Antibacterial activity (Inhibition zone in mm) of herbal residues (*in vitro*)

| Herbal residues | *Escherichia coli** | *Staphylococcus aureus** | *Salmonella typhimurium** | *Bacillus cereus** | *Campylobacter jejuni* | *Listeria monocytogenes** | *Streptococcus pyogenes** | *Methicillin resistant Staphylococcus aureus*** |
|---|---|---|---|---|---|---|---|---|
| Amla | 18.00$^{b}$ ± 1.15 | 19.33$^{b}$ ±0.33 | 13.33$^{b}$ ±0.33 | 14.00$^{b}$ ±1.15 | 13.00 ± 0.58 | 16.67$^{b}$ ±2.33 | 12.00$^{b}$ ± 0.00 | 13.00$^{c}$ ± 0.57 |
| Turmeric | 21.00$^{ab}$ ±2.31 | 25.00$^{ab}$ ±2.88 | 22.00$^{ab}$ ±3.46 | 12.00$^{b}$ ±1.15 | 13.00 ± 0.58 | 21.00$^{ab}$ ±1.73 | 18.00$^{ab}$ ±2.31 | 18.66$^{b}$ ±0.68 |
| Ginger | 26.00$^{a}$ ±1.15 | 30.67$^{a}$ ±3.48 | 24.33$^{a}$ ±3.48 | 18.00$^{a}$ ±1.15 | 13.00 ±1.15 | 25.00$^{a}$ ± 1.15 | 20.33$^{a}$ ± 4.09 | 22.67$^{a}$ ± 1.21 |

$^{abc}$ values in a column not sharing common superscripts differ significantly ** (P<0.01) * (P<0.05)

The anti bacterial activity of herbal residues was judged by measuring the length of the inhibition zone formed by each residue for each pathogenic bacterium. Except for *Campylobacter jejuni*, amla showed least antibacterial activity and ginger showed the maximum (P<0.05) activity. Ginger residue was effective in preventing the growth of pathogens in the gut followed by turmeric and amla.

*Effect of dietary treatments on pathogenic bacteria of large intestinal contents*

The total viable count (CFU/gm) was significantly (table 6) higher (P<0.01) in $T_1$ or $T_2$ than in $T_3$, $T_4$ or $T_5$ fed pigs and the values were 143.08 ($T_1$), 109.17 ($T_2$), 29.58($T_3$), 67.33($T_4$) and 19.75($T_5$). Feeding diets containing herbal residues ($T_3$ to $T_5$) reduced (P<0.01) the Coliform, *Staphylococci* and *Salmonella* and the values (CFU/gm) were 68.58, 77.50, 26.83, 52.33 and 14.00 (Coliforms); 60.67, 59.50, 27.83, 36.58 and 10.08 (*Staphylococcus)* and 46.25, 54.67, 29.42, 34.50 and 13.08 (*Salmonella*) in pigs fed $T_1$ to $T_5$, respectively.

**Table 6:** Effect of dietary treatments on pathogenic bacteria (cfu/g) of large intestinal contents

| | $T_1$ | $T_2$ | $T_3$ | $T_4$ | $T_5$ |
|---|---|---|---|---|---|
| Total viable count * * | 143.08$^{a}$ ±10.64 | 109.17$^{b}$ ±5.70 | 29.58$^{d}$ ±1.69 | 67.33$^{c}$ ±2.77 | 19.75$^{d}$ ±0.60 |
| Coliforms * * | 68.58$^{b}$ ± 3.42 | 77.50$^{a}$ ±3.05 | 26.83$^{d}$ ±0.94 | 52.33$^{c}$ ±1.45 | 14.00$^{e}$ ±1.13 |
| *Staphylococcus* * * | 60.67$^{a}$ ± 3.49 | 59.50$^{a}$ ±1.73 | 27.83$^{c}$ ±0.99 | 36.58$^{b}$ ±1.05 | 10.08$^{d}$ ±1.19 |
| *Salmonella* * * | 46.25$^{b}$ ± 1.20 | 54.67$^{a}$ ±2.37 | 29.42$^{d}$ ±0.94 | 34.50$^{c}$ ±1.14 | 13.08$^{e}$ ±0.89 |

$^{abcd}$ Values in a row not bearing common superscripts differ significantly ** (P<0.01)

Similar to the present results (Table 6), earlier reports also indicated antimicrobial effects of plants extracts (Newbold *et al*., 2004). The antimicrobial property was attributed to the hydrophobicity of plant extracts which facilitates their union to the bacterial surface inducing unstabilization (Tsuchiya *et al*., 1996; Zhang and Lewis, 1997) or the inactivation of different molecules of the bacteria such as enzymes or receptors through their union to the specific site (Sharon and Ofek, 1986; Ya *et al*., 1988; Stern *et al*., 1996).

It was reported that phytogenic feed additives have a strong antibacterial and to some extent antifungal properties. They inhibit the growth of *Escherichia coli*, *Proteus sp*, *Staphylococci*, *Streptococci* and *Salmonella* (Aruoma *et al*., 1996; Benencia and Courreges, 2000; Garcia *et al*., 2003) which otherwise compete with the host for nutrients.

## Conclusion

Herbal residues when included in the pig diets at 2% level were able to inhibit the growth of pathogens in the gut and thereby reducing the competition by the microbes for the nutrients leading to a better utilization and performance.

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# Effects of dietary protein level on growth and body composition of asian catfish, *Clarias batrachus* fry

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**Abstract**

This study aimed to evaluate the protein requirements of *Clarias batrachus* fry, were estimated by feeding isocaloric diets. The influence of dietary protein level on weight gain, survival, food utilization and body composition were estimated. The Asian catfish, *C. batrachus* fry were fed four diets containing 25% (diet 1), 30% (diet 2), 35% (diet 3) and 40% (diet 4) protein levels and 3580 kcal $kg^{-1}$ of energy level for 30 days. Fry fed with diet 3 containing 35% protein showed the highest final body weight, survival, specific growth rate and protein efficiency ratio and were significantly ($P < 0.05$) affected by dietary treatments. *C. batrachus* fry raised at 25% protein (diet 1) had higher apparent feed conversion ratio (AFCR) (1.30±0.13) than 35% dietary protein level (0.63±0.07). Higher protein efficiency ratio (4.52±0.4) observed at higher protein level (35%) than 25% protein level (3.08±0.6). Body lipid and ash content observed to have been influenced ($P < 0.05$) by diets with increasing protein level. Further, crude protein was not affected ($P > 0.05$) with increase in dietary protein level. The study concluded that the diet 3 containing 35% protein was optimal for growth of *C. batrachus* fry.

## Introduction

In aquaculture practices, more emphases are generally given to the dietary protein requirements to achieve optimal fish growth and production. Protein is one of the major dietary nutrients, affecting growth performance of fish and also feed cost (Lovell 1989). Regular intake of protein is required by fish to build new tissues and to repair old tissues. Protein deficiency in the diet result in reduction of growth and loss of weight due to utilization of body protein to maintain the functions like building of new tissues and repairing of new tissues (Rath 1993; Singh et al. 2009). Hence, fish require high levels of protein in their diets, which vary from fish species to species. In addition, extremely high protein levels may result in poor growth and increased susceptibility of fish to diseases and parasites due to poor water quality. When fed to fish, diets containing excessive amounts of protein cause toxicity since the fish tend to excrete high amounts of ammonia in the rearing water which may lead to growth depression (Zeitoun et al. 1976). On the other hand, reduction in fish growth could be due to lack of non-protein energy in diets containing high amounts of protein (Jauncey 1982), in which case some protein would be used for energy rather than growth. Moreover, excess dietary protein which cannot be stored are catabolized preferentially over carbohydrates and fats and used for energy instead of growth by some fishes (Wilson 1989). Requirements of protein in the diets for silver carp, were reported to be between 37% and 42% (Singh 1989), for common carp between 31% and 54% (Varghese et al. 1976; Sen et al. 1978; Pandian 1989) and for pearl gourami 45% (Singh et al. 2003).

The Asian catfish, *Clarias batrachus*, is widely distributed throughout Indian continents and is known as a species of aquaculture importance. The

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twin qualities; rapid growth and high marketability, make it a good candidate fish species for aquaculture production (Goda et al. 2007; Singh et al. 2009). Commercially available artificial feeds are used to supplement the protein requirements in the mass production of fry. However, this use is based more on availability than suitability (Tidwell et al. 1992; Gupta et al. 2007); the available artificial feeds are not designed specifically for catfish and fail to meet the actual protein requirements of the fry. An economically viable commercial production of *C. batrachus* requires reliable diets that will support nutritional requirements for optimal growth and survival. Studies on the protein requirements of *C. batrachus* have been focused on juveniles (Khan et al. 1993) and information regarding the optimum protein level in fry diet is scarce. For sustainable aquaculture of this species, knowledge of optimal dietary protein requirements, growth and feed utilization need to be investigated. Therefore, the objective of this study was to determine the optimum level of dietary protein, growth, feed utilization and body composition of Asian catfish, *C. batrachus* fry.

## Materials and Methods

*Test animals and experimental system*

Fry of Asian catfish *C. batrachus* were procured from a local fish seed supplier (M/s Jawhar fish seed supplier, Fazilka, Punjab) and acclimatized to laboratory conditions for one week in two plastic trough containing 50 L water. Fry were fed to satiation, a mixture of groundnut oil cake and wheat flour (1:1). Daily removal of 50% water from plastic trough was done to remove uneaten food and excreta and replenishment of same volume of fresh water. Groups of 30 catfish fry of average 0.2 g body weight were stocked in 12 plastic troughs of 15 litre capacity. Triplicate troughs were allotted randomly for each feeding treatment. The water quality was maintained through daily 50% water exchange and siphoning out of the waste material. Continuous aeration was provided by air stones using an air compressor to ensure oxygen saturation. The experiment was lasted for 30 days.

Water quality parameters were determined daily and there were no significant differences between dietary treatments at any sampling period. Ranges of temperature, pH, dissolved oxygen, ammonia, nitrates and nitrites were from 34.2 to 34.6°C, 8.0 to 8.37, 7.62 to 8.50 mg $L^{-1}$, 0.12 to 0.16 mg $L^{-1}$, 0.09 to 0.16 mg $L^{-1}$ and 0.04 to 0.07 mg $L^{-1}$, respectively, during the experimental period. The water quality parameters were within the acceptable range as reported for *C. batrachus* (Rao et al. 1994) rearing and culture.

*Diet preparation and feeding trial*

Four isocaloric diets were formulated with different proportions of ingredients to contain 25% (diet 1), 30% (diet 2), 35% (diet 3) and 40% (diet 4) protein level. The ingredients and proximate composition of the experimental diets are shown in Table 1. The ingredients of each diet were mixed thoroughly and an aliquot of water were added to the mixture. The resulting dough was cooked for 20 min in a pressure cooker. After cooling, the dough was pelletized by using a hand pelletizer. Pellets of 1mm diameter size were dried at 50°C. The dried diets were then stored in airtight plastic containers. Experimental diets were fed to the fry at 7% of body weight, twice a day (09:00 and 17:00 h) for a period of 30 days. After every 10 days, ten fishes from each trough were weight individually for recording weight and calculating daily food ration.

*Diet and body composition analysis*

At the end of the experiment, ten fishes from each replicate were sacrificed for the analysis of chemical composition of whole body. Diet and body tissue at the end of the experiment were analysed for dry matter (DM), crude protein (CP), lipid and total ash according to the method of AOAC (2000). Organic matter (OM) of diets was calculated by subtracting the total ash value from DM. Total carbohydrates was estimated by subtracting CP and lipid values from OM. Gross energy of diets was calculated on the basis of gross energy values of crude protein, total carbohydrate and lipid of the respective diets. The digestible energy values of the diets were calculated as 14.6 MJ $kg^{-1}$ proteins, 33.9 MJ $kg^{-1}$ lipids and 10.5 MJ $kg^{-1}$ carbohydrate on the basis of values adapted for channel catfish (NRC 1993).

*Growth parameters*

At the end of the feeding trial, the water from each plastic trough was siphoned out and the total number of fry in each trough was counted. Randomly the individual body weights of fry were recorded and the mean final body weight was multiplied with total number of fry to arrive at final biomass production from each trough. Feed conversion ratio was considered to be apparent as no correction was made for uneaten food left (if any). The following formulae were used to calculate growth performance in *C. batrachus* fry:

1. Specific growth rate (SGR) = 100 ($\log_e$ final body weight - $\log_e$ initial body weight)/culture period (days).

2. Apparent feed conversion ratio (AFCR) = total dry feed fed (g)/total live weight gain (g).
3. Protein efficiency ratio (PER) = weight gain/ protein intake.

*Analysis of data*

The experimental data were subjected to one-way ANOVA (Snedecor & Cochran 1968) and results were considered to be statistically significant at 0.05 probability level or smaller.

## Results

*Growth performance*

The diets are formulated in such a way that gross energy level remains almost same in all the experimental diets. Estimated digestible energy for different experimental diets ranged from 11.13 to 11.46 kJ $g^{-1}$ of diet (Table 1). The data of final body weight, biomass gain, percent survival, feed intake, specific growth rate (SGR), apparent feed conversion ratio (AFCR) and protein efficiency ratio (PER) are summarised in Table 2.

The initial body weight (0.2 g) of the fish fry was similar among the treatments. The final body weight attained by diet 1 treatment was the lowest (0.68±0.08 g), followed by diet 2 (0.82±0.05 g), however, both were lower than that of diet 3 (0.99±0.07 g) and diet 4 (0.91±0.05 g) fed fish treatments (Table 2). It was observed that net body weight gain in fish fed on 35% and 40% protein diet was significantly higher ($P < 0.05$) than that of fish fed on 30% protein diet, which was also significantly higher than that of 25% protein diet fed fish. Dietary protein levels had significant effect on AFCR and PER values in catfish fry. AFCR value of 25% protein diet was significantly higher ($P < 0.05$). Fish fed diet containing 35% protein showed significantly lower ($P < 0.05$) AFCR value and better than those obtained with the diets containing 40% and 30% level protein (Table 2). Fry fed the 35% (diet 3) protein diet showed the highest PER followed by diet 2, 4 and 1 treatment.

*Body composition*

Whole body composition of *C. batrachus* fry is shown in Table 3. There were significant differences ($P < 0.05$) for whole-body lipid and ash contents. Both body lipid and ash content decreases significantly with increase in protein content of the diet, whereas, protein values were increased. However, fry fed 25% protein (diet 1) recorded lower protein content ($P > 0.05$) compared with the other treatments.

**Table 1:** Ingredient and chemical composition of the experimental diets

| | Diet | | | |
|---|---|---|---|---|
| **Item** | **1** | **2** | **3** | **4** |
| **Ingredient (g $kg^{-1}$ as-is basis)** | | | | |
| Fish meal | 175 | 220 | 285 | 325 |
| Meat meal | 50 | 50 | 50 | 50 |
| Soybean meal | 100 | 170 | 220 | 290 |
| Groundnut oilcake | 50 | 50 | 50 | 50 |
| Wheat flour | 150 | 110 | 80 | 50 |
| Rice bran | 250 | 190 | 120 | 85 |
| Corn starch | 185 | 170 | 155 | 110 |
| Sunflower oil | 20 | 20 | 20 | 20 |
| Vitamin & mineral mixture * | 15 | 15 | 15 | 15 |
| Iodized salt | 5 | 5 | 5 | 5 |
| **Proximate composition (% dry weight) #** | | | | |
| Dry matter | 89.33 | 89.49 | 89.62 | 89.84 |
| Crude protein (calculated) | 25.00 | 30.00 | 35.00 | 40.00 |
| Crude protein (analyzed) | 25.41 | 30.21 | 35.03 | 39.92 |
| Lipid | 7.70 | 6.96 | 6.16 | 5.81 |
| Ash | 7.24 | 8.13 | 10.47 | 12.35 |
| Organic matter † | 82.09 | 81.36 | 79.15 | 77.49 |
| Total carbohydrate § | 48.98 | 44.19 | 37.96 | 31.76 |
| Gross energy (kJ $g^{-1}$) $ | 1746.5 | 1748.5 | 1723.9 | 1719.3 |
| Digestible energy (kJ $g^{-1}$) ¶ | 11.46 | 11.41 | 11.18 | 11.13 |

* Vitamin & mineral per gram of premix contained: Vitamin A 8000 i.u., vitamin B2 2.8 mg, vitamin B12 5 mg, vitamin D3 1500 i.u., vitamin E 5 mg, vitamin K3 5 mg, vitamin PP 12.5 mg, D calcium pantothenate 5 mg, copper sulphate 0.7 mg, zinc sulphate 2.5 mg, ferrous sulphate 6.2 mg, potassium iodide 0.4 mg, manganese sulphate 3.8 mg, sorbitol 20 mg.
# Values are the mean of three determinations.
† Organic matter = dry matter – total ash.
§ Total carbohydrates = organic matter – (crude protein + total lipid).
$ Calculated using gross caloric values of 5.65, 9.45 and 4.1 kcal $g^{-1}$ for protein, fat and carbohydrate, respectively, according to Brett (1973).
¶ Calculated as 14.6 MJ $kg^{-1}$ protein, 33.9 MJ $kg^{-1}$ lipid and 10.5 MJ $kg^{-1}$ carbohydrate, according to NRC (1993).

