ICAR-CIBA Technology Series: No. 20

CIBA-Plankton^{Plus} an effective plankton booster for aquaculture developed from fish-waste

ICAR-CENTRAL INSTITUTE OF BRACKISHWATER AQUACULTURE
भा.कृ.अनु.प.–केन्द्रीय खारा जलजीव पालन अनुसंधान संस्थान

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Published by

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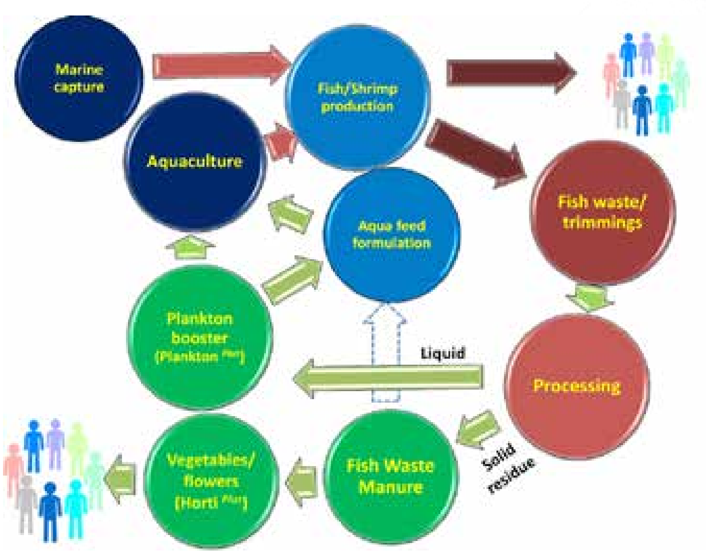
Introduction

Fish waste represents 25-50% the total production that is estimated to be approximately 21.72 million tons (FAO, 2014) and is a source of low-cost nutrients (Oetterer, 2002). Due to lack of adequate disposition for the waste from the fish industry it creates sanitary and environmental problems (Ferraz de Arruda et al., 2007). A way of minimizing the environmental problems generated by the high amount of fish waste is its transformation in a product which can be used as ingredient in animal/fish/shrimp rations (Ristic et al., 2002 De et al.,2017), or can be used for boosting plankton in aquaculture pond or as nutrient rich manure for agriculture/horticulture. Research work has been done on preparation of fish silage from fish waste using organic acids and use of the fish silage as protein rich ingredient to formulate animal/aqua feed (Ferraz de Arruda et al., 2007). Ample studies were also done for converting fish waste into fish protein hydrolysate (FPH) by utilizing proteolytic enzymes to hydrolyze the fish protein (Kristinsson and Rasco, 2000). Generally, production of fish protein hydrolysate involves use of temperature and centrifugation. But, here the fish waste has been converted to a product named Plankton booster by a unique method. The product has been tested for its efficiency in boosting plankton and growth performance in fish and shellfish in outdoor tank and pond system and showed promising performance.



Advantage of Plankton^{Plus} Technology

- In fish silage and fish protein hydrolysate, degree of hydrolysis of protein is restricted to less than 15 %. In the present technology degree of protein hydrolysis is more than 20%
- Production of fish protein hydrolysate involves high temperature for incubation and drying of the product which needs more expenditure. The present technology does not need any equipment for providing external temperature as incubation is done at ambient temperature and physical state of the product is liquid for easy use for plankton boosting in aquaculture ponds.
- In fish silage and Fish Protein Hydrolysate, liquefaction is limited which restricts the product for its quick dispersal and easy availability of nutrients in water. As present product is in complete liquid phase, its better dispersal in water and easy availability of nutrients to plankton and shrimp/fish are good for enhancing the aquaculture production.

Preparation method

Fish trimmings/waste was collected and minced through wet grinder and was subjected to processing through novel technology. Minced fish trimmings was converted to a liquid containing free amino acids, peptides, fish oils rich in polyunsaturated fatty acids and minerals. The liquid is branded as Plankton*Plus* and ready to use as plankton booster in aquaculture systems. As a byproduct, dry powder was also produced, which is branded as Horti*Plus*, used as manure/ fertilizer in horticulture.