**Table 2:** Growth performance of *Clarias batrachus* fry fed with increasing levels of dietary protein for 30 days*

| Nutritional indices | Diet 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| Initial weight (g) | 0.2±0.02 [a] | 0.2±0.01 [a] | 0.2±0.01 [a] | 0.2±0.02 [a] |
| Final weight (g) | 0.68±0.08 [c] | 0.82±0.05 [b] | 0.99±0.07 [a] | 0.91±0.05 [a] |
| Net weight gain (g) | 0.48±0.03 [c] | 0.62±0.05 [b] | 0.79±0.06 [a] | 0.71±0.02 [a] |
| Survival (%) | 45.6±11.2 [c] | 62.3±8.1 [b] | 76.5±7.2 [a] | 65.8±9.5 [b] |
| Biomass gain (g) | 33.2±9.5 [d] | 93.3±10.9 [c] | 167.7±13.6 [a] | 120.2±7.4 [b] |
| Total feed intake (g) | 43.1 | 74.6 | 106.0 | 82.9 |
| SGR [$] | 2.45±0.4 [c] | 2.82±0.3 [b] | 3.2±0.2 [a] | 3.03±0.3 [a] |
| AFCR [†] | 1.30±0.13 [a] | 0.80±0.11 [b] | 0.63±0.07 [c] | 0.69±0.09 [b] |
| PER [#] | 3.08±0.6 [d] | 4.16±0.7 [b] | 4.52±0.4 [a] | 3.62±0.6 [c] |

* Values are the mean of triplicate groups of ten random fishes. Mean values in the rows with different superscripts are significantly different ($P < 0.05$).
[$] Specific growth rate (SGR) = 100 ($\log_e$ final body weight - $\log_e$ initial body weight)/culture period (days).
[†] Apparent feed conversion ratio (AFCR) = total dry feed fed (g)/total live weight gain (g).
[#] Protein efficiency ratio (PER) = weight gain/protein intake.

**Table 3:** Carcass composition of *Clarias batrachus* fry (% dry weight) fed increasing levels of dietary protein for 30 days*

| Constituents | Diet 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| Dry matter | 23.8±2.6 [a] | 22.5±1.4 [a] | 21.0±3.0 [a] | 20.2±1.2 [a] |
| Crude protein | 13.53±1.3 [a] | 13.90±2.6 [a] | 14.25±0.98 [a] | 14.31±1.9 [a] |
| Total lipids | 4.77±0.82 [a] | 3.56±0.56 [b] | 2.55±0.27 [c] | 2.07±0.31 [d] |
| Total ash | 2.54±0.06 [a] | 2.26±0.11 [b] | 2.02±0.05 [c] | 1.87±0.08 [d] |

* Values are the mean of triplicate groups of ten random fishes. Mean values in the rows with different superscripts are significantly different ($P < 0.05$).

## Discussion

The protein level was increased steadily from diet 1 to diet 4, which was required to study the protein requirements of fish. Because of the protein level increased, some other constituent must vary among the dietary treatments, and in the present study total carbohydrate concentration decreased steadily from diet 1 (48.98%) to diet 4 (31.76%). The decrease in carbohydrate concentration was due to decreased levels of rice bran and wheat flour in the diets. The increased ash content in diet 4 was due to the presence of higher level of fish meal and soybean meal as a major feed ingredient. Fish meal and soybean meal are generally considered as good quality protein sources with essential amino acid index (EAAI) of around 0.90. The initial body weight of the *Clarias* fry was similar among the treatments. The final body weight attained by diet 1 was the lowest, followed by diet 2; however, both were lower than that of diet 3 and 4 fed fish ($P < 0.05$). Since the diets were offered at 7% of the wet body weight and the fish consumed the diets within 25 min, voluntary intake of the diets was not a factor that affected growth, which corroborates the observations of Moon and Gatlin (1994); Giri et al. (2000) and Giri et al. (2003). The inferior growth performance of *Clarias* fry fed diet 1 and 2 might be due to the presence of higher level of carbohydrates in the diets, which agrees with the observation of Venkatesh et al. (1986) in *Clarias batrachus* fed a diet containing more than 50% carbohydrate. It is seen in the present study that fry were capable possibly of tolerating carbohydrate up to 37.96% diet without exhibiting growth reduction, which was much higher than the values reported for channel catfish (28%) (Garling & Wilson 1977), but less than the values reported for Nile tilapia (40%) (Anderson et al. 1984), hybrid catfish of *C. macrocephalus* × *C. gariepinus* (50%) (Jantrarotai et al. 1994), and *C. batrachus* (51%) (Giri et al. 2000). The poor performance of fry on diets with lower protein levels may have been influenced by the relationship between the protein and energy in the diets. Information on the gross energy requirements of the test fish is lacking, but the energy level used in the present study was based on the available information from other studies on catfish. Earlier, Khan et al. (1993) concluded that the optimum protein requirement of juvenile *M. nemurus* is 42% with protein to digestible energy ratio of 113.82 mg $kcal^{-1}$. The 40% protein diet used in this study has protein to gross energy ratio of 111.7 mg $kcal^{-1}$. The protein and energy level in fish diets is important because fish, like any other animal, eat primarily to satisfy their energy requirements and they tend to adjust their feed intake in accordance with their energy requirements (Cho & Kaushik 1985; Smith 1989; Kim et al. 1991). Excessive energy levels also have deleterious effects such as deposition of fat (Hajra et al. 1988) which is undesirable.

A significant decrease ($P < 0.05$) in net biomass gain in diet 2 and 1 treatment with decreasing dietary protein was observed. Low dietary protein was also associated with decreased survival of the fry. The works of Kandasami et al. (1987) and Fiogbe et al. (1996) also indicated similar decrease in growth as well as survival of fish, fed low levels of protein (less than 30% diet). The low fish survival in diet 1 (45.6±11.2 %) and diet 2 (62.3±8.1 %) as compared to diet 3 (76.5±7.2 %) can be attributed to the dietary treatments. Fish in diet 1 and 2 treatments were observed to feed well on artificial diets but increased mortalities were observed in these treatments during the latter part of the experiment. Cannibalism was also noted in the aforementioned treatments. Cannibalism is known to be triggered by internal factors such as variable sizes and weak state of the fry in the culture system, which is influenced by the fry diet (Ehrlich et al. 1989; Qin & Fast 1996; Watanabe et al. 1996).

There was a significant ($P < 0.05$) increase in SGR and PER, together with an improved AFCR with increased dietary protein (Table 2). Earlier work in *C. batrachus* fry (Chuapoehuk 1987) also indicated that dietary low level of protein (less than 35% diet) decreased the PER values in catûsh, which is also supported by others (Shyong et al. 1998; Giri et al. 2003).

Using the final body weights as the indicator it was estimated that the dietary protein requirement was 35% for *Clarias batrachus* fry with a corresponding energy value of about 1719.3-1748.5 kJ $g^{-1}$ diet. This dietary protein requirement value for catfish fry is much higher than that reported for *Clarias batrachus* fry (30%) and *Pangasius* sp. (30%) by Chuapoehuk (1987) and Aizam et al. (1980), respectively, but lower than those found for other tropical catfishes, particularly *C. gariepinus*, 40% (Degani et al. 1989), hybrid catûsh of *C. macrocephalus* × *C. gariepinus*, 40% (Jantrarotai et al. 1998) and *Mystus nemurus*, 42% diet (Khan et al. 1996).

Result of present study, indicated that dietary protein level influenced the whole body DM, CP, lipid and ash content (Table 3). Protein concentration in the carcass of catfish increased with increase in dietary protein levels from 25% to 40% but these changes were not significant ($P > 0.05$). A similar increasing trend of tissue protein has also been observed for other fish species, Zeitter et al. (1984) in *Cyprinus carpio*; Mohanty & Samantaray (1996) in *Channa striatus*; Shyong et al. (1998) in *Zacco barbata* and Giri et al. (2003) in hybrid post-larvae of *Clarias batrachus* x *Clarias gariepinus*. Shearer (1994) pointed out that the proximate composition of fish is influenced by both endogenous factors such as fish size and sex as well as exogenous factors such as diet composition. This may partly explain the lack of agreement concerning the influence of various levels of dietary protein on the protein content of the fish body.

The carcass ash content decreased significantly ($P < 0.05$) with increase of dietary CP content. There was a direct relation between whole body DM and lipid with dietary protein levels, which was reported earlier also by Juancey (1982) for juveniles of tilapia *Sarotherodon mosambicus*; Shiau & Huang (1988) for hybrid tilapia *Oreochromis niloticus* x *O. aurea*; Khan et al. (1996) for catfish *Mystus nemurus* and Giri et al. (2003) for hybrid post-larvae of *Clarias batrachus* x *C. gariepinus*. The increased level of dietary carbohydrate in fish fed low protein diets might have stimulated several tissue lipogenic enzyme activities and converted dietary carbohydrate into fat. The resultant fat thus synthesised might have been deposited in the adipose tissues. Similar studies of Likimani & Wilson (1982) and Giri et al. (2003) also observed that feeding higher level of carbohydrate increased tissue DM as well as lipid levels in channel catfish and hybrid catfish, respectively.

In conclusion, based on the growth performance, best feed utilisation and highest survival observed in this study, a diet with dietary protein level of 35% is required for *C. batrachus* fry, when fish meal and soybean meal were used as the primary sources of protein and the diets were fed at 7% of the wet body weight.

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# Effect of various feed rations on the growth, survival and body composition of gold spot mullet (*Liza parsia*) fry reared in cages

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**Abstract**

*Liza parsia* fry were trained on dry feed, were kept in pond cages and fed with 4, 6, 8 and 10% of their actual body weight. The effect of the daily ration on the growth, survival, condition factor, feed conversion and body composition were observed until fry nursed in cages reached one gram. The appropriate daily ration of 6% of body weight seemed to be advisable for the practices as it achieved the highest average length of 4.46cm, average weight of 1.03g and SGRof 7.03%day$^{-1}$. There was no significant difference between the conditional factors or survivals of the groups. The best feed conversion (FCR) and protein efficiency ratio (PER) was achieved at the 6% daily ration group and the worst at 10% group in every week of the experiment.

## Introduction

Aquaculture of mullets has great potential in brackish waters; its farming is still at infant stage in India compare to rest of world. Mullet culture is a good alternative to direct towards the intensification of production as it has gained the importance in several countries of South East Asia, because these fishes are considered of high quality priced. The mullet usually occur in coastal waters and estuaries throughout the tropical and subtropical belts of the world and sometimes even in temperate zones. They are known to ascend in schools to the shallow littoral areas and connected creeks, channels etc., with the high tide for feeding purposes and this characteristic habit is utilized while collecting them, using almost similar gears throughout the world. Active gears such as scoop nets, skimming nets and beach seines are commonly used to collect wild fry (Sadek and Mires, 2000; Liao, 1994).

*Liza parsia* is one of the important cultivable species in brackish water fish farming available along the West coast of India. As the culture of gold-spot mullet, *L. parsia* becomes more popular, strategies for supplementary feeding will have to be assessed to reap maximum economic returns. Feeding is one of the most important considerations, because it can affect growth and the efficiency of feed utilization. In this type of farming supplementary feeding has become an integral means of achieving greater productivity.

Supplementary feeding is the single most critical and expensive variable cost in semi-intensive and intensive culture. The economic success of production control in aquaculture depends to a large extent on reasonable feeding costs. One way of reducing feeding costs is to estimate the daily optimal ration and formulate a feeding chart that will best suit local farming conditions. Minimization of the amount of feeding may have the effect not only of reducing the cost of feeding but also the biological loading of recirculation systems and effluent production in flow-through systems (Woods 2005).

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Some studies have been conducted on the effects of manipulating feeding regimes for several cultured brackish water fish species to improve culture efficiency (Chiu et al. 1987; Saether and Jobling 1999; Mihelakakis et al. 2001).

Determination of appropriate feeding rations for cultured fish is important to achieve maximum productivity, because feeding rate affects nutrient requirements in fish, such knowledge is regarded as a pre-requisite for estimation of the amounts of nutrients they should receive (Talbut, 1985). When food intake levels are higher than the optimum, growth increase is negligible (Tsevis et al., 1992) whereas sub-optimum rations may result in reduced growth and increased size variation (Johnston *et al*., 2003).

In any selective culture, a constant supply of nursery reared healthy fingerlings is the most essential prerequisite. The fry of *L. parsia* directly stocked in culture pond leads to high mortality. Therefore, an attempt was made to rear the fry of *L. parsia* in cages as it has advantages as compared to pond.

The aim of the present work was to investigate the effect of daily feeding rations on the growth, survival, body composition, condition, feed conversion and protein efficiency of *L. parsia* fry during nursery rearing in pond cages.

## Materials and methods

### *Fish and experimental procedure*

Fry of *L. parsia* were collected during low tide with the help of dragnet from the Kasarveli creek, situated at Sakhartar, Ratnagiri, Maharashtra State, Republic of India (16° 59′ 10" N and 73° 16′ 25" E). Collected fry was transported to the laboratory in plastic containers (20 litre capacity). Fry of *L. parsia* were identified by using the taxonomic key (Barve, 1987). The cages were installed in a brackish water pond located at College of Fisheries, Shirgaon, Ratnagiri campus. Area of the pond was 450 $m^2$ (30 m x 15 m). During high tides, the depth of pond water was up to 110 cm while 90 cm at low tides. Rectangular shaped cages were constructed for the fry of *L. parsia* as described by Yu *et al.* (1979). Cage with dimension of 1 m (L) x 1 m (B) x 0.5 m (H) was with volume of 0.5 $m^3$. Mosquito net cloth of polyamide (PA) with 24 mesh $inch^{-1}$ mesh size was used for preparation of cage bag. Two loops were attached at each corner of the cage bag to fix the bag with the bamboo. Loops were made from the extra mosquito net material; each loop was 6 cm in length. The top cover was connected with the cage bag for opening or closing the cage for feeding and maintenance. The cage was fixed by submerging $3/4^{th}$ part in water. Fry with average initial length of 1.3 $\pm$ 0.2 mm and average initial weight of 0.07 $\pm$ 0.02 mg were stocked at 50 fry $m^{-2}$ and were fed at 4%, 6%, 8% and 10% of body weight with 5 replicates each. Diet was given twice a day (9:00 h and 17:00 h) directly into cages. No special feeding area was provided in the cage.

### *Diet Formulation*

Diet was formulated containing about 30% protein by using different ingredients as given by Sawant *et al.* (2005). The ingredients and proximate composition of the test diets are given (Table 1). The moisture, crude protein, lipid and ash content in the test diets were analyzed, according to standard procedures of Association of Official Analytical Chemist (AOAC, 1995).

**Table 1:** Proportion of ingredients and proximate composition of diet used in experiment

| Proportion of ingredients | |
|---|---|
| **Ingredients** | **Quantity (%)** |
| Wheat flour | 12.18 |
| Rice bran | 12.18 |
| Whole poultry egg | 37.82 |
| Mustard Oil Cake | 37.82 |
| **Proximate composition** | |
| Crude Protein (%) | 31.57 |
| Crude Fat (%) | 9.61 |
| Moisture | 8.91 |
| Ash (%) | 5.82 |
| Carbohydrate* (%) | 44.09 |
| Gross energy (kcal $g^{-1}$)** | 450.43 |

*Carbohydrate (%) = (100 %) – [(% Protein) + (% Fat) + (% Moisture) + (% Ash)] … (Woods and Aurand, 1977).
**Gross energy (Kcal $g^{-1}$)=(Crude protein x 5.65) + (Crude fat x 9.5)+(Carbohydrate x 4.1) … (El – Sayed, 2002).

### *Water parameters*

Water parameters such as temperature, pH, salinity, dissolved oxygen, carbon dioxide and alkalinity were ranged from 28.4 to 30.2°C, 7.4 to 8.0, 26 to 29 g $L^{-1}$, 3.5 to 4.4 mg $L^{-1}$, 8.2 to 9.4 mg $L^{-1}$ and 120 to 138 mg $L^{-1}$ respectively, were analyzed every week outside and inside the cages according to standard methods APHA (2005).

### *Statistical Analysis*

All data on growth and survival were analysed by one-way ANOVA followed by Least Significant

Difference (LSD) test. Differences were considered significant at $P < 0.05$ according to standard statistical methods by (Snedecor & Cochran 1967; Zar 2004). Quadratic regression analysis (Zeitoun et al. 1976) was used to determine break-points in the growth data. The break-points obtained represented the optimum rations for growth.

## Results

*Growth and Survival*

Different feed rations were found to significantly affect the growth of *L.parsia* (Table 2). After five weeks of the feeding trial the weight gain of fish fed at 6% ration was signiûcantly higher ($P$<0.05) than those fed the 4, 8 and 10% rations.

**Table 2:** Effects of feeding ration on the growth and survival of *L. parsia* fry in cages during the experiment.

| Feeding ration (%) | Initial weight (g) | Final weight (g) | Weight gain (%) | Survival (%) |
|---|---|---|---|---|
| 4 | 0.084±0.002 | 0.53±0.03$^a$ | 537.95±12.59$^a$ | 64.0±1.41$^a$ |
| 6 | 0.082±0.003 | 1.03±0.02$^d$ | 1167.02±65.06$^d$ | 76.8±2.41$^c$ |
| 8 | 0.092±0.007 | 0.85±0.02$^c$ | 840.73±44.24$^c$ | 69.2±1.20$^b$ |
| 10 | 0.070±0.002 | 0.66±0.02$^b$ | 650.61±23.76$^b$ | 67.2±1.62$^b$ |

Data with different letters in the same column means significant differences ($P$<0.05) between treatments.

*Food Conversion Ratio (FCR) and Protein Efficiency Ratio (PER)*

Best FCR, and highest specific growth and protein efficiency ratio (PER) where obtained for fish fed 6% and 8% rations. Feed conversion ratio (FCR) decreased with increasing ration up to 6%. No signiûcant ($P$>0.05) improvement in FCR was evident for fish fed at 6% ration and increasing the ration further resulted in no improvement or even in poor FCR. PER was also found to be significantly higher for the 6% ration than form the 4 and 10% ration, and not significantly different ($P$>0.05) from that for the 8% ration (Table 3).