Nutrient profile of Plankton*Plus*

The liquid product is having protein content of 45-55% and lipid of 15-22% (Fig 1). It is rich in histidine (4.92±0.04 %), Glutamic acid (4.58±0.10 %), Cysteine (4.40±0.08 %) and Lysine (4.12±0.07 %) (Fig 2). It contained 9.13 % Docosa Hexaenoic Acid (DHA), 3.65 % Eicosa Pentaenoic Acid (EPA) and 3.58 % Arachidonic Acid. It is also a high source of calcium (Ca-4.03%), phosphorous (P-1.43%) and iron (Fe-3.54 %).

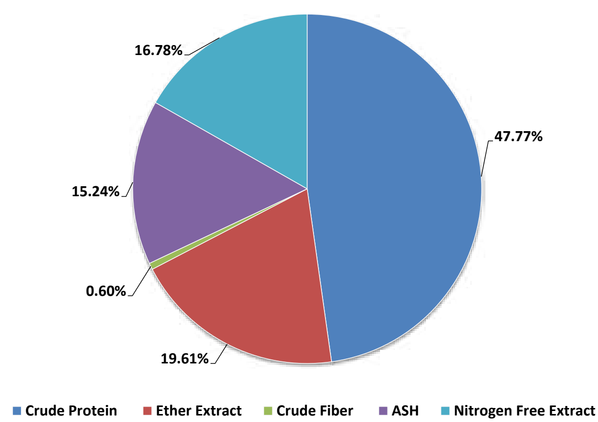


Fig 1. Proximate composition of Plankton *Plus*

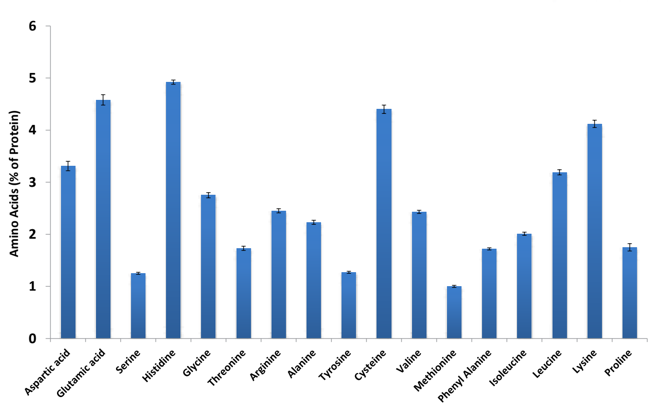
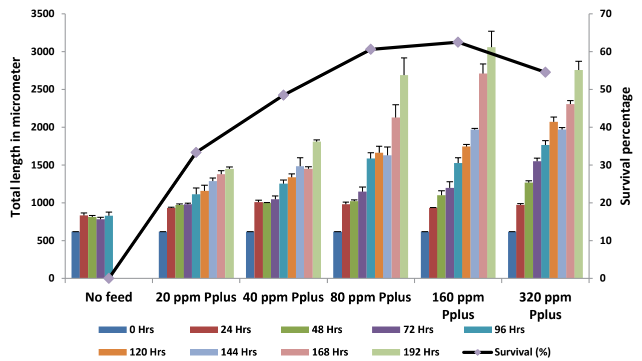


Fig 2. Amino acid profile of Plankton *Plus*

Plankton^{Plus} as zooplankton growth promoter

Artemia

Potential of Plankton ^{Plus} (PPlus) for culturing the zooplankton (Artemia, copepod and cladocerons) was evaluated by feeding them with different concentrations of PPlus. Study was conducted to reduce use of microalgae by supplementing PPlus for Artemia culture. The dose of Pplus is optimized and 80 ppm was giving better growth compared to others (Fig 3). In the next experiment PPlus (80 ppm) was added along with microalgae (*Nannochloropsis occulata*) at different frequency of application, i.e., one time (first day) application, alternate day application and daily application. The growth and survival of Artemia were significantly ($p<0.05$) higher when PPlus was used with daily application of microalgae (Fig 4). Interestingly, the growth and survival of Artemia were also higher when PPlus was supplemented with alternate day algae application compared to daily algae feeding without PPlus. The experiments clearly revealed that Plankton ^{Plus} supplementation @ 80 ppm with microalgae improved Artemia biomass production and can reduce the usage of algae by 50 % for its culture.