**Table 3:** Effects of feeding ration on the specific growth rate, conditional factor, feed conversion and protein efficiency performance of *L. parsia* fry in cages during the experiment [1].

| Feeding ration (%) | SGR[2] | Condition[3] Factor ($K_f$) | FCR[4] | PER[5] |
|---|---|---|---|---|
| 4 | 5.14±0.07$^a$ | 1.13±0.17 | 0.53±0.03$^a$ | 0.14±0.01$^a$ |
| 6 | 7.03±0.17$^d$ | 1.15±0.12 | 1.03±0.02$^d$ | 0.30±0.01$^d$ |
| 8 | 6.21±0.16$^c$ | 1.13±0.23 | 0.85±0.02$^c$ | 0.24±0.02$^c$ |
| 10 | 5.59±0.11$^b$ | 1.15±0.18 | 0.66±0.02$^b$ | 0.18±0.01$^b$ |

[1]Mean values ± SEM from five replicate analyses; [2]SGR = {(In mean final weight) - (In mean initial weight)/No. of days}*100; [3]Kf= weight of fish (g)*100/length$^3$ (cm); [4]FCR = dry food fed (g)/wet weight gain (g); [5]PER =Weight gain (g,wet weight basis)/Protein intake (g,dry weight basis). Data with different letters in the same column means significant differences ($P$<0.05) between treatments.

The relationship between FCR (Y) and dietary ration (X) was best described by the second-degree polynomial equation:

$Y = 0.0084x^2 – 0.1203x + 1.527$

($r = 0.918$; $P < 0.05$)

The relationship between PER (Y) and dietary ration (X) was best described by the second-degree polynomial equation:

$Y = -0.0137x^2 + 0.1939x - 0.4046$

($r = 0.919$; $P < 0.05$)

On the basis of these equations the best values for FCR and PER were obtained for 6.55 and 6.60% rations, respectively.

*Body Composition*

There were marked differences between whole-body compositions among the fish fed different rations (Table 4). There were no significant differences ($P$>0.05) between body moisture content for fish fed at different rations except for fish fed under 2% of their body weight ($P$<0.05) where higher moisture content was evident. Whole-body protein content was found to be significantly higher ($P$<0.05) for 4% ration compared with fish fed other rations. The whole-body fat content of fish fed different rations gradually increased with the increasing ration and was found to be signiûcantly ($P$<0.05) higher for 6% and 8% rations. Body ash did not differ among fish fed different rations, except for the 2% ration, for which ash content was significantly higher ($P$<0.05).

**Table 4:** Body composition of *L. parsia* fry fed at different rations

| Feeding Ration | Moisture (%) | Protein (%) | Fat (%) | Ash (%) |
|---|---|---|---|---|
| Initial | 79.57±3.81 | 10.2±0.43 | 6.24±0.04 | 3.99±0.13 |
| 2 | 41.21±4.32 | 14.23±0.28$^{a}$ | 5.58±0.10$^{a}$ | 3.42±0.18$^{b}$ |
| 4 | 35.91±5.25 | 18.56±0.64$^{c}$ | 5.82±0.05$^{a}$ | 2.46±0.27$^{a}$ |
| 6 | 37.82±5.69 | 16.69±0.88$^{b}$ | 6.08±0.07$^{c}$ | 2.61±0.32$^{a}$ |
| 8 | 39.62±5.48 | 15.96±0.75$^{b}$ | 6.16±0.08$^{c}$ | 2.85±0.22$^{a}$ |

Data with different letters in the same column means significant differences ($P$<0.05) between treatments.

## Discussions

Feeding ration is an important factor governing the growth of fish (Chiu et al. 1987). The relationship between growth rate and ration in fish is very important, because feed accounts for 50% of the cost of the intensive ûsh culture (Tacon and Metian, 2008). Growth rate and ration interact to determine FCR and are used to estimate the daily ration for a particular fish stock. Similar to all animals, fish will lose weight when their nutrient intake rate falls below that required for daily maintenance. As food availability increases, the quantity consumed by the fish will also increase, giving a linear increase in specific growth rate (SGR %) up to the point of maximum voluntary food intake. Growth rate is linearly correlated to food intake (Peres and Oliva-Teles 2005). If fish are fed above their appetite, the extra food will be wasted and a high FCR will result. High FCRs result from both over and under feeding. Beyond a certain level, overfeeding has no effect on growth, and results in a poor growth (De Silva and Andersson 1995) and will also cause water pollution from aquaculture (Storebakken and Austreng 1987).

It is apparent from the results of this study that growth of *L. parsia* fry fed at different rations varied significantly. It was found that, feeding fish in the range of 6% body weight (bw) per day results in maximum utilization of food for growth. On subjecting FCR and PER to second-degree polynomial regression analysis, however, break-points occurred for rations of 6.35 and 6.45%, respectively. These break-points indicate that rations in the range 6.0–6.5% bw per day is optimum for growth of *L. parsia*.

Significantly poor FCR for higher rations can be the result of loss of nutrients and wastage of food, because fish took longer to consume food to reach satiation. Hassan and Jafri (1994) reported a gradual decline in conversion efficiency for Asian catfish, *Clarias batrachus* fed higher rations. In this study a similar trend in feed-conversion efficiency was also noticed for *L. parsia* fed higher rations than the optimum. Poor growth and FCR for fish fed at lower ration of 4% bw per day suggests that, this ration is approximate to maintenance requirements only and that most of the ingested nutrients are used to maintain life and a small portion is available for growth. Present finings for *L. parsia* also seem to be in agreement with the observations of Ahmed (2007) for Rohu, *Labio rohita*. Ration level is an important factor affecting feed utilization and the requirements are affected by fish age and size. Diet composition and numerous other factors (Siddique, 2009) also play a significant role in this regard.

The whole-body composition of fish is often used as an indicator of fish quality. Several factors, including growth and diet are known to affect the body composition of ûsh. Body composition is also significantly affected by feeding rate (Cho et al. 1976; Storebakken and Austreng 1987; Hassan and Jafri 1994; Khan et al. 2004). The whole-body composition of *L. parsia* fed different rations in this study varied substantially. Body moisture content decreased significantly with increasing rations up to 6%; further increasing the rations did not result in any signiûcant difference in moisture content. Body protein content increased with increasing rations levels up to 6%; thereafter a significant fall of body protein was noticed. The fat content of fish fed different rations gradually increased with the increasing rations and was found to be significantly higher for 10% ration. This corresponds with findings for rainbow trout, *Oncorhynchus mykiss* (Storebakken and Austreng 1987). When rations were lower the amount of fat was slightly lower, although at the same time the fish managed to maintain relatively higher and constant amounts of protein in their body tissue over the initial value, suggesting that in this fish body fat is preferred to protein as an energy source. A similar result for body fat was also reported by Hung and Lutes (1987).

The optimum ration recommended in this study for *L. parsia* (6.0%) is similar to that reported for sole, *Solea vulgaris* (7% bw per day; Lagardere 1987) and higher than that reported for other Indian major carp, mrigal, C. mrigala (5.5%; Khan et al. 2004).

In this experiment we did not find a difference between the condition factors of the groups. The feeding rates have not significantly inûuenced the survivals.

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# Seasonality of nutrient content in epilimnion zone of a small reservoir in punjab (India)

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**Keywords:**
Seasonality
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**Abstract**

The nutrients occur in the reservoir, however, majority as well as increased quantity of the nutrient elements get stacked in the hypolimnion zone of the reservoir. With a view to know the status of various nutrients in the epilimnion zone of reservoir, some elements such as phosphate-phosphorus ($PO_4$-P), nitrate-nitrogen ($NO_3$-N), calcium (Ca), magnesium (Mg), potassium (K) and sodium (Na) were analyzed on monthly basis after collection of water in 2.5litre polyethylene bottle from epilimnion zone of a small reservoir (Chohal) during December 2000 to November 2001 in Punjab. The nutrient parameters show considerable monthly and seasonal variation, which is also linked to the hydrological cycle. There were sharp changes observed in the seasonality of nutrient content in Chohal water as recorded after analyses. The maximum average monthly value of $PO_4$-P and Na was observed during summer whereas Ca and Mg in monsoon. The maximum average value of $NO_3$-N and K was recorded during winter season. The average monthly minimum value of $PO_4$-P, K and Na was recorded during monsoon whereas Mg in winter season. The value of $NO_3$-N and Ca was found to be minimum during summer. The overall range of $NO_3$-N was recorded from traces to 1.774 mg/l whereas other nutrient contents such as $PO_4$-P fluctuated from traces to 0.527 mg/l, Ca (9.0 to 15.9 mg/l ), Mg (3.43 to 26.49 mg/l), K (2.1 to 7.8 mg/l), and Na (6.6 to 37.5 mg/l) in Chohal reservoir. The findings revealed that the circulation of nutrient occurs in the epilimnion zone of the reservoir and the nutrient content varies season to season, however, the nutrients are important determinants of biogenic productivity of the reservoir.

## Introduction

The reservoir is characterized by fluviatile and lentic conditions co-exist along with certain unique features of their own. It offers enough scope for stock manipulation through ecological manoeuvring, paving way for production hikes at a relatively low capital investment. Reservoirs are man-made ecosystems without a parallel in nature. Man-made impoundments created by erecting a dam across a river to obstruct its flow. The developmental activities of post-independent India have been of harnessing rivers for irrigation and hydroelectric power. Consequently a good number of reservoirs have come into existence all over India, which constitutes an important inland fishery resources having immense potential. Our country holds over 3.15 million ha of tropical reservoirs (Sugunnan, 1995) and nearly half the reservoirs belong to small category (<1000 ha) which form common features of Indian rural landscape (Sugunan and Sinha, 2000). The fish-production systems of open-water have been classified as capture fisheries of rivers and estuaries; culture fisheries of ponds; culture-based fisheries of small reservoirs and floodplain wetlands; and enhancement fisheries of medium and large reservoirs. Fishery in small reservoirs that is almost like ponds and the bulk production of open waters is

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from reservoirs. The present fish production rate in small reservoir of Bihar is only 3.9kg/ha/year. The average national yield from small reservoirs in India is nearly 50 Kg/ha/yr. The present average fish production rate from Indian reservoir is very low (20kg/ha/ya) whereas it is surprisingly high in China (743kg/ha) and Indonesia (177kg/ha).

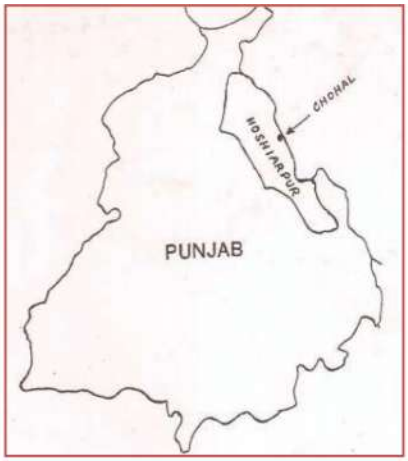
Punjab is a land locked state of India and recognized as agriculturally dominated food bowl of India. Punjab state is lying between 20 30° – 32 30° N longitude and 73 55° – 76 55°E latitude and covering an area of 50,360 $km^2$ is bestowed with vast aquatic resources in the form of lotic and lentic water bodies. Dams have been raised on rivers Satluj, Ravi and Beas to create multipurpose reservoirs for irrigation and controlling flood. These include Ranjit Sagar reservoir (3535 ha) on river Ravi, Nangal Lake (200ha) and Ropar barrage (80 ha) on River Satluj and Harike barrage (200ha) on the confluence of river Ravi and Satluj (Aggarwal, 1998). Also, there are many streams which flow from the foothills of lower shivalik range located in the Northeastern periphery of Punjab state. These are different from alluvial streams/rivers as there flow occurs only during monsoon season (June to September) or in the months of January/February. The flood peaks are of short duration. With an object of using flood peaks, reducing associated flood damage and for meeting the demand for irrigation water, the state is harnessing these flashy streams. Small dams have been raised at Dholwaha, Janauri, Damsal, Maili, Saleran, Chohal (District Hoshiarpur), Mirzapur, Perch, Siswan and Durgapur (District Roopnagar) on these streams. Some of the reservoirs have been studied for its water quality and fish productivity. However, the rate of fish production reflects around 61.2kg/ha/yr in Chohal reservoir (Sehgal, 2003).

Today almost every country has statutory regulations to ensure safety in use of fertilizers, chemicals and pesticides and there is also increasing choice to select a safer product for a given benefit. In recent time, there is growing emphasis on rational use of chemicals including pesticides thus arises out of the basic fact that technological progress is inevitable in our country and the challenges like pollution and hazards must be met to ensure progressive growth and prosperity (Hassan, 2002). Interestingly, variety of pollution hazards flows in freshwater resources in Punjab. The inflow and outflow of water for irrigation and power generation and sudden fluctuation in water level cause changes in standing crop of reservoirs affecting production process. The quality of impounded water varies from reservoir to reservoir and even within the same reservoir depending on the soil, climate and human activities. It also varies with morphometric characters, shape and size of the reservoir basin, photoperiod, wind action and amount of water change. However, the status of nutrient content varies from reservoir to reservoir. Such variability necessitates for separate evaluation of the water quality, nutrient status and productivity of different reservoir ecosystem prevailing in varied agro-climatic zones of Punjab. It is characteristics that fertility and productivity of reservoir improves after ageing compare to newly created reservoir. The productivity of reservoir depends upon the available nutrient content in the water as all aquatic plant and animal organisms require optimum amount of nutrient for their growth and survival. The nutrients occur in the reservoir, however majority as well as increased quantity of the nutrient elements get stacked in the hypolimnion zone of the reservoir. With a view to know the status of various nutrients in the epilimnion zone of Chohal reservoir, some elements were examined.

## Materials and Methods

The salient features of irrigation dam and morphometric features of Chohal reservoir were studied through survey in reservoir area as well as visit in irrigation department, Punjab. Water samples from epilimnion zone were collected from small reservoir i.e. Chohal reservoir for knowing the dynamics of available nutrient constituents, supporting other chemical and physical parameters their seasonal fluctuations as well as impact on the living organisms. Samples were analyzed on monthly basis after collection of water in a 2.5 litre polyethylene bottle from epilimnion zone of a small reservoir (Chohal), District- Hoshiarpur, three meters away from the bank to avoid any interference as well as mid-zone of the reservoir (Fig. 1 & 2). The water samples were collected using indigenously made boat (Fig.3). The estimation of light intensity, ambient temperature, water temperature, secchi disc transparency, specific conductivity, pH, salinity, was done at the sampling sites. For immediate analyses of some elements such as phosphate-phosphorus ($PO_4$-P), nitrate-nitrogen ($NO_3$-N), calcium (Ca), magnesium (Mg), potassium (K) and sodium (Na) the samples were brought to the laboratory. These analyses were done with the use of Thermometer, Pen Type Pocket pH Meter, Conductivity Meter, Salinity Meter, Lux Meter and Field Water Analyzer Kit, Flame- photometer, Spectrophotometer following Standard Methods (APHA, AWWA and WEF, 1992; Trivedi and Goel, 1984). Available phosphate-

**Fig. 1:** Map of Punjab state showing Hoshiarpur




phosphorus was analyzed by stannous chloride method using standard phosphate solution and spectrophotometer (Model: Systronics - 106). The optical density (OD) of $PO_4$-P was read at 690 nm. Similarly, nitrate-nitrogen was analyzed by phenol disulphonic acid method using standard nitrate solution and spectrophotometer. The OD of $NO_3$-N was read at 410 nm. Sodium and potassium was measured by flamephotometer (Model: Systronics) where as calcium, and magnesium by EDTA titrimetric method. Samples of existing ichthyofauna were also collected from Chohal reservoir and identified following Talwar and Jhingran (1991).

**Fig. 2:** Outline map of Chohal reservoir (Hoshiarpur) Showing depths at various range lines

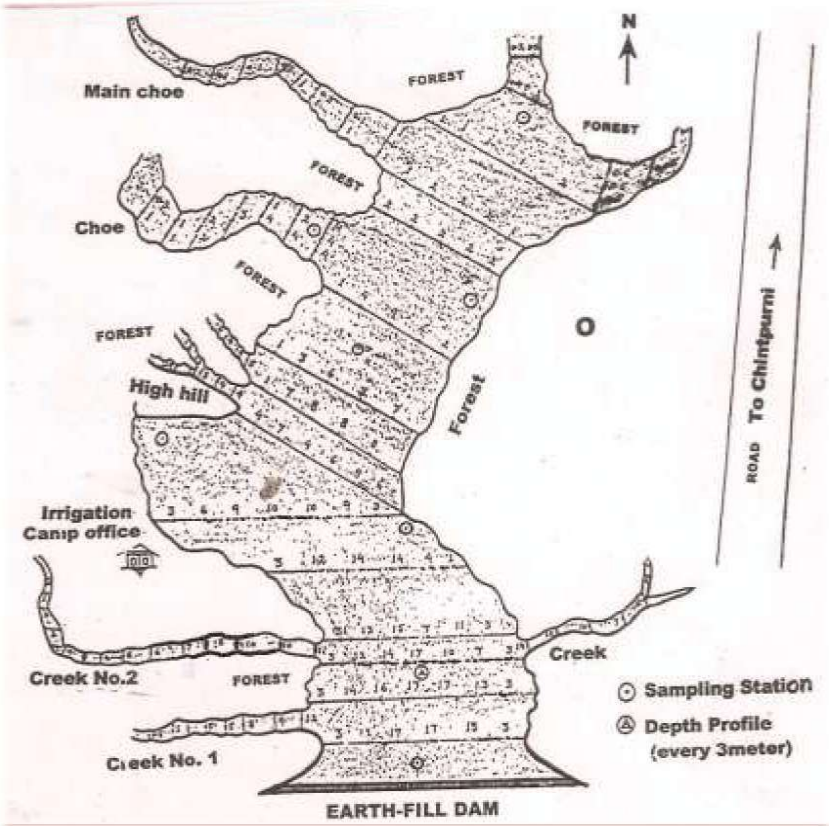



**Fig. 3:** Collection of water and soil samples from Chohal reservoir

## Results and Discussion

*Geo-morphology and hydrography features of Chohal Reservoir*

The Chohal irrigation dam is located in the Kandi area of Punjab under the Kandi Watershed Development Program of the World Bank. It consists of reservoir, spillway, irrigation outlets, and irrigation distribution system as its main component. The reservoir construction was started in January 1991 and completed in March 1993 with expenditure cost of Rs 1248 lakh. Chohal reservoir is located at a distance of about 91 km away from Ludhiana district. The reservoir occupies an area of 61 ha. It is 12 km away from Hoshiarpur district headquarter and situated on the Northeastern periphery of the Punjab state. The bathymetry, morphometric characters and special features of the irrigation dam are mentioned below and also simply depicted in figure-1.