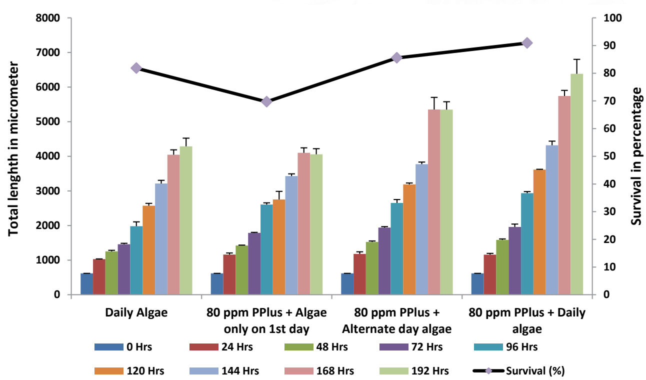
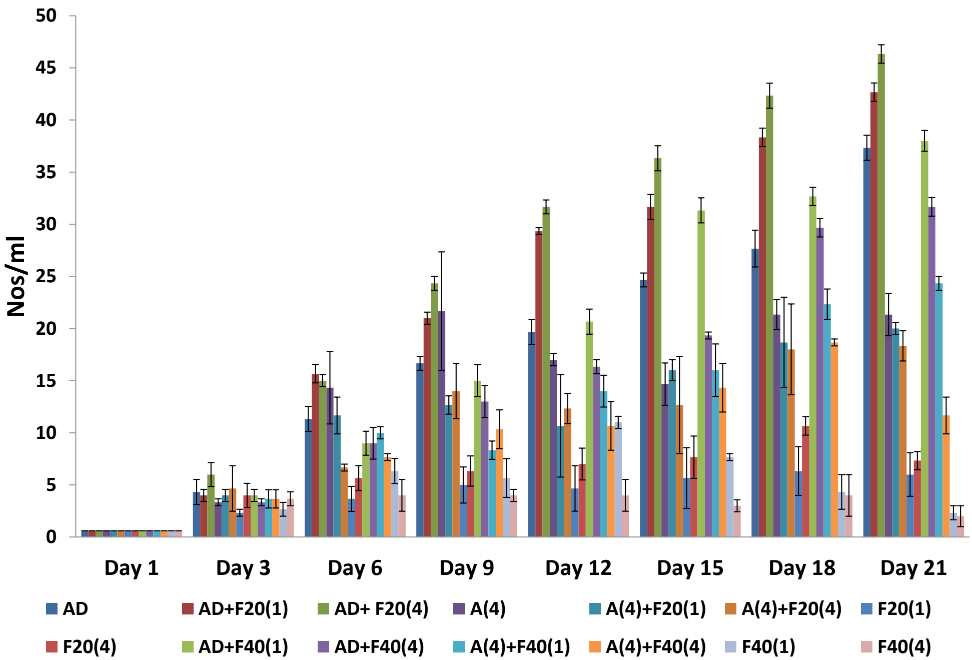


Fig 3 & 4.Growth and survival of artemia fed with Plankton ^{Plus} and algae

Copepod

An experiment was conducted using Cyclopoid copepod added with plankton plus at concentration of 20 and 40 ppm on daily basis and 4 days interval in combination with *Thalassiosira* sp and *Nannochloropsis* sp for a period of 20 days in 20L Plastic tubs. Copepod was added at the rate of 600 nos/l and counting was done in 3 days interval. Maximum density was observed in the treatment added with 20 ppm Pplus on 4 days interval (46±0.9 nos/ml) followed by treatment with single dose (20 ppm) i.e. 43±0.9nos/ml followed by treatment with single dosage of 40ppm i.e.38±1nos/ml with daily inoculation of algae and control treatment (37+1.2nos/ml). There is a significant difference was observed in the treatment with control and treatment added with 20 ppm Pplus on 4 days interval dosage ($p<0.05$). Minimum density was observed in the treatment with Pplus alone (Fig 5). Over all result indicates that Pplus can be used as plankton promoter in combination with microalgae at a concentration of 20 ppm.