The top level and height of dam above foundation is 388.6m and 28.6m where as length and width of top is 330m and 6m respectively. The maximum depth of reservoir is 17.08m during the normal reservoir level whereas 20.08m during the full reservoir level. The mean depth of the reservoir was calculated to be 6.5m. The gross storage capacity of the reservoir is 396.71ha m or 3267.55 AF or 39,67,100$m^3$ whereas the dead storage capacity is 44.80ha m or 369 AF or 4,48,000$m^3$. The dead storage level is 366m and the depth at dead storage level is 1.6 m. The discharge capacity of the carrier is 675 cusec. The gross culturable areas (GCA) and culturable command area of the reservoir is 1125 ha and 900 ha respectively. The dam is an earthen fill dam, which is 330m long. The width of the dam is 200m at the base and 6 m at the top. The characteristics of spillway of Chohal irrigation dam having crest level is 381.5m, width at crest (20m) and design flood (275 cusec) (Jindal *et al*, 2000).

*Physico-chemical and nutrient status of Chohal reservoir*

Various physico-chemical and nutrient parameters of Chohal reservoir were analysed with sufficient degree of accuracy. After the analyses of various physicochemical and nutrient parameters during different season revealed that water quality is the major determinants of biogenic productivity of Chohal reservoir. There were sharp changes in the seasonality of physico-chemical and nutrient features observed after analyses. The nutrient content in epilimnion zone of a small reservoir i.e., Chohal in Punjab is depicted in Table – 3 to 5.

The range of light intensity varies from 28000 to 39800 lux in the reservoir. The overall range of ambient and water temperature varies from 16.0 to 44.0 C and 13.5 to 36.0°C respectively. The other physicochemical characteristics such as water transparency fluctuated from 21.0 to 244.0cm, salinity (0.3 to 0.7ppt), conductivity (0.144 to 0.469mS), total dissolved solids (66.4 to 243.0ppm), pH (7.5 to 9.0), $CO_3$ (8.8 to 28mg/l), $HCO_3$ (104 to 162mg/l), total alkalinity (126.6 to 162.0 mg/l), chloride (2.0 to 11.5mg/l), and hardness (30 to 118mg/l) in the reservoir water. The content of dissolved oxygen was found to be in the range of 4.87 – 11.61mg/l. The range of pH showed the Chohal reservoir water is alkaline throughout the year and maintained high buffering capacity. Similar pH observation also reported in floodplain-wetland and Ganges River (Hassan *et al,* 1998a & 1998b). The alkalinity is mainly caused due to carbonate, bicarbonate and hydroxyl ions where as hardness may be due to Ca and Mg content in the water (Hassan, 2000). The available dissolved oxygen in water depends on the balance mechanism of respiration by reservoir fauna and putrefaction of organic matter. Concentration of high DO content is an indication of healthy system, which is good for distribution, behavior, and physiological growth of aquatic organisms including fisheries (Hassan *et al,* 1998a & 1998b; Hassan, 2000).

The nutrient parameters show considerable monthly and seasonal variation, which is also linked to the hydrological cycle.

*Nutrient content of Chohal reservoir*

The inorganic solids when in solution consists of anions like carbonates, chlorides, sulphates, phosphates, nitrates etc in combination with metallic cations such as calcium, sodium, potassium, magnesium, iron etc. The dissolved solids in water mass influence the chemical density of the environment, and abundance and composition of the biotic community (Jhingran, 1977). These nutrients are considered important in aquaculture and freshwater ecosystem, but their amount if excess in the medium, is harmful to the aquatic organisms including fish species. Phosphorus and nitrogen have been identified as the growth limiting nutrients in most water bodies (Vollenweider, 1978; Sugunan, 1995).

*Phosphate-phosphorus ($PO_4$-P)*

Phosphorus is least abundant but it most commonly limits the biological productivity in a vast majority of aquatic ecosystems. It is clearly realized

that among the nutrients, nitrates and phosphates seem to be limiting to some extent in nearly all the freshwater ecosystems. While in tropical waters nitrogen often limits the metabolic processes, phosphorus generally does this in temperate waters. In natural waters phosphorus is available in three forms i.e. inorganic or soluble phosphate phosphorus, soluble organic phosphorus and particulate organic phosphorus of seston contained in plankton, detritus, etc. The only significant form of inorganic phosphorus is orthophosphate. Hence in aquatic ecosystems only inorganic phosphorus as soluble orthophosphate plays significant role. The major source of phosphate in water is domestic sewage, agriculture effluents and industrial waste waters. The high concentration of phosphate is therefore indicative of pollution. There were sharp changes observed in the seasonality of nutrient content in Chohal water as recorded after analyses. The maximum average monthly value of phosphate-phosphorus was recorded to be 0.2395 mg/l during summer followed by 0.013 in winter where as average monthly minimum value of $PO_4$-P was observed to be 0.002 mg/l during monsoon. However, traces of phosphate-phosphorus were noticed during the month of January, February, July, August and November. The overall range of phosphate-phosphorus was recorded from traces to 0.527 mg/l in the epilimnion zone of Chohal reservoir (Table 3 to 5). Sinha *et al* (1998) revealed higher $PO_4$-P content in downstream of the River Ganga during monsoon period. The value analyzed in Chohal reservoir was found to be under the limit during investigation period.

*Nitrate-nitrogen ($NO_3$-N)*

The concentration of nitrate-nitrogen ($NO_3$-N) range from undetectable levels to nearly 10 mg/l in unpolluted freshwaters, but are highly variable seasonally and spatially (Wetzel, 1983). The overall range of nitrate-nitrogen was recorded from traces to 1.774 mg/l in the epilimnion zone of Chohal reservoir which also shows to a good natural condition of the water. The maximum average monthly value of nitrate-nitrogen (0.803 mg/l) was observed during winter followed by 0.228 mg/l during monsoon, whereas average monthly minimum value of $NO_3$-N was recorded to be 0.1435 mg/l during summer (Table - 3 to 5). However, traces of $NO_3$-N were noticed during the month of June, July and November. In freshwater systems close to land, nitrate can reach high levels that can potentially cause the death of fish. While nitrate is much less toxic than ammonia or nitrite, levels over 30ppm of nitrate can inhibit growth, impair the immune system and cause stress in some aquatic species. In most cases of excess nitrate concentrations in aquatic systems, the primary source is surface runoff from agricultural or landscaped areas which have received excess nitrate fertilizer. These levels of nitrate can also lead to algal blooms, and when nutrients become limiting (such as potassium, phosphate or nitrate) then eutrophication can occur. As well as leading to water anoxia, these blooms may cause other changes to ecosystem function, favoring some groups of organisms over others. Consequently, as nitrates form a component of total dissolved solids, they are widely used as an indicator of water quality. Alderfer and Lovelace (1977) believed that inorganic nitrogen above 0.03 mg/l stimulates algal growth.

*Calcium (Ca)*

Calcium is one of the most abundant substances of natural water being present in high quantities in rocks. The disposal of sewage and industrial wastes are also important source of calcium. Calcium, magnesium, iron and manganese cations contribute to the hardness of water (Shrivastava and Patil, 2002). It was reported that hard waters are more productive than the soft water from fisheries point of view (Barrett, 1953). Plankton usually needs calcium as a micronutrient (Wetzel, 1983). Various species have different sensitivity for calcium. Species of the larger genera are divisible into those adapted to acidic (pH < 6), calcium deficient waters (< 10 mg Ca/L), through a series of those adapted to increasingly alkaline, calcium-rich waters (Hutchinson, 1967). The overall range of calcium was fluctuated from 9.0 to 15.9 mg/l in the epilimnion zone of Chohal reservoir. The maximum average monthly value of calcium (12.73 mg/l) was recorded during monsoon followed by 7.37mg/l in winter, whereas average monthly value of calcium (11.63mg/l) was found to be minimum during summer (Table 3 to 5). Calcium is essential for living organisms, particularly in cell physiology, where movement of the calcium ion $Ca^{2+}$ into and out of the cytoplasm functions as a signal for many cellular processes. Calcium metal reacts with water, evolving hydrogen gas at a rate rapid. Part of the slowness of the calcium-water reaction results from the metal being partly protected by insoluble white calcium hydroxide. Calcium acts as neurotransmitter and help in muscle contraction of fish body. Calcium poisoning in fish are similar to those of sodium salts. NaCl and MgCl reduce the toxicity of calcium chloride. The value of calcium in reservoir was found to be under the limit during the study period.

*Magnesium (Mg)*

The overall range of magnesium was fluctuated from 3.43 to 26.49 mg/l in the epilimnion zone of Chohal reservoir. The maximum average monthly value of magnesium (17.0 mg/l) was observed during monsoon followed by 9.69 mg/l of Mg in summer, whereas average monthly minimum value (7.37 mg/l) in winter season (Table - 3 to 5). Magnesium ion's high solubility in water helps ensure that it is the third most abundant element dissolved in water. Magnesium compounds are much more soluble than their calcium counterparts. The monocarbonates of hard waters are usually more than 95% $CaCO_3$ under ordinary $CO_2$ pressures, and $MgCO_3$ and $Mg(OH)_2$ precipitate significantly only at very high pH values (>10) under most natural conditions (Wetzel, 1983). Magnesium is also the metallic ion at the center of chlorophyll, porphyrins and is thus a common additive to fertilizers. Magnesium ion is necessary for all life. Due to the important interaction between phosphate and magnesium ions, magnesium ions are essential to the basic nucleic acid chemistry of life, and thus are essential to all cells of all known living organisms. Magnesium poisoning in fish causes similar symptoms to those of poisoning in sodium salts. The sluggish eye movement and subsequent turn on their sides in fish are the indication of magnesium poisoning. The value of magnesium in reservoir was found to be under the limit during the study period.

*Potassium (K)*

Potassium in nature occurs only as ionic salt. As such, it is found dissolved in water, and as part of many minerals. Potassium ion is necessary for the function of all living cells, and is thus present in all plant and animal tissues. It is found in especially high concentrations in plant cells. Potassium compounds generally have excellent water solubility, due to the high hydration energy of the $K^+$ ion. The potassium ion is colorless in water. Potassium cations are important in neurons function, and in influencing osmotic balance between cells and the interstitial fluids, with their distribution mediated in all animals by the so-called $Na^+/K^+$-ATPase pump. Potassium is also important in allowing muscle contraction and the sending of all nerve impulses in animals through action potentials. It is the major cation inside animal cells, and it is thus important in maintaining fluid and electrolyte balance in the body. Potassium also produces potassium hydroxide in the reaction with water. Potassium hydroxide is a strong alkali and so is a caustic hazard, causing burns. The coloration of fish body is lighter in case of potassium poisoning. The epithelium of fish gill swells, disintegrates, undergoes lysis leading to disruption of the gas exchange. Symptoms of potassium poisoning are analogous to those of poisoning with sodium. Potassium salts are more toxic than sodium salts (Metelev *et al*, 1983). Moderate epilimnetic reduction in potassium concentration, which has been observed in extremely productive lakes, is presumably related to potassium utilization by the massive algal populations and by submerged macrophytes (Mickle and Wetzel, 1978). The overall range of potassium was recorded from 2.1 to 7.8mg/l in the epilimnion zone of Chohal reservoir. The maximum average monthly value of potassium (4.73mg/l) was observed during winter season followed by 3.875mg/l of K in summer, where as average monthly minimum value (2.53mg/l) was recorded during monsoon (Table 3 to 5). The value of potassium was found to be under the limit during the study period which is not harmful for the fish species exist in the Chohal reservoir.

*Sodium (Na)*

Sodium is one of the important cation occurring naturally. Sodium is present in great quantities in the Earth's oceans as sodium chloride. It is also a component of many minerals, and it is an essential element for animal including fish life. Sodium ions are necessary for regulation of blood and body fluids, transmission of nerve impulses, heart activity, and certain metabolic functions. Sodium is needed by animals, which maintain a high blood sodium concentration and extracellular fluid sodium concentration. Excitable animal cells, for example, rely on the entry of $Na^+$ to cause a depolarization. System for maintaining optimal salt and water balance in the body is a complex one. Excess sodium toxicity supports erratic behavior of fish swimming, fish respond poorly to mechanical stimuli; sometimes turn on their side or abdomen upward and paralytic phenomena leading to death. The dark coloration of fish body is the characteristic symptoms of sodium toxicity. A threshold level of sodium (4 mg/l) is required for near optimal growth of several species (Kratz and Myers, 1955). The overall range of sodium was recorded from 6.6 to 37.5 mg/l in the epilimnion zone of Chohal reservoir. The maximum average monthly value of sodium (30.8 mg/l) was observed during summer followed by 30.1 mg/l of Na in winter, where as average monthly minimum value (12.97 mg/l) was found during monsoon (Table - 3 to 5). The value of sodium was found to be under the limit during the study period which is not harmful for the fish species exist in the Chohal reservoir. However, sodium concentration in irrigation water and soil is of great interest as high sodium contents makes soil hard to plough and unsuitable for seedling emergence.

**Table 1:** Nutrient Content in Epilimnion Zone of a Small Reservoir in Punjab during winter

| Reservoir Nutrient | Month | | | | Average |
|---|---|---|---|---|---|
| | November | December | January | February | |
| $PO_4$-P (mg/l) | Trace | 0.052 | Trace | Trace | 0.013 |
| $NO_3$-N (mg/l) | Trace | 1.774 | 0.503 | 0.935 | 0.803 |
| Calcium (mg/l) | 15.9 | 12.2 | 10.8 | 9.8 | 12.18 |
| Magnesium (mg/l) | 3.43 | 8.7 | 9.04 | 8.31 | 7.37 |
| Potassium (mg/l) | 7.8 | 3.9 | 3.6 | 3.6 | 4.73 |
| Sodium (mg/l) | 37.5 | 29.3 | 25.6 | 28.0 | 30.1 |
| Other supporting parameters : | | | | | |
| Luxmeter readingx100 | 395 | - | - | - | 395 |
| Ambient Temp. (°C) | 30.5 | 19.5 | 16.0 | 27.0 | 23.25 |
| Water Temp. (°C) | 21.5 | 16.0 | 13.5 | 20.5 | 17.875 |
| Transparency (cm) | 111.0 | 132.0 | - | 212.0 | 151.7 |
| Sp. Conductivity (mS) | 0.404 | 0.448 | 0.144 | 0.147 | 0.286 |
| pH | 7.5 | 8.32 | 8.5 | 8.7 | 8.26 |
| Salinity (ppt) | 0.4 | 0.5 | 0.3 | 0.3 | 0.375 |

**Table 2:** Nutrient Content in Epilimnion Zone of a Small Reservoir in Punjab during summer

| Reservoir Nutrient | Month | | | | Average |
|---|---|---|---|---|---|
| | March | April | May | June | |
| $PO_4$-P (mg/l) | 0.010 | 0.384 | 0.527 | 0.037 | 0.2395 |
| $NO_3$-N (mg/l) | 0.479 | 0.077 | 0.018 | Trace | 0.1435 |
| Calcium (mg/l) | 12.6 | 13.3 | 10.6 | 10.0 | 11.63 |
| Magnesium (mg/l) | 7.63 | 7.95 | 12.98 | 10.21 | 9.69 |
| Potassium (mg/l) | 4.1 | 3.7 | 4.0 | 3.7 | 3.875 |
| Sodium (mg/l) | 30.1 | 30.0 | 32.0 | 31.1 | 30.8 |
| Other supporting parameters : | | | | | |
| Luxmeter readingx100 | - | - | - | 362 | 362 |
| Ambient Temp. (°C) | 18.0 | 42.0 | 42.0 | 39.0 | 35.3 |
| Water Temp. (°C) | 20.5 | 31.0 | 36.0 | 35.5 | 30.8 |
| Transparency (cm) | - | 244 | 140 | 127.0 | 170.3 |
| Sp. Conductivity (mS) | 0.371 | 0.319 | 0.415 | 0.469 | 0.394 |
| pH | 8.9 | 9.0 | 8.6 | 8.5 | 8.75 |
| Salinity (ppt) | 0.4 | 0.4 | 0.7 | 0.5 | 0.5 |

**Table 3:** Nutrient Content in Epilimnion Zone of a Small Reservoir in Punjab during monsoon

| Reservoir Nutrient | Month | | | | Average |
|---|---|---|---|---|---|
| | July | August | September | October | |
| $PO_4$-P (mg/l) | Trace | Trace | - | 0.006 | 0.002 |
| $NO_3$-N (mg/l) | Trace | 0.551 | - | 0.132 | 0.228 |
| Calcium (mg/l) | 15.9 | 13.3 | - | 9.0 | 12.73 |
| Magnesium (mg/l) | 8.29 | 16.21 | - | 26.49 | 17.0 |
| Potassium (mg/l) | 2.1 | 2.9 | - | 2.6 | 2.53 |
| Sodium (mg/l) | 17.7 | 14.6 | - | 6.6 | 12.97 |
| Other supporting parameters : | | | | | |
| Luxmeter readingx100 | 280 | 398 | - | 387 | 355 |
| Ambient Temp. (°C) | 39.0 | 42.0 | - | 44.0 | 41.7 |
| Water Temp. (°C) | 35.0 | 34.5 | - | 29.5 | 33.0 |
| Transparency (cm) | 175.0 | 186.0 | - | 21.0 | 127.3 |
| Sp. Conductivity (mS) | 0.405 | 0.262 | - | 0.271 | 0.313 |
| pH | 8.2 | 8.0 | - | 7.9 | 8.03 |
| Salinity (ppt) | 0.5 | 0.4 | - | 0.4 | 0.43 |

The nutrient contents and physico-chemical constituents of Chohal reservoir at Hoshiarpur district showed significant monthly/seasonal variations. Also, variety of aquatic flora and fauna such as phytoplankton, zooplankton, macro-phytes, benthic macro invertebrates, nekton communities including fish species was monitored from Chohal reservoir. Chohal reservoir does not receive any waste water. Available flora and fauna indicate that quality of reservoir water is suitable for their survival. Thus it is apparent that several environmental factors influence quality and productivity of water. The need of the day is to bring greater awareness for harmonizing population dynamics, adopting enhancement norms, advancement in eco-friendly technology like cage and pen culture, socio-economic development and harnessing of natural resources (Hassan, 2000; Hassan, 2005).