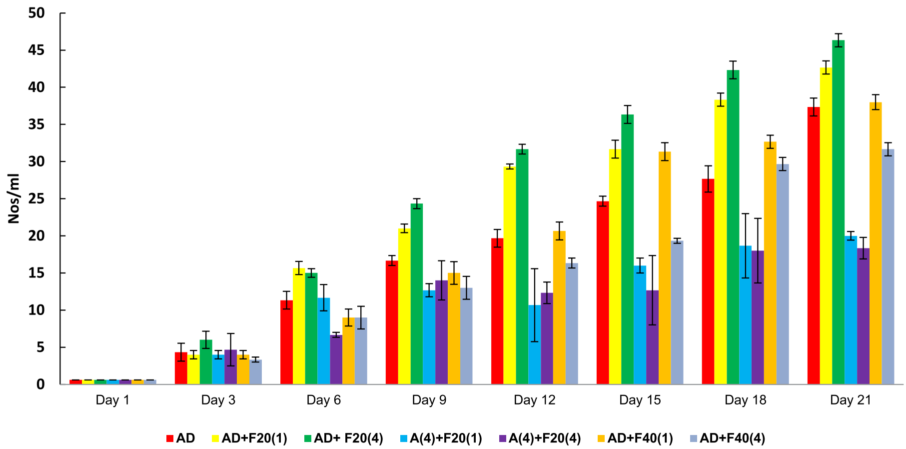


AD- Daily algae; FX(1)- Pplus @ X ppm at single dose; FX(4)- Pplus @ X ppm at 4 days interval; A(4)- Algae added 4 days interval

Fig 5. Density of copepod/ml in different experimental treatments

Cladoceran *sp*

An experiment was conducted in Cladoceran sp (*Eurycercus berinji*) using Plankton ^{Plus} at a concentration of 20, 40, 80, 160 and 320ppm on daily basis and in 4 days interval in combination with *Thalassiosira* sp and *Nannochloropsis* sp for a period of 20 days. Cladoceran was inoculated at a rate of 600 Nos/L and counting was done on 2 days interval. Maximum density was observed in the treatment added with 40ppm Plankton ^{Plus} (23±0.9nos/ml) on 4days interval dosage compared to control (17±1.2nos/ml) with daily dosage of algae on 11th day (Fig 6). Overall result indicated that Plankton ^{Plus} @ 40 ppm on 4 days interval can be used in combination with microalgae for production of Cladoceran species.



AD- Daily algae (*Nannochloropsis* +*Thalassiosira*); FX(1)- Pplus @ X ppm at single dose; FX(4)- Pplus @ X ppm at 4 days interval; FX- Pplus @ X ppm

Fig 6: Abundance of Cladoceran sp (Nos) fed with Plankton *Plus*

Effect of Plankton*Plus* on growth performance in *Penaeus indicus* and plankton dynamics

Outdoor experiment was conducted to study its effect on growth performance of *Penaeus indicus*. There was four treatments in the experiment; Control (without any juice), T1 (water soaked Ground nut cake at 2 g/100L water), T2 (Plankton *Plus* @1 ml/100L water) and T3 (Plankton *Plus* @ 10 ml/100L water). Shrimps of all the treatments were offered feed (CP-39.57%) at 10 % of biomass twice (10 am & 4 pm) daily. After 30 days of experiment it was found that average body weight and average daily gain and survival% were significantly (P<0.05) higher in Plankton *Plus* supplemented groups.

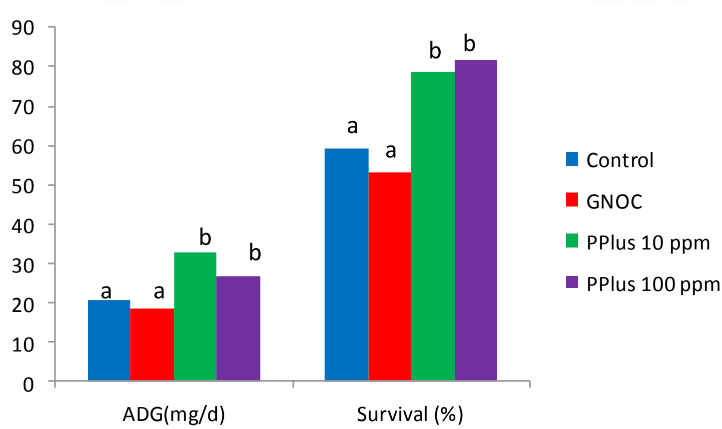


Fig 7: Growth and survival of *Penaeus indicus* supplemented with Pplus

Optimization of dose of Plankton^{Plus} in tank rearing system

Effect of Plankton^{Plus} (PPlus) supplementation at different dose (5, 10, 20, 40, & 80 ppm) on plankton status and growth performance of *Penaeus indicus* (ABW 0.32±0.01g) was studied and compared with other conventionally used materials (Cattle manure 20 g/100 L, Mustard cake 2 g/100 L, Juice prepared with fermentation of yeast 0.04 g+ molasses 0.3 g+ paddy dust 0.6 g / 100 L) for boosting plankton in tank rearing system. Weight gain and survival of shrimp was found to be higher in Plankton^{Plus} supplemented (10, 20 & 40 ppm) groups. Prasinophyceae group of phytoplankton (*Tetraselmis* sp and *Halosphaera* sp) dominated in Plankton^{Plus} supplemented groups and abundance was increasing with increased concentrations of Plankton^{Plus}. Cyanobacterial percentage was invariably less in all Plankton^{Plus} supplemented tanks.

Influence of Plankton^{Plus} on diversity and dynamics of microalgae were studied. Plankton ^{Plus} supplementation increased plankton density and improved plankton diversity. Prasinophyceae (*Tetraselmis* and *Halosphaera)* population increased due to Plankton ^{Plus} supplementation, while it decreased in control and traditional fertilizer supplemented groups. Clear dominance of microalgae like *Tetraselmis* sp, *Halosphaera sp., Isochrysis* sp, *Chlorella* sp, *Nitzschia* sp etc was found in Plankton^{Plus} supplemented tanks. Since these microalgae are nutritionally rich and digestible to shrimp, they will contribute considerably for the growth of shrimp. Moreover, unwanted cyanobacterial (BGA, Chain BGA) percentage was invariably less in all Plankton ^{Plus} supplemented tanks compared to other treatment and control.