## Conclusion

The available nutrient constituents in reservoir show considerable monthly and seasonal variation, which are linked to the hydrological cycle. The findings revealed that the circulation of

nutrient occurs in the epilimnion zone of the reservoir and the nutrient content varies season to season, however, the nutrients are important determinants of biogenic productivity of the reservoir. The nutrient features of chohal reservoir considered for the study revealed that reservoir water can serve as a good habitat for many aquatic fauna including fisheries. Therefore, nutrient status of small reservoir can be improved by input of fertilizers for the enhancement of productivity compare to natural carrying capacity of the reservoir. The fertility and productivity of reservoir may improve after ageing. However, productivity of reservoir mainly depends upon water qualities and availability of nutrients as well as existence of flora and fauna in the reservoir water.

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# Effect of different supplementary diets on length-weight relationship and condition factor of stinging catfish, *Heteropneustes fossilis* (Bloch.) under captive conditions

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**Keywords:**
Catfish
Condition Factor
*Heteropnuestes fossilis*
Length-Weight Relationship
Soybean Meal
Groundnut Meal.

**Abstract**

An experimental study was conducted to observe the efficacy of different supplementary formulated diets on growth of *Heteropneustes fossilis* in terms of length-weight relationship and condition factor for 150 days. Four diets (Control - $D_1$; Experimental - $D_2$ - $D_4$) were prepared by using plant protein sources i.e. soybean meal and groundnut meal in addition to traditional feed components i.e. rice bran and mustard meal of control diet. Significantly higher ($p<0.05$) average final body weight (23.92g) and specific growth rate (0.66) was recorded with diet $D_2$ having major contribution from the soybean meal (24% rice bran, 24% mustard cake, 50% soybean meal). Maximum value of exponential 'b' (2.73), correlation coefficient 'r' (0.8510) and condition factor 'K' (0.67) in the diet $D_2$ further showed the positive effect of soybean meal on fish growth. However, the range of exponential 'b' from 1.99 to 2.73 and condition factor 'K' from 0.59 - 0.67 in all the diets indicate robustness or well being of fish throughout the study period. Thus, the results of the study showed the acceptability of soybean meal by *H. fossilis* in terms of higher growth and condition factor.

## Introduction

Stinging catfish, *Heteropneustes fossilis* (Bloch.), commonly known as Singhi, is considered as one of the highly demanded freshwater air breathing fish species in the Indian subcontinent and Southeast Asian region. It is one of the hardy fish and needs less management practices for commercial production [12]. Moreover, it is very popular and highly priced due to its high digestibility, palatability, medicinal and nutritive value and lesser spines as well as fat. In-spite of all these advantages, the culture of singhi is not commercialized till now in India due to less or non-availability of quality seed and feed. In context to nutritionally balanced feed for *H. fossilis*, protein is by far the most important nutrient required for optimum growth of fish, which can be supplied from various animal and plant protein sources [34, 13, 32]. Among major plant protein sources, soybean meal (SBM) is the product obtained after oil extraction and reported to have crude protein levels of 44-48 % [25] along with balanced amino acids profile. Further, it has more than 90% digestibility in various freshwater omnivorous fish species. Several experiments revealed that the diets containing 28-32% crude protein primarily from SBM provide growth equivalent to diets containing animal protein such as fish meal and meat and bone meal [31, 33, 28] without requiring supplementation of any crystalline amino acids. Likewise, groundnut meal (GNM) is also considered as one of the important plant based protein supplement for promoting fish growth. It is highly palatable and has better binding properties for feed pelleting than soybean. It is one of the richest sources of $B_1$ and niacin, which are otherwise low in cereals.

Though a good deal of work has been carried out to study the effect of supplementary diets on different aspects of survival and growth of *H. fossilis* [14,27,4,19,21], but limited studies had been conducted on growth and culture potentiality of *H.*

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*fossilis* in terms of length-weight relationship (LWR) and condition factor (K). Therefore, the study has been carried out under captive conditions to know the effect of SBM and GNM on growth *H. fossilis* with respect to both these parameters.

## Materials and Methods

*Experimental design*

The experiment was carried out in triplicate for 150 days in FRP pools (500liters capacity), having two inch soil layer at the bottom. After initial filling of pools with water, fingerlings of *H. fossilis* (Bloch.) were stocked @ 10/pool. At the time of stocking, the average total body length (cm) and weight (g) of fish ranged from 10.58 - 10.82 and 8.23 - 8.87 respectively.

*Experimental diets and feeding of fish*

For preparation of the diets (Control - $D_1$; Experimental - $D_2$ - $D_4$), ingredients were grinded, mixed and steam cooked to prepare the pellets (Table 1). The proximate analysis of different feed ingredients and prepared diets (Table 2) was done as per [2]. Nitrogen free extract was calculated by subtracting the values of crude protein, ether extract, crude fat and ash from 100. Gross energy ($kcalg^{-1}$) was calculated by using the energy factor 5 for proteins [38], 9 for fats and 4 for carbohydrates [16].

Fish were fed with formulated diets @ 5% of body weight once a day in evening hours throughout the experimental period. The feed quantity was regulated based on the fortnightly sampling of experimental fish.

**Table 1:** Percent Composition of supplementary diets

| Ingredients | Experimental diets | | | |
|---|---|---|---|---|
| | $D_1$ | $D_2$ | $D_3$ | $D_4$ |
| Rice bran* | 49 | 24 | 24 | 24 |
| Mustard meal* | 49 | 24 | 24 | 24 |
| Soybean meal* | - | 50 | - | 25 |
| Groundnut meal* | - | - | 50 | 25 |
| Vitamin-mineral mixture | 1.0 | 1.0 | 1.0 | 1.0 |
| Salt | 0.5 | 0.5 | 0.5 | 0.5 |

* Solvent extracted; Molasses was added as binder @ 0.5 % to all the diets

**Table 2:** Percent Proximate composition (DM basis) and gross energy of different feed ingredients and prepared diets

| Feed/ feed ingredient | Crude protein | Crude fibre | Moisture | Ash | Ether extract | Nitrogen free extract | Gross energy ($kcalg^{-1}$) |
|---|---|---|---|---|---|---|---|
| Rice bran | 14.88 | 23.10 | 11.60 | 8.60 | 0.50 | 41.32 | 2.44 |
| Mustard meal | 36.58 | 24.80 | 10.30 | 7.50 | 1.16 | 19.66 | 2.71 |
| Soybean meal | 37.83 | 10.85 | 9.30 | 7.50 | 1.67 | 32.85 | 3.35 |
| Groundnut meal | 31.90 | 27.30 | 9.90 | 8.50 | 0.83 | 21.57 | 2.53 |
| $D_1$ | 24.73 | 12.85 | 7.80 | 8.10 | 0.83 | 39.69 | 3.15 |
| $D_2$ | 30.88 | 12.85 | 8.40 | 5.95 | 1.33 | 40.59 | 3.28 |
| $D_3$ | 30.63 | 21.42 | 9.20 | 8.07 | 0.87 | 29.81 | 2.80 |
| $D_4$ | 30.89 | 16.05 | 15.80 | 7.98 | 1.03 | 28.25 | 2.76 |

*Water quality analysis*

Water quality parameters (temperature, pH, dissolved oxygen, total alkalinity, ammonical - nitrogen, nitrite - nitrogen) were recorded at fortnightly intervals throughout the experiment according to [3].

*Growth analysis*

Fish were measured in terms of total body length and weight at fortnightly intervals. *Following growth parameters were calculated*

$$\textit{Specific growth rate (SGR)} = \frac{\text{In Av. final body weight - In Av. initial body weight}}{\text{Culture days}} \times 100$$

ln = Natural logrithum

*Length-weight relationship*

The length-weight (log-transformed) relationships were determined by linear regression analysis and scatter diagrams of length and weight. The length-weight relationship of the experimented fish is worked out as per cube law given by [23].

$$W = aL^b$$

Where, W=Weight of fish (g), L is total length (cm), 'a' is the regression intercept and 'b' is the regression slope.

The logarithmic transformation of the above formula is: Log W = log a + b log L

*Fulton's condition factor (K):* Fulton's condition factor (K) was calculated according to equation: $W/L^3 \times 100$ [17]

Where, W=weight of fish (g), L=Length of fish (cm).

*Statistical analysis*

One way ANOVA was applied to work out the effect of different diets on water quality parameters and growth of fish and two ways ANOVA to determined differences among the treatments and culture period ($p<0.05$). Bray Curtis similarity metrics, Principle Component Analysis (PCA) were conducted through PAST, SPSS (v 16.0) software.

## Results

The values for all the water quality parameters were within the optimum range throughout the experimental period (Table 3). Moreover, the differences for these parameters among different treatments were not significant.

Average final body weight (23.92 g) and SGR was (0.66) found to be significantly higher ($p<0.05$) in $D_2$ followed by $D_1$, $D_3$ and $D_4$. The maximum value of condition factor 'K' was also recorded in diet $D_2$ (0.67) followed by $D_1$, (0.64), $D_3$ (0.60) and $D_4$ (0.59). The highest values of 'K' in $D_2$ suggested that fish fed with diet containing soybean meal were much more robust than the fish fed with other diets. Length – weight relationship of fishes in terms of regression co-efficient 'b' (2.73), Correlation co-efficient 'r' (0.8510) was also found to be highest in $D_2$ (Table 4 and Fig.1-4).

**Table 3:** Water quality parameters in different treatments

| Parameters | Experimental Diets | | | |
|---|---|---|---|---|
| | $D_1$ | $D_2$ | $D_3$ | $D_4$ |
| Temperature ($^0$C) | 30.17$^a$ ±0.34 | 30.07$^a$ ±0.56 | 29.70$^a$ ±0.55 | 30.15$^a$ ±0.55 |
| pH | 7.79$^a$ ±0.12 | 7.97$^a$ ±0.15 | 8.08$^a$ ±0.15 | 8.03$^a$ ±0.12 |
| Total Hardness (mgl$^{-1}$) | 224.00$^a$ ±19.68 | 212.54$^a$ ±15.49 | 217.63$^a$ ±17.51 | 238.81$^a$ ±22.33 |
| Dissolved Oxygen (mgl$^{-1}$) | 7.39$^a$ ±1.63 | 6.93$^a$ ±1.66 | 7.81$^a$ ±1.26 | 8.33$^a$ ±1.33 |
| Total Alkalinity (mgl$^{-1}$) | 248.63$^a$ ±18.90 | 251.81$^a$ ±18.93 | 264.31$^a$ ±16.44 | 292.04$^a$ ±18.34 |
| Ammonical nitrogen (mgl$^{-1}$) | 0.18$^a$ ±0.07 | 0.10$^a$ ±0.03 | 0.18$^a$ ±0.04 | 0.16$^a$ ±0.05 |
| Nitrite nitrogen (mgl$^{-1}$) | 0.03$^a$ ±0.003 | 0.02$^a$ ±0.002 | 0.02$^a$ ±0.003 | 0.02$^a$ ±0.029 |

*Values (mean±S.E.) with same superscripts in a row/column do not differ significantly ($P<0.05$)

**Table 4:** Length weight relationship of *H. fossilis* in different treatments

| Parameters | Experimental diets | | | |
|---|---|---|---|---|
| | $D_1$ | $D_2$ | $D_3$ | $D_4$ |
| Av. initial total body length (cm) | 10.68$^a$ ± 0.10 | 10.58$^a$ ±0.08 | 10.69$^a$ ± 0.04 | 10.82$^a$ ± 0.11 |
| Av. final total body length (cm) | 14.13$^b$ ± 0.31 | 14.85$^a$ ± 0.39 | 14.30$^b$ ± 0.29 | 14.05$^b$ ± 0.63 |
| Av. initial body weight (g) | 8.63$^a$ ±0.17 | 8.87$^a$ ±0.18 | 8.23$^a$ ±0.23 | 8.55$^a$ ±0.17 |
| Av. final body weight (g) | 18.58$^{bc}$ ±1.68 | 23.92$^a$ ±0.93 | 16.61$^{bc}$ ±1.45 | 15.88$^c$ ±1.99 |
| SGR %/day | 0.51 | 0.66 | 0.47 | 0.41 |
| Logarithm equation Log W= Log a+ b Log L | Log W= Log 0.000588 + 2.28 LogL | Log W= Log 0.000457 + 2.73 LogL | Log W= Log 0.000489 + 2.21 LogL | Log W= Log 0.00269 + 1.99 Log L |
| b Regression co-efficient | 2.28 | 2.73 | 2.21 | 1.99 |
| r | 0.8057 | 0.8510 | 0.7903 | 0.7168 |
| Coefficient of determination $r^2$ | 0.6492 | 0.7243 | 0.6246 | 0.5139 |
| Condition factor K | 0.64 | 0.67 | 0.60 | 0.59 |

*Values (mean±S.E.) with same superscripts in a row/column do not differ significantly ($P<0.05$)

**Fig. 1-4:** Logarithmic relationship between length and weight with regression equation of *H. fossilis* in different treatments ($D_1$ – $D_4$)

**Fig. 1**

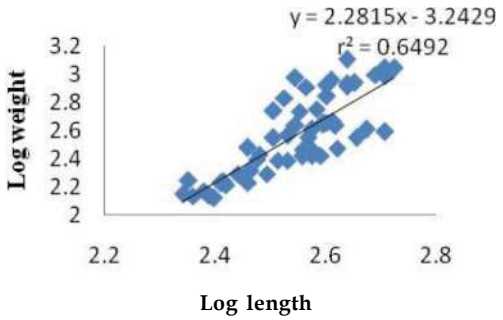



**Fig.2**

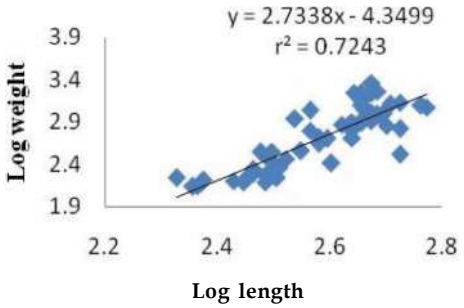



**Fig. 3**

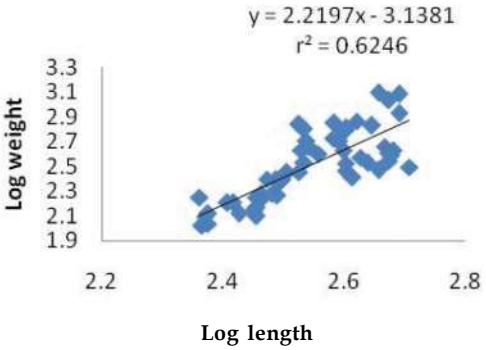
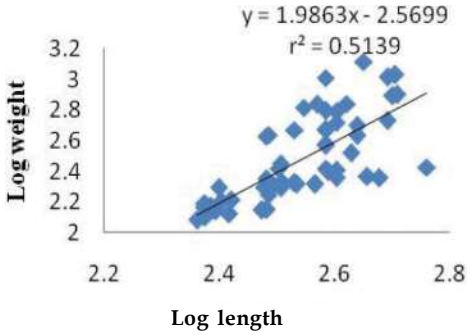



**Fig. 4**




## Discussion

Growth, feed efficacy, feed consumption and overall well being of the fish depends on physico-chemical parameters of water [30] in relation to various environmental factors [9]. All the water quality parameters fluctuated during the experimental period, but were within the favourable range reported for fish culture as suggested by [7-8, 35].

The growth parameters revealed acceptability of SBM by *H. fossilis* (Bloch.), which can be attributed to its higher digestibility. [24 Also reported more than 90% digestibility of SBM in common carp, channel catfish, tilapia etc., [26] used SBM as sole protein source in the diet of *Oreochromis karongae* and obtained higher growth rate in term of SGR, FCR and fish yield. SBM is one of the most efficiently utilized protein sources for catfishes like *H. fossilis*, *C. batrachus* and *C. gariepinus* [37]. The acceptability of SBM by *H. fossilis* even at its highest level in present study also demonstrates its efficient utilization without any deteriorating effect on fish growth. Groundnut meal (GNM) alone or in combination ($D_3$ and $D_4$) with soybean meal did not showed encouraging results due to the deficiency of sulphur containing amino acids i.e. methionine and cysteine followed by lysine [18] along with deficient amount of vitamin $B_{12}$ and calcium. The inclusion rates of GNM in experimental and practical diets for aquatic animals reported to be in the range of 5% to 61% [18]. The low feed intake, feed utilization and poor palatability [1] of GNM incorporated diets had also resulted in poor growth performance in *H. fossilis* in present study and therefore must be supplemented appropriately with lysine and methionine to have beneficial effect on fish growth [10].

The application of length-weight relationship (LWR) provide simple alternative to estimate body weight from length measurements that are less variable and more easily measured in the field. Growth is said to be positive allometric, when the weight of an organism increases more than length (b>3) and negative allometric when length increases more than weight (b<3) [39]. When total body length regressed with body weight, the slope value get significantly lower than critical isometric value i.e. 3. In the present study 'b' varied between 1.25 to 2.73 in all the treatments indicating negative allometric growth, which indicates that fish become slender as it increases in length [29]. The results of the study are in conformity with the views of [23, 11] that a fish normally do not retain the same shape or body outline

throughout the lifespan and specific gravity of tissue may not remain constant and the actual relationship may depart significantly from Cube Law. Negative allometric growth has also been reported in previous studies in *H. fossilis* [19] and *Channa punctatus* [5,15]. Variation in slope may be attributed to sample size variation, life stages and environmental factors [20]. Highest slope and coefficients of determination ($r^2$) of *H. fossilis* in $D_2$ reflected the faster growth of fish compared to other diets.