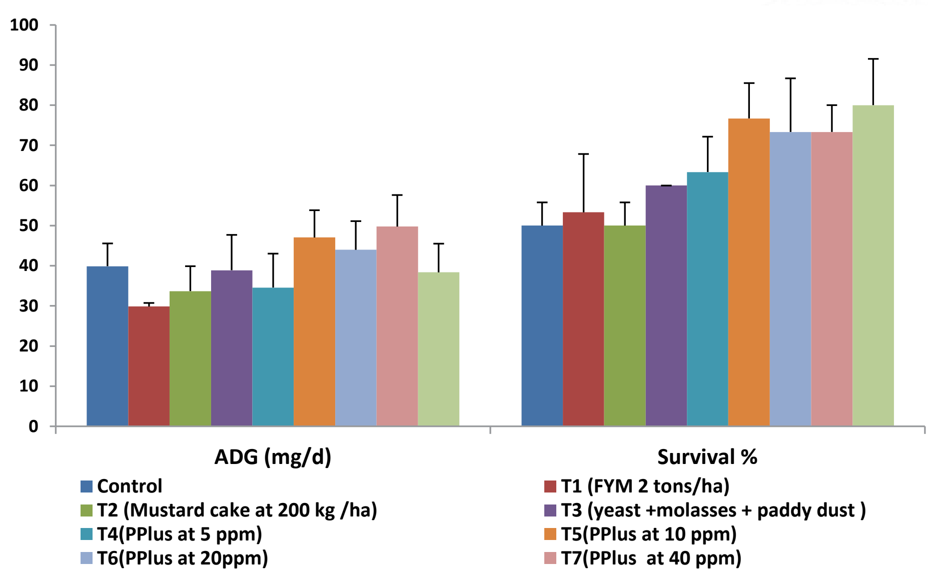


Fig 8: Growth and survival of *Penaeus indicus* supplemented with Pplus

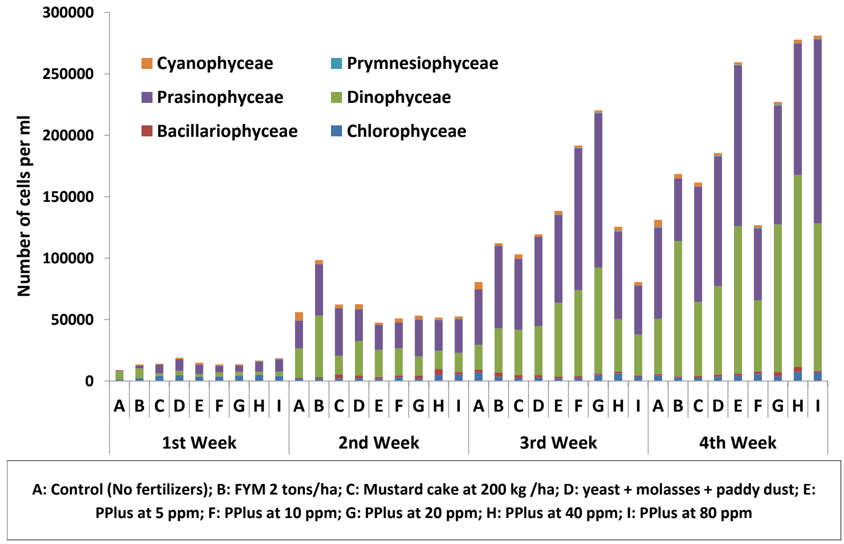
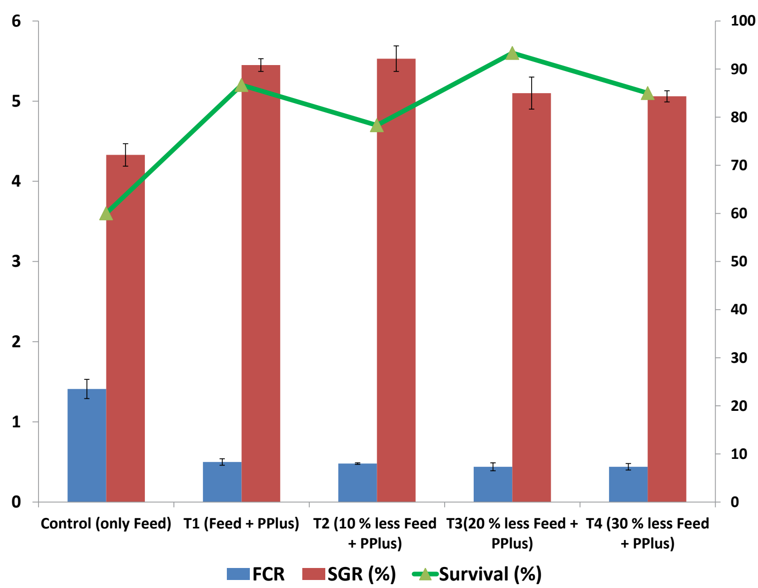


Fig 9: Number of cells of microalgae groups in **PPlus** tanks

Study on the potential of Plankton*Plus* on reduction of feed requirement in *P. vannamei*

A 45 days outdoor experiment was conducted to study the potential of Plankton*Plus