The condition factor (K) of a fish reflects physical and biological characteristics and fluctuations by interaction among feeding conditions, parasitic infections and physiological factors [23] along with assessment of fish condition [22] based on weight at a given length (indicating energy reserves in fish). It indicates the changes in food reserves and thus the general health condition without undergoing *in vitro* proximate analysis of tissues [36]. Highest condition factor in $D_2$ showed the positive effect of SBM diet in terms of well being of fish under culture conditions [6].

## Conclusion

Wild stock of *H. fossilis* under captive conditions reared on plant protein based formulated diets indicated a favourable response in terms of growth showing easy ecological transition from the wild habitat to the experimental environment. Hence, acceptability for plant based supplementary diets by *H. fossilis* confirms its potentiality for culture under captive conditions.

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# Probiotics application in aquaculture: improving nutrition and heath

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**Abstract**

Biotechnology has immense potential in enhancing animal nutrition, heath-status and productivity. One of such application of biotechnology in aquaculture is probiotics. Probiotics are micro-organisms that are beneficial to host in improving its health, digestive enzyme activity, immunity and nutrition. Probiotics may be administered to fish as a food supplement or as a water additive. These may prove as a boon to aquaculture as these not only improve heath of animal but are also helpful in bioremediation of water, and are eco-friendly. These also compete with the pathogenic bacteria; decrease their virulence, hence, limiting the use of antibiotics. The present review paper highlights the application of probiotics in aquaculture. It also summarizes the development and research highlights of the probiotic status and mode of action, which are of great significance from an eco-friendly and sustainable aquaculture point of view.

## Introduction

Growing population of a developing country and to meet the requirements of food for such a large population is an issue of major concern. Aquaculture has gained much momentum in fulfilling demands of seafood for a considerable part of population. According to a recent data published by the Food and Agriculture Organization, Fisheries and Aquaculture Department, the world aquaculture production of food fish reached 62.7 million tonnes in 2011, up by 6.2% from 59 million tonnes in 2010 and contributing about 40.1% to the world's total fish production (FAO, 2011). Indian aquaculture production mainly consists (~ 87%) of 3 native major carps and 3 exotic carps. Besides this, fish and its products are trending much now-a-days due to its rich nutritive value and awareness among people. The use of vaccines, antimicrobial agents and disinfectants for increasing fish and shrimp production, has led to the expansion of antibiotic resistance among the microorganisms which have become a global concern (Esiobu et al., 2002).

Infectious diseases are considered to be of paramount importance to the development and sustainability of commercial aquaculture, in terms of direct losses of biomass and productivity as well as indirectly as trade restrictions and poor water quality (Verschuere et al., 2000; Sharifuzzaman et al., 2014). The pathogens, however, get congenial environment for multiplication causing disease manifestations, when the fishes constantly suffer from stress due to adverse conditions in the pond ecosystem like higher temperature, higher stocking densities, less oxygen and heavy organic load etc. Hence, semi-intensive and intensive systems are very much prone to disease outbreak. Bacterial infections are one of the important causes of disease problems in Indian aquaculture (Sahoo et al., 2011). *Aeromonas hydrophila* is the most common pathogen, and it can easily spread through accidental abrasions and causes haemorrhagic septicaemia, ulcers, exophthalmia, abdominal distension (Austin and Austin, 2012).

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Until now, prevention or controlling aquatic disease has mainly depended on antibiotics and disinfectants. However, the massive use of these chemicals has led to antibiotic resistance in some instances (Verschuere et al., 2000). Also the rapid expansion of intensive aquaculture industry, are often acccompanied by rotted uneaten feed, sedimentation of faeces and organic residue. The water quality rapidly deteriorates as a result. In particular, nitrogenous compounds such as ammonia and nitrite quickly built up, which are both harmful to fish even at low concentrations (Mohapatra et al., 2012; Xie et al., 2013). Water exchange can be applied to maintain good water quality, however frequent exchange is not only laborious and costly, but also may incur disease causing agents and pollute nearby water bodies (Mohapatra et al., 2012). Therefore, there is an urgent demand for cost-effective and environment-friendly approaches for remediation of aquaculture water.

In order to mitigate the antibiotic resistance, disease problem and match the demand of seafood, biotechnological interventions have been employed. One of the successful biotechnological applications in the field of aquaculture is the use of probiotics. Probiotics are harmless live microorganisms that help the wellbeing of the host animal by competitive exclusion of pathogenic bacteria through the production of inhibitory compounds, enhancing nutrition and immune response of host species and improving water quality (Thompson et al., 1999; Verschuere et al., 2000; Gupta and Dhawan, 2011, 2012).

According to the definition of probiotic, effective probiotic treatments might provide broader-spectrum and greater disease protection as a result of immunity enhancement (Kesarcodi-Watson et al., 2008). Many micro-organisms like *Bacillus, Lactobacillus, Enterococcus, Bifidobacterium* are regarded as safe probiotics and have been commercialised for use. As *Bacillus* bacteria secrete many exoenzymes (Moriarty, 1996, 1998), these have been used widely as putative probiotics. Studies have shown that administration of probiotics in the freshwater prawn *M. rosenbergii* led to improvement in growth and survival as well as enhancement in immunity (Suralikar and Sahu, 2001; Venkat et al., 2004; Keysami et al., 2007; Shinde et al., 2008; Saad et al., 2009; Seenivasan et al., 2012). In aquaculture, probiotics can be administered either as a food supplement or as an additive to the water (Moriarty, 1998). The efficacy of a probiotic application depends on many factors such as species composition and viability, administration level, application method, frequency of application, diet, fish age, overall culture management and environmental stress factors (Lara-Flores, 2011).

## Beneficial effects of probiotics on host

Probiotics may act in several ways as shown in Fig.1 and exerts beneficial effects on the host by:

a. Enhancing growth performance through establishment of healthy gut micro-environment (Merrifield et al., 2011; Gupta et al., 2014).

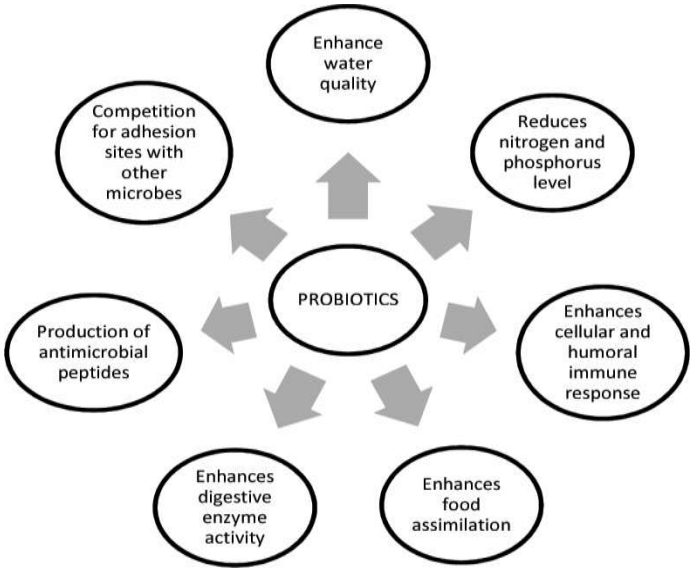
b. Providing nutrients to digestion through production of exogenous digestive enzymes and vitamins (Tinh et al., 2008; Gupta et al., 2015b).

c. Inhibiting pathogenic microorganisms through competition (Verschuere et al., 2000; Irianto and Austin., 2002; Gupta et al., 2014).

d. Enhancing immune response through elevating specific and innate immunity (Irianto and Austin., 2002; Gupta et al., 2014, 2015a); and

e. Improving water quality through water nitrogen remediation (Verschuere et al., 2000; Zhou et al., 2009; Gupta et al., 2015a; 2015b).

**Fig. 1:** Beneficial effects of probiotics



**Mechanism of probiotic action**

Probiotics modulate the growth of intestinal microbiota, suppress potentially harmful bacteria and reinforce the body's natural defense mechanisms (Giorgio et al. 2010; Bidhan et al. 2014), thus improving resistance against infectious diseases (Gildberg et al. 1997). Bacterial probiotics do not have specific mode of action but act on species specific or even strain-specific and immune responses of the animal, and their interaction with intestinal bacterial communities plays a key role (Simon 2010). Probiotics produce inhibitory substances that may be antagonistic to the growth of pathogens in the intestine. The ability of some probiotics to adhere to the intestinal mucus may block the intestinal infection route common to many pathogens (Ringo et al. 2010; Gatesoupe 1999). They can also stimulate the appetite and improve nutrition by the production of vitamins, detoxification of compounds in the diet and breakdown of indigestible components (Abdelhamid et al. 2009; Bidhan et al. 2014). Due to multiple advantages, they have gained a lot of importance in area of research in aquaculture. Some of micro-organisms used as a probiotic are listed in Table 1. However, yeast, algae and mixed strains has also been used. Mixed cultures of micro-organisms used as probiotic are listed in Table 2.

**Table 1:** List of micro-organisms used as probiotics in aquaculture.

| Group/ Genus of probiont | Micro-organism as probiotic | Target species | Method of administration | Function(s) | Reference(s) |
|---|---|---|---|---|---|
| *Bacillus* | *B. coagulans* | *Cyprinus carpio* koi | Feed additive | Growth promoter, immunostimulant | Lin et al., 2012; Wang and Xu (2006) |
| | *B.coagulans* SC8168 | *Pennaeus vannamei* | Water additive | Enhancement of water quality and growth promoter | Zhou et al., 2009 |
| | *Bacillus sp.* Commercial product (DMS) | *P. monodon* | Water additive | *Immunostimulant* | Moriarty (1998) |
| | *Bacillus sp. S11* | *P. monodon* | Feed additive | Growth promoter, immunostimulant | Rengpipat et al. (1998) |
| | *B. subtilis* | *Poecilia reticulata, Xiphophorus maculatus* | Feed additive | Enhancement of reproductive performance | Ghosh et al., 2007. |
| | *B. subtilis E20* | *Litopenaeus monodon* | Feed additive | Immunostimulant | Liu et al., 2010 |
| | *B. subtilis* □ *UTM 126* | *Litopenaeus vannamei* | Feed additive | Immunostimulant | J. L. Balc´azar and T. Rojas-Luna, 2007 |
| | *B. subtilis* | *Penaeus monodon,* | Water additive | Growth promoter, immunostimulant | Utiswannakul et al., 2011 |
| | *B. licheniformis* | *Penaeus monodon* | Water additive | Enhancement of water quality, growth promotor | Moriarty and Decamp 2005. |
| | *B. cereus* | *Farfantepenaeus brasiliensis* | Water additive | Growth promoter | Moreira et al., 2011 |
| | *B. circulans* | *L. rohita* | Feed additive | Growth promoter | Ghosh et al. (2004) |
| | *B. subtilis* | Indian major carps | Feed additive | Growth promoter | Kumar et al. (2006) |
| *Lactobacillus* | *Lactobacillus casei* | *Poeciliopsis gracilis* | Enriched *Artemia nauplii* | Growth promoter, stress tolerant | Hernandez et al., 2010 |
| | *L. rhamnosus* | *Danio rerio* | Feed additive | Enhancement of fecundity | Gioacchini et al., 2010 |
| | *Lactobacillus lactis* AR21 | *Brachionus plicatilis* | Feed additive | Growth promoter | Shiri Harzevili et al., 1998. |
| | *Lactobacillus Plantarum* | *Rotifer* | Feed additive | Immunostimulant and growth promoter | Gatesoupe, 1991 |
| | *Lactobacillus spp.* | *P. monodon* | Feed additive | Growth promoter | Phianphak et al. (1999) |
| | *Lactobacillus rhamnosus* ATCC 53103 | Rainbow trout | Feed additive | Immunostimulant | Nikoskelainen et al. (2001) |
| | *L. rhamnosus* JCM 1136 | Rainbow trout | Feed additive | Immunostimulant | Panigrahi et al. (2004) |
| *Arthrobacter* | Arthrobacter XE-7 | Shrimp larvae | Water additive | *Immunostimulant* | Li et al. (2006) |
| *Streptomyces* | Streptomyces | Penaeus monodon | Feed additive | Growth promoter, water quality enhancement | Das et al., 2006 |
| | *Streptomyces* | *Xiphophorus helleri* | Feed additive | Groeth promoter, pathogen inhibition | Dharmaraj & Dhevendaran 2010 |
| *Streptococcus* | *Streptococcus* sp. | *Fenneropenaeus indicus* | Feed additive | Immunostimulant | Ajitha et al., 2010 |
| *Carnobacterium* | *Carnobacterium divergens* | *Gadus morhua* | Feed additive | Growth promoter | Gildberg et al., 1997 |
| | *Carnobacterium divergens* | Atlantic cod (*Gadus morhua*) | Feed additive | Growth promoter | Gildberg & Mikkelsen, 1998 |
| | *Carnobacterium sp* | Atlantic salmon, Rainbow trout | Feed additive | Growth promter | Robertson et al. (2000) |
| *Alteromonas* | *Alteromonas* CA2 | *Crassostrea gigas* | Feed additive | Growth promoter | Douillet and Langdon 1994. |
| | *Alteromonas* | *Argopecten* | Water additive | Immunostimulant | Riquelme et al. (2000) |

| | | | | | |
|---|---|---|---|---|---|
| | *haloplanktis* | *purpuratus* | | | |
| *Aeromonas* | A. hydrophila | Goldfish | Feed additive | Immunostimulant | Irianto et al., 2003 |
| | *Aeromonas media* A 199 | Pacific oyster larvae *(Crassostreagigas)* | Water additive | Growth promoter | Gibson et al., 1998 |
| *Enterococcus* | *Enterococcus faecium* SF68 | *Anguilla anguilla* | Feed additive | Immunostimulant | Chang and Liu 2002 |
| *Pseudomonas* | Fluorescent *pseudomonad* F19/3 | Atlantic salmon presmolts | Bathing in bacterial suspension | Growth promoter | Smith and Davey 1993 |
| | *Pseudomonas spp.* | Rainbow trout | Water additive | Immunostimulant | Spanggaard et al., 2001 |
| | *Pseudomonas fluorescens* AH2 | Rainbow trout juveniles | Water additive | Growth promoter | Gram et al., 1999 |
| | *Pseudomonas fluorescens* | Rainbow trout *(O. mykiss)* | Water additive | Growth promoter | Gram et al., 1999 |
| *Lactococcus* | *Lactococcus lactis* AR21 | Rotifers | Water additive | Growth promoter | Harzevili et al., 1998 |
| *Pediococcus* | *Pediococcus acidilactici* | *Artemia* | Water additive | Growth promoter | Gatesoupe 2002 |
| | *Pediococcus acidilactici* | *Artemia* | Feed additive | Immunostimulant | Villami et al., 2003 |
| *Roseobacter* | *Roseobacter sp.* BS107 | *Pecten maximus* | Water additive | Growth promoter | Ruiz-Ponte et al. (1999) |
| | *Roseobacter sp.* strain 27-4 | Turbot larvae | Water additive | Growth promoter | Hjelm et al., 2004 |
| *Yeast (Saccharomyces)* | *Saccharomyces cerevisiae* | *Litopenaeus vannamei* | Feed additive | Immunostimulant | Scholz et al., 1999 |
| Unicellular alga *(Tetraselmis)* | *Tetraselmis suecica* | Atlantic Salmon juveniles | Feed additive | Growth promoter | Austin et al.,1992 |
| *Vibrio* | *Vibrio alginolyticus* | *L. vannamei* | Water additive | Growth promoter | Garriques and Arevalo 1995 |
| | *Vibrio alginolyticus* | Atlantic Salmon juveniles | Bathing in bacterial suspension | Growth promoter | Austin et al., 1995 |
| | *Vibrio pelagius* | Turbot | Water additive | Growth promoter | Ringø and Vadstein 1998 |
| | *Vibrio alginolyticus* C14 | *Artemia* nauplii | Water additive | Growth promoter | Gomez-Gil et al., 1998 |

**Table 2:** List of mixed cultures (various strains of micro-organisms) used as probiotics in aquaculture.

| **Mixed cultures** | **Target species** | **Mode of administration** | **Function(s)** | **Reference** |
|---|---|---|---|---|
| *Bacillus coagulans, Paenibacillus polymyxa* and *B. licheniformes* | *Cyprinus carpio* | Feed additive | Growth promoter, immunostimulant | Gupta et al., 2014 |
| *Bacillus megaterium, B. polymyxa, B. licheniformis, B. subtilis* (Biostart) | *Channel catfish* | Water additive | *Growth promotor* | Queiroz and Boyd 1998 |
| *Cytophaga sp., Roseobacter sp., Ruergeria sp., Paracoccus sp., Aeromonas sp., Shewanella sp.* | Gilthead sea bream larvae *(Sparus aurata)* | Water additive | *Growth promotor* | Makridis et al. (2005) |
| *Vibrio spp., Micrococcus sp.* | Gilthead sea bream | Feed additive | *Immunostimulant* | Chabrillón et al. (2006) |
| *Vibrio P62, Vibrio P63, Bacillus P64* | *P. vannamei* | Water additive | Immunostimulant | Gullian et al. (2004) |
| *Vibrio hepatarius, Vibrio sp., Bacillus sp.* | *P. vannamei* | Feed additive | Growth promotor | Balca´zar (2003) |
| *Streptococcus lactis and Lactobacillus bulgaricus* | Turbot larvae *(Scophthalmus maximus)* | Enrichment of live food | Growth promotor | Garcı´a de la Banda et al. (1992) |
| *Lactobacillus sp. and Carnobacterium sp.* | Turbot larvae | Enrichment of rotifers | Growth promotor | Gatesoupe (1994) |
| *Aeromonas hydrophila, Vibrio fluvialis, Carnobacterium sp., Micrococcus luteus* | Rainbow trout | Feed addititve | Disease resistance | Irianto and Austin (2002) |
| *Saccharomyces cerevisiae, S. exiguus, Phaffia rhodozyma* | *Penaeus vannamei* | Feed additive | Disease resistance | Scholz et al. (1999) |
| *Pseudomonas sp., Vibrio fluvialis* | *P. monodon* | Water additive | Immunostimulant | Alavandi et al. (2004) |
| *Lactobacillus casei, L. brevis, L. helveticus, Lactococcus lactic spp. lactis, Leuconostoc, Mesenteroides spp. mesenteroides, Pediococcus acidilactici* | *Artemia nauplii* | Water additive | Immunostimulant | Villamil et al. (2003) |
| *Streptococcus faecium, L. acidophilus, S. cerevisiae* | *Nile tilapia* | Feed additive | Growth promotor | Lara-Flores et al. (2003) |
| Commercial product: Bactocell (*Pediococcus acidilactici*), Levucell (*Saccaromyces cerevisiae*) | *Pollack* | Enriched Artemia | Growth promotor | Gatesoupe (2002) |
| Improval (*L. sporogenes* and *Saccharomyces cerevisiae*) | *Macrobrachium rosenbergii* | Feed additive | Growth promotor | Gupta and Dhawan 2012 |
| *Bacillus* NL 110, *Vibrio* NE 17 | *M. rosenbergii* | Feed additive | Enhancement of water quality, growth promotor | Rahiman et al., 2010 |

*Improvement in water quality*

Remediation of aquaculture water using microorganisms is a burgeoning trend for the sustainable development of aquaculture industries (Verschuere et al., 2000). It is very important to provide fish with a healthy environment and probiotics has great deal of potential (Zhou et al., 2009). *Bacillus* species are widely used for water remediation because they are stable for long period due to spore formation, easily prepared by fermentation and possess antagonistic effects on pathogens (Xie et al., 2013; Zhou et al., 2009; Hong et al., 2005). Screening strains with good remediation characteristic in conjunction with their influence on survival, immune response and disease resistance still remains a fundamental step towards developing commercial microbial agents. Very few reports are available on spore forming bacteria to describe the remediation effect in freshwater aquaculture. Wang and He (2009) observed that the application of probiotics would mitigate the nitrogen and phosphate pollution in ponds sediments. Xie et al. (2013) demonstrated the use of *Bacillus amyloliquefaciens* for remediation of aquaculture water. Wang et al. (2005) investigated the effect of commercial probiotics on water quality of *P. vannamei* ponds and the results showed that probiotics could significantly reduce the concentrations of nitrogen in pond water. Similarly, Ma et al. (2009) evaluated the feasibility of *Lactobacillus* strains for the removal of nitrogen and observed simultaneous removal of ammonia, nitrite and nitrate. However, Zhou et al. (2009) observed inconsistence results by using different concentrations of *B. coagulans* as water additive in the culture of shrimp *P. vannamei*. Gupta et al. (2015a) used varied concentrations of *P. polymyxa* in common carp as water additive and found no effect of probiotics on the water quality improvement.

*Enhancement of growth performance*

Probiotics administration also has been shown to increase animal survival by enhancing resistance to pathogens by activating both cellular and humoral immune defences. The enhanced growth performance of animal might be induced by the probiotics via synthesis of vitamins and cofactors or enzymatic improvement (Gatesoupe, 1999). *Bacillus* strains are widely used in aquaculture industry through dietary supplementations for the improvement of growth (Ghosh et al., 2008) and feed utilization through digestive enzymes enhancement (Zhou et al., 2009). Gupta et al. (2015b) studied that inoculation of varied concentrations of *P. polymyxa* in water resulted in significant improvements in growth performance in terms of weight gain, specific growth rate, survival, relative per cent survival and feed utilization in terms of food conversion ratio and protein efficiency ratio of *C. carpio*. Previous study showed that *Bacillus* sp when applied as probiotic was able to colonize both in the culture water and the fish digestive tract, thereby increasing the fish survival (Rengpipat et al., 1998; Zhou et al., 2009; Gupta et al. 2014). Similar experiments had previously been documented in preliminary trials on Nile tilapia *Oreochromis niloticus* (Lara-Flores et al., 2003; Haroun et al., 2006), Indian carp *Cirrhinus mrigala* (Swain et al., 1996), Persian sturgeon *Acipenser persicus* (Faramarzi et al. 2011), Chinese carp *Cyprinus carpio* (Ramkrishnan et al., 2008) and giant freshwater prawn *Macrobrachium rosenbergii* (Gupta and Dhawan, 2011, 2012). However, Shariff et al. (2001) found that treatment of *P. monodon* with a commercial *Bacillus* probiotic did not significantly increase growth and survival. It was difficult to directly assess different studies using probiotics, because the efficacy of probiotic application depended on many factors (Gomez-Gil et al., 2000; Gupta et al., 2014) such as species composition, application level, frequency of application and environmental conditions.

*Enhancement of digestive enzyme activity*

Studies indicate that some probiotics increase the content of digestive enzymes in the gastro-intestinal tract (GIT) and thereby facilitate nutrient utilization and digestion (Haroun et al., 2006; Abdelhamid et al., 2009). The increased activities of digestive enzymes in fish fed probiotics are due to the improvement in the digestion of protein, starch, fat and cellulose and increase in absorption of food, which in turn contributed to the improved growth and survival. Effects have been reported for fish and shrimp, in which digestion was shown to increase considerably in response to probiotics in the diet (Lara-Flores et al., 2003; Ziaei-Nejad et al., 2006; Wang, 2007). Bacteria, particularly members of the genus *Bacillus* secreted a wide range of exoenzymes (Moriarty, 1998). The exogenous enzymes produced by the probiotic would represent, at most, only a small contribution to the total enzyme activity of the gut (Ding et al., 2004; Zhou et al., 2009), and the presence of the probiotic might stimulate the production of endogenous enzymes by the fish. The increase in specific activities of digestive enzymes in probiotic inoculated fish led to enhanced digestion and increased absorption of food, which in turn contributed to the improved growth and survival in fish (Gupta et al. 2015b).

*Enhancement of immune responses*

The innate immune system, comprising physical barriers, and cellular and humoral components, serves as a defense weapon in aquatic organisms (Magnadóttir, 2006). The beneficial effects of probiotics as immunostimulants have already been studied in several freshwater fish for example *Labeo rohita* (Nayak et al., 2007), *Oreochromis niloticus* (Aly et al., 2008), *O. mykiss* (Sharifuzzaman and Austin, 2010) and *C. carpio* (Gupta et al., 2014). Lysozyme is a cationic enzyme that attack the â-1, 4 glycosidic bond between N-acetylmuramic acid and N-acetylglucosamine in the peptidoglycan of bacterial cell walls. This enables lysozyme to lyse certain Gram-positive bacteria, and in conjunction with complement, even some Gram-negative bacteria (Alexander and Ingram, 1992). Administration of *Bacillus* strains could significantly enhance serum lysozyme activity of rainbow trout, *O. mykiss* (Merrifield et al., 2009) and common carp, *C. carpio* fry (Gupta et al., 2014). In contrast, the serum lysozyme content of tilapia (*O. niloticus*) was not affected by treatment with *B. subtilis* B10 and *B. coagulans* B16 as water additive for 40 days (Zhou et al., 2009). The differences in the effects of lysozyme activity can be due to the inclusion levels as well as the fish species under study. Respiratory bursts are produced by phagocytes to attack invasive pathogens during phagocytosis and have been widely used to evaluate host defense capabilities against pathogens; however, excessive accumulation of reactive oxygen intermediates (ROIs) is extremely toxic to host cells (Dalmo et al., 1997). The stimulation of respiratory burst activity after dietary probiotic supplementation involving feeding regimes and feeding durations have been previously reported in various fish (Aly et al., 2008; Kumar et al., 2008; Giri et al., 2012; Geng et al., 2011; Sun et al., 2010; Gupta et al., 2014). Studies have also demonstrated that dietary administration of high levels of probiotics for longer periods affects respiratory burst activity in *L. rohita* (Kumar et al., 2008; Giri et al., 2012); *O. niloticus* (Aly et al., 2008) and *R. canadum* (Geng et al., 2012). The myeloperoxidase is an important enzyme that utilizes oxidative radicals to produce hypochlorous acid to kill pathogens. During oxidative respiratory burst, it is mostly released by the azurophilic granules of neutrophils (Dalmo et al., 1997). Result of elevated myeloperoxidase level in serum was observed for *B. amyloliquifaciens* in carp, *C. catla* (Das et al., 2013); *B. subtilis* in rainbow trout, *O. mykiss* (Newaj-Fyzul et al., 2007); *L. plantarum* in grouper, *E. coioides* (Son et al., 2009) and *P. polymyxa* in common carp, *C. carpio* (Gupta et al., 2014).

In vertebrates, phagocytic process is followed by the production of reactive oxygen molecules, such as superoxide anion ($O_2$), hydrogen peroxide ($H_2O_2$) and hydroxyl radical (OH), all of which are highly microbicidal (Sun et al., 2010). The main enzymes which detoxify reactive oxygen molecules are superoxide dismutase (SOD), catalase and glutathione peroxidase, all of them abundant in fish tissues (Di Giulio et al., 1993). SOD catalyses the dismutation of the highly reactive $O_2$ to the less reactive $H_2O_2$ and belongs to the main antioxidant defence pathways in response to oxidative stress (Fridovich, 1995). Catalase is the primary cellular enzymatic defense against $H_2O_2$, converting it into $H_2O$ and $O_2$, and is critical for the process of scavenging free radicals (Dorval et al., 2003). Zhou et al. (2009) and Sun et al. (2010) demonstrated that the SOD activities of tilapia (*O. niloticus*) and grouper (*E. coioides*), respectively increased significantly after treated with *Bacillus* spp. However, Son et al. (2009) found that dietary administration of different levels of *L. plantarum* significantly decreased the SOD activity. The authors hypothesized that the decreased SOD in those fish fed *L. plantarum*-supplemented diets may occur in order to retain the superoxide anion level or to convert it into the singlet oxygen ($^1O_2$) and/or hydroxyl radicals (OH) via a metal-catalyzed interaction to enhance the microbial-killing capacity of phagocytes. Therefore, further study is needed to illustrate the effects of probiotics on the antioxidant enzymes and their related immune function in fish.

*Protection against infectious diseases*

The activation of non-specific immunity by immunostimulants is associated with increased protection against infectious disease (Sakai, 1999). Probiotics help in achieving natural resistance and controlling disease-related loss among farmed fish (Abraham et al., 2007). Gupta et al. (2015a) examined application of probiotic *P. polymyxa* as water additive and its effect on fish injected with *A. hydrophilla in vivo*. Bacterium *A. hydrophilla* is the causative agent of haemorrhagic septicaemia in a wide range of commercially important fish species including carps (Zhang et al., 2012). Occurrence of antibiotic resistant strains of *A. hydrophilla* in fish was reported (Giri et al., 2012). Therefore, prophylactic against this bacterium by the inclusion of immunostimulants in water becomes more practical to implement in a fish farm. In a previous study protection was achieved in eel (*A. anguilla*) and Indian carp (*C. catla*) against *A. hydrophila* infection after the fish were fed with diet supplemented with *B. amyloliquefaciens* (Cao et al., 2011; Das et al., 2013). Protection against

edwardsiellosis (Nayak et al., 2007); enteric red mouth disease (Kim and Austin, 2006); furunculosis (Irianto and Austin, 2002); lactococossi and streptococcosis (Brunt and Austin, 2005) and aeromoniasis (Newaj-Fyzul et al., 2007) have successfully been accomplished through probiotics feeding. Increased non-specific immune responses such as lysozyme, RBA, MPO, SOD by the application of probiotics resulted in enhancement of fish disease resistance.

## Conclusions

The present review highlighted the application of probiotics in aquaculture. Probiotics improve nutrition as well as the health of fish, prawn and molluscs. The supplementation of probiotics through feed is a better method to ensure the efficiency of the probiotic bacteria in the gastro-intestine of fish without interacting with the surrounding medium. The negative environment effects on fish could be mitigated through the use of probiotics as water additive. The use of a probiotic in culture water is a special focus of environmental research.

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# Cost and earning analysis of ice plant of fishery industry of Ratnagiri

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**Keywords:**
Ice Plants
Capital Cost
Variable Cost
Fixed Cost
Net Profit
Capital Turnover Ratio
Gross Ratio
Variable Cost Ratio
Fixed Cost Ratio

**Abstract**

This paper attempts to explore the cost and earning analysis of ice plant of fishery industry of Ratnagiri. Cost and earning analysis of any business gives an idea about the economic feasibility. Considering vast differences in the production capacities of ice plants, costs and earning analysis was performed separately for the two categories of ice plants, first with less than 50 tonne and second with more than 50 tonne capacity. The net profit earned by less than 50 tonne capacity ice plant was much more less than that of more than 50 tonne capacity. All the economic indicators estimated clearly showed that the more than 50 tonne capacity ice plants were more profitable than that of less than 50 tonne capacity ice plants.

## Introduction

Fish is highly perishable food commodity; its spoilage begins as soon as the fish is dead after catching. Various biochemical and microbiological changes (Gopakumar, 2002) take place in fish after death, due to which it become inedible. Biochemical and microbiological process can be reduced by lowering the temperature of fish. Ice is an effective and ideal cooling medium commonly used for lowering the temperature, which absorbs heat from fish and prevents spoilage. Fish is to be marketed at lower temperature in cold chain from the time of harvest till it is consumed. India being a developing country, cold chains are not well established and ice is commonly used as a cheapest source for preservation of fish while marketing.

Fishing voyages at present are of several days duration and the use of ice on-board fishing vessels is a common practice to keep the fish in good condition till it is landed. Similarly, use of ice has become a common practice in the marketing of fish to get better price. Usage of quantity of ice for preservation of fish depends on quantity and quality of fish to be marketed. Requirement of ice for on-board fish preservation varies according to type of fishing operation. Quantity of ice required in the fishing industry varies season-wise as quantity and kind of fish landed varies from season to season. Ice plants were established in Ratnagiri since the inception of mechanized fishing. Mechanized fishing during those periods was shrimp targeted, as the shrimp was the major commodity of export during that period.

Marine fishing industry in Maharashtra has witnessed rapid development due to intensive mechanization program, which have led to the rise in seafood production. Maharashtra has five maritime districts namely Mumbai, Thane, Raigad, Ratnagiri and Sindhudurg. Ratnagiri district is one of the major fish contributors having 167 km of coastline. Mirkarwada is one of the important minor fishing harbors situated in Ratnagiri city, the head

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quarters of Ratnagiri district. In addition to Mirkarwada fishing harbor, there are 18 fish landing centers in Ratnagiri block. There are 344 trawlers, 161 purse-seiners and 570 gill-netters (Anon, 2008) operated along the coast of Ratnagiri block. Marine fish landed in Ratnagiri block is either processed for export or sold in fresh condition in local markets through various marketing personnel. There are total 18 numbers of ice plants situated in Ratnagiri city and one ice plant situated in Jaigad village. These ice plants are fulfilling the present requirement of ice of the fishing industry of the Ratnagiri block. As per the vast requirement of ice in fishery industry, it is necessary to carry out economic feasibility of various production capacity of ice plant. Earlier, Indian Institute of Management, Ahmedabad studied state-wise economics of various capacity of ice plant (Gupta *et al.*, 1984). However, the economic feasibility of various production capacities of ice plants of fishery industry of Ratnagiri is unknown. Considering the importance of ice plants in fishery industry of Ratnagiri, the present study was undertaken to understand the cost and earning analysis of various production capacity of ice plants in Ratnagiri block.

## Materials and Methods

The study was carried out in the year 2007-08 in Ratnagiri block. The study was employed along the 67 km stretch of Ratnagiri block extended from 17°18′50.89" N and 73°11′15.90" E to 16°45′37.70" N and 73°18′20.00" E (Fig.1). There were the vast differences in the production capacities of ice plants, costs and earning analysis was performed separately for the two categories of ice plants, first with less than 50 tonne and second with more than 50 tonne capacity, which were operating in Ratnagiri block. The averages of different economic factors of these ice plants were estimated separately. Interview schedules were constructed for collection of required information about different economic factors of ice plant were formulated (McGoodwin, 2001).

Data related all economic parameters of ice plants were collected from all the 19 ice plants functioning in Ratnagiri block. Expenditure on land, construction of building, electrification, machinery, fabrication of block ice cans, plumbing and furniture were included in capital cost, whereas the expenses on electricity, machinery maintenance, salary, office expenses and water charges were the major components of variable cost. Capital costs and variable costs for less than and more than 50 tonne capacity ice plants were calculated separately for each component by averaging cost incurred by sampled units (Dewey, 1975). The fixed cost per annum was calculated by adding the interest on capital cost and variable cost, depreciation on capital cost and insurance (Dewey, 1975). Monthly revenue was raised by multiplying the total monthly ice production with the average sale price for the respective month. Total revenue was estimated by summing the revenue of each month. The annual net profit for both categories of ice plant was obtained separately by subtracting the total expenditure from the revenue in a year.

Capital turnover ratio, rate of return to capital, gross ratio, variable cost ratio, fixed cost ratio (Salim and Biradar, 2001) and pay back period (Bensam, 1999) were some of the key economic indicators estimated separately for both the categories of ice plants on the basis of costs and earning analysis of ice plants operating in Ratnagiri block.

## Results and Discussion

The ice plants operating in Ratnagiri block were mostly established during the period from 1972 to 2008. Considering the vast differences in the production capacities of ice plants, costs and earning analysis was performed separately for the two categories of ice plants, first with less than 50 tonne and second with more than 50 tonne capacity (Table 1 and 2). The total capital cost of less than and more than 50 tonne capacity ice plant was Rs. 34,02,841/- and Rs. 59,01,456/- respectively. The major share of capital investment was construction cost, followed by machinery cost, block ice cans etc (Fig. 1 and 3). The variable cost was Rs. 17,70,075/- and Rs. 31,40,500/- for less than and more than 50 tonne capacity ice plant respectively whereas, the project cost of respective plants were Rs. 51,72,916/- and Rs. 90,41,956/-. The major expenditure among the variable costs was on electricity charges, salaries and water charges etc (Fig. 2 and 4). Similar observation about the variable costs was also reported by Gupta *et al.* (1984) for 18 tonne capacity ice plants in Maharashtra. The annual revenue of less than and more than 50 tonne capacity ice plants was Rs. 34,52,400/- and Rs. 86,31,000/- respectively whereas, the net profit calculated for respective ice plants were Rs. 7,15,091/- and Rs. 38,04,519/-. Gupta *et al*. (1984) have also reported the costs and earning analysis of 18 tonne capacity ice plant. The most of the costs, revenue and net profits reported by them were much more less than the values estimated for the respective factors during the present study. These differences are observed due to the escalation of prices, as both the studies are carried out in different periods.

Capital turn over ratio was estimated at 1.01 and 1.46 for less than and more than 50 tonne capacity ice plant respectively (Table 3). It has indicated that a rupee earned per rupee invested was more in second category ice plant than first category ice plant. The gross ratio, variable cost ratio and fixed cost ratios were estimated at 0.79, 0.51 and 0.28 respectively for less than 50 tonne capacity ice plant and for more

**Table 1:** Costs and earning analysis for ice plant of less than 50 tonne capacity in Ratnagiri Block

**A. Capital cost**

| Particulars | Quantity | Amount |
|---|---|---|
| 1. Land cost | 10 are @ Rs. 18,000/- per are | Rs. 1,80,000.00 |
| 2.Construction Cost | a. Construction cost 223 sq. m. building @ Rs. 3,768/- per sq. m | Rs. 8,40,264.00 |
| | b. Brine tank, 40 sq. m. @ Rs. 3,768/- per sq. m. | Rs. 1,50,720.00 |
| | c. Overhead tank (25,000 lit) | Rs. 1,50,000.00 |
| | d. Water storage tank (40,000 lit) | Rs. 1,50,000.00 |
| 3. Machinery cost | a. Compressor (KC3) 2 numbers @ Rs. 2,50,000/- each | Rs. 5,00,000.00 |
| | b. Condenser | Rs. 1,50,000.00 |
| | c. Receiver | Rs. 27,000.00 |
| | d. Electric host (crane) with 1 hp pump | Rs. 50,000.00 |
| | e. Ice crusher 2 numbers @ Rs. 25,000/- each | Rs. 50,000.00 |
| | f. Agitator | Rs. 10,000.00 |
| | g. Compressor motor 2 numbers @ Rs. 1,00,000 each | Rs. 2,00,000.00 |
| | h. Valves | Rs. 15,000.00 |
| | i. Oil separator | Rs. 5,000.00 |
| 4. Plumbing | | Rs. 70,000.00 |
| 5. Block ice cans | 360 numbers @ Rs. 1,500/- per one can | Rs. 5,40,000.00 |
| 6. Furniture cost | a. Table and chairs | Rs. 10,000.00 |
| | b. Cupboard 2 numbers @ Rs. 6,000/- | Rs. 12,000.00 |
| 7. Electrification | | Rs. 2,92,857.00 |
| **Total Capital cost** | | **Rs 34,02,841.00** |
| **B. Variable cost** | | |
| 1. Salary | a. Labors (4 numbers): @ Rs. 3,500/- per labour for 12 months | Rs. 1,68,000.00 |
| | b. Operators (3 numbers): @ Rs. 4,000/- per operator for 12 months | Rs. 1,44,000.00 |
| | c. Watchman (2 numbers): @ Rs. 2,000/- per watchman for 12 months | Rs. 48,000.00 |
| 2. Electricity charges | for 24,500 units/month @ Rs. 5/- per unit (9 month) | Rs.11,02,500.00 |
| 3. Water charges | 9,450 tonne water @ Rs. 13.5/- per tonne | Rs. 1,27,575.00 |
| 4. Maintenance charges of machinery | | Rs. 1,00,000.00 |
| 5. Maintenance charges of building | | Rs. 50,000.00 |
| 6. Office expenses | | Rs. 30,000.00 |
| **Total variable cost** | | **Rs. 17,70,075.00** |
| **C. Total Project cost (A+B)** | | Rs. 51,72,916.00 |
| **D. Total loan amount at 80 per cent of the total project cost** | | **Rs. 41,38,333.00** |
| **E. Fixed cost** | a. Interest on total loan amount @ 15% | Rs. 6,20,750.00 |
| | b. Depreciation on Capital cost @ 10% | Rs. 3,40,284.00 |
| | c. Insurance on total assets (for one year) | Rs. 6,200.00 |
| **Total Fixed cost** | | **Rs. 9,67,234.00** |
| **F. Total cost (B+E)** | | **Rs. 27,37,309.00** |
| **G. Revenue** | | **Rs. 34,52,400.00** |
| **H. Profit/Loss (G-F)** | | **Rs. 7,15,091.00** |
| **I. Profit** | | **Rs. 7,15,091.00** |

**Table 2:** Costs and earning analysis for ice plant of more than 50 tonne capacity in Ratnagiri Block

**A. Capital cost**

| Particulars | Quantity | Amount |
|---|---|---|
| 1. Land cost | 20 are @ Rs. 18,000/- per are | Rs. 3,60,000.00 |
| 2. Construction Cost | a. Construction cost 372 sq. m. building @ Rs. 3,768/- per sq. m | Rs. 14,01,696.00 |
| | b. Brine tank, 70 sq. m. @ Rs. 3,768/- per sq. m. | Rs. 2,63,760.00 |
| | c. Overhead tank (60,000 lit) | Rs. 2,50,000.00 |
| | d. Water storage tank (1 lakh lit) | Rs. 3,00,000.00 |
| 3. Machinery cost | a. Compressor (KC3) 1 number @ Rs. 2,50,000/- | Rs. 2,50,000.00 |
| | (KC4) 1 number @ Rs. 5,00,000/- | Rs. 5,00,000.00 |
| | b. Condenser | Rs. 2,50,000.00 |
| | c. Receiver | Rs. 54,000.00 |
| | d. Electric host (crane) with 1 hp pump | Rs. 1,00,000.00 |
| | e. Ice crusher 2 numbers @ Rs. 25,000/- each | Rs. 75,000.00 |
| | f. Agitator | Rs. 10,000.00 |
| | g. Compressor motor 2 numbers @ Rs. 1,00,000 each | Rs. 3,00,000.00 |
| | h. Valves | Rs. 25,000.00 |
| | i. Oil separator | Rs. 7,500.00 |
| 4. Plumbing | | Rs. 1,57,500.00 |
| 5. Block ice cans | 500 numbers @ Rs. 2,500/- per one can | Rs. 12,50,000.00 |
| 6. Furniture cost | a. Table and chairs | Rs. 10,000.00 |
| | b. Cupboard 2 numbers @ Rs. 6,000/- | Rs. 12,000.00 |
| 7. Electrification | | Rs. 3,25,000.00 |
| **Total Capital cost** | | **Rs. 59,01,456.00** |
| **B. Variable cost** | | |
| 1. Salary | a. Labors (7 numbers): @ Rs. 3,500/- per labour for 12 months | Rs. 2,94,000.00 |
| | b. Operators (4 numbers): @ Rs. 4,000/- per operator for 12 months | Rs. 1,92,000.00 |
| | c. Watchman (3 numbers): @ Rs. 2,000/- per watchman for 12 months | Rs. 72,000.00 |
| 2. Electricity charges | for 42,500 units/month @ Rs. 5/- per unit (9 month) | Rs. 19,12,500.00 |
| 3. Water charges | 20,000 tonne water @ Rs. 13.5/- per tonne | Rs. 2,70,000.00 |
| 4. Maintenance charges of machinery | | Rs. 2,00,000.00 |
| 5. Maintenance charges of building | | Rs. 1,50,000.00 |
| 6. Office expenses | | Rs. 50,000.00 |
| **Total variable cost** | | **Rs. 31,40,500.00** |
| **C. Total Project cost (A+B)** | | **Rs. 90,41,956.00** |
| **D. Total loan amount at 80 per cent of the total project cost** | | **Rs. 72,33,565.00** |
| **E. Fixed cost** | a. Interest on total loan amount @ 15% | Rs. 10,85,035.00 |
| | b. Depreciation on Capital cost @ 10% | Rs. 5,90,146.00 |
| | c. Insurance on total assets (for one year) | Rs. 10,800.00 |
| **Total Fixed cost** | | **Rs. 16,85,981.00** |
| **F. Total cost (B+E)** | | **Rs. 48,26,481.00** |
| **G. Revenue** | | **Rs. 86,31,000.00** |
| **H. Profit/Loss (G-F)** | | **Rs. 38,04,519.00** |
| **I. Profit** | | **Rs. 38,04,519.00** |

**Table 3:** Economic indicators for less than and more than 50 tonne capacity ice plants

| | Particulars | <50 tonne | >50 tonne |
|---|---|---|---|
| **Aggregate measure** | | | |
| 1 | Total cost (Rs.) | Rs. 27,37,309.00 | Rs. 48,26,481.00 |
| 2 | Total capital cost (Rs.) | Rs. 34,02,841.00 | Rs. 59,01,456.00 |
| 3 | Total variable cost (Rs.) | Rs. 17,70,075.00 | Rs. 31,40,500.00 |
| 4 | Fixed cost (Rs.) | Rs. 9,67,234.00 | Rs. 16,85,981.00 |
| 5 | Income over variable cost (Rs.) | Rs. 16,82,325.00 | Rs. 54,90,500.00 |
| 6 | Annual ice production (tonne) | 4,932 | 12,330 |
| 7 | Annual revenue (Rs.) | Rs. 34,52,400.00 | Rs. 86,31,000.00 |
| 8 | Total number of production days | 274 | 274 |
| 9 | Net annual profit (Rs.) | Rs. 7,15,091.00 | Rs. 38,04,519.00 |
| 10 | Cost of electrification (Rs.) | Rs. 2,92,857.00 | Rs. 3,25,000.00 |
| 11 | Construction cost (Rs.) | Rs. 12,90,984.00 | Rs. 22,15,456.00 |
| 12 | Machinery cost (Rs.) | Rs. 10,77,000.00 | Rs. 17,29,000.00 |
| **Efficiency ratio** | | | |
| 1 | Capital turnover ratio | 1.01 | 1.46 |
| 2 | Rate of return to loan amount (%) | 32.28 | 67.6 |
| 3 | Gross ratio | 0.79 | 0.55 |
| 4 | Variable cost ratio | 0.51 | 0.36 |
| 5 | Fixed cost ratio | 0.28 | 0.2 |
| 6 | Payback period (yrs) | 3.22 | 1.34 |
| **Economic efficiency measures Per production day** | | | |
| 1 | Production per day (tonne) | 18 | 45 |
| 2 | Revenue (Rs.) | Rs. 12,600.00 | Rs. 31,500.00 |
| 3 | Variable cost (Rs.) | Rs. 6,460.13 | Rs. 11,461.68 |
| 4 | Income over variable cost (Rs.) | Rs. 6,139.87 | Rs. 20,038.32 |
| 5 | Net profit (Rs.) | Rs. 2,609.82 | Rs. 13,885.11 |

**Fig. 1:** Proportion of constituent components of capital cost for ice plant of less than 50 tonne capacity

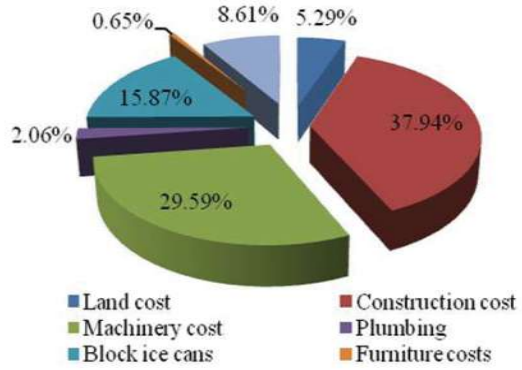



**Fig. 2:** Proportion of constituent components of variable cost for ice plant of less than 50 tonne capacity

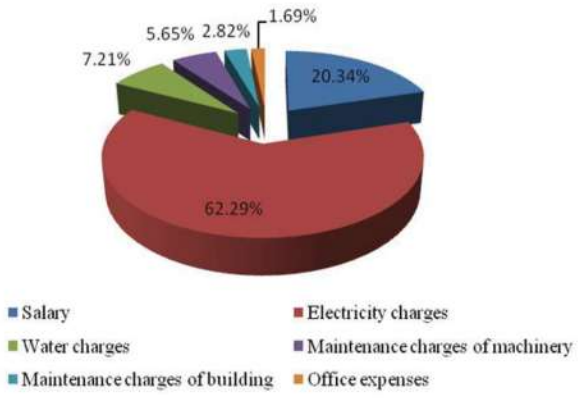



**Fig. 3:** Proportion of constituent components of capital cost for ice plant of more than 50 tonne capacity

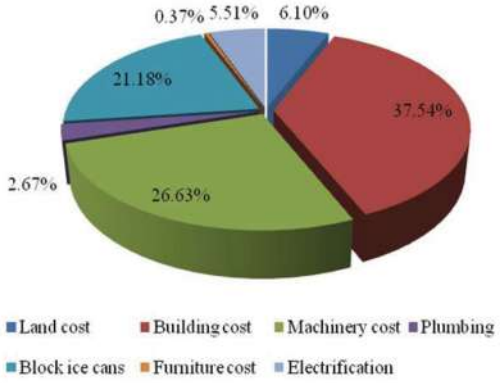
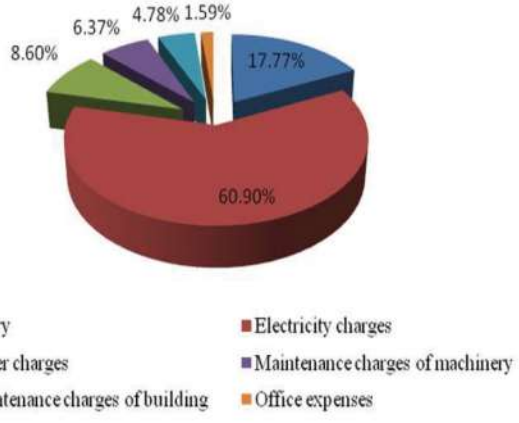



**Fig. 4:** Proportion of constituent components of variable cost for ice plant of more than 50 tonne capacity



than 50 tonne capacity ice plant, these were 0.55, 0.36 and 0.20 respectively. The value of gross ratio of both ice plants are below one, which signifies that the ice plants of both categories are profitable in the first year itself, as 79 and 55 per cent amount from the revenue is spent towards the total cost for less than and more than 50 tonne ice production capacity ice plants respectively. The revenue spent towards the variable cost for less than and more than 50 tonne capacity ice plant was 51 and 36 per cent respectively and only 28 and 20 per cent of revenue was spent towards fixed cost for the same capacity ice plant respectively. The pay back period was 3.22 and 1.34 years for less than and more than 50 tonne capacity ice plant respectively, which indicated that more than 50 tonne capacity ice plant required one and half year to recover the initial investment whereas, less than 50 tonne capacity ice plant required more than three and half years. The per day net profit recorded for less than and more than 50 tonne capacity ice plants were Rs. 2,609.82/- and Rs. 13,885.11/- respectively. There is no report available to compare the result of the present study therefore; the result of the present study cannot be compared with others.

## Conclusion

It could be concluded from the present study that instead of low production capacity ice plants, owner should have to prefer to establish ice plant with more production capacity for good economic feasibility. In the present study, it exposed that the less than 50 tonne capacity ice plant earned lesser net profit as compare to more than 50 tonne capacity ice plant. It is clear from all the economic indicators that more than 50 tonne capacity ice plants were more profitable as compared to less than 50 tonne capacity ice plants.

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**Standard journal article**

[1] Flink H, Tegelberg Å, Thörn M, Lagerlöf F. Effect of oral iron supplementation on unstimulated salivary flow rate: A randomized, double-blind, placebo-controlled trial. J Oral Pathol Med 2006; 35: 540-7.

[2] Twetman S, Axelsson S, Dahlgren H, Holm AK, Källestål C, Lagerlöf F, et al. Caries-preventive effect of fluoride toothpaste: A systematic review. Acta Odontol Scand 2003; 61: 347-55.

**Article in supplement or special issue**

[3] Fleischer W, Reimer K. Povidone iodine antisepsis. State of the art. Dermatology 1997; 195 Suppl 2: 3-9.

**Corporate (collective) author**

[4] American Academy of Periodontology. Sonic and ultrasonic scalers in periodontics. J Periodontol 2000; 71: 1792-801.

**Unpublished article**

[5] Garoushi S, Lassila LV, Tezvergil A, Vallittu PK. Static and fatigue compression test for particulate filler composite resin with fiber-reinforced composite substructure. Dent Mater 2006.

**Personal author(s)**

[6] Hosmer D, Lemeshow S. Applied logistic regression, 2nd edn. New York: Wiley-Interscience; 2000.

**Chapter in book**

[7] Nauntofte B, Tenovuo J, Lagerlöf F. Secretion and composition of saliva. In: Fejerskov O, Kidd EAM,

editors. Dental caries: The disease and its clinical management. Oxford: Blackwell Munksgaard; 2003. p. 7-27.

**No author given**

[8] World Health Organization. Oral health surveys - basic methods, 4th edn. Geneva: World Health Organization; 1997.

**Reference from electronic media**

[9] National Statistics Online—Trends in suicide by method in England and Wales, 1979-2001. www.statistics.gov.uk/downloads/theme_health/HSQ 20.pdf (accessed Jan 24, 2005): 7-18. Only verified references against the original documents should be cited. Authors are responsible for the accuracy and completeness of their references and for correct text citation. The number of reference should be kept limited to 20 in case of major communications and 10 for short communications.

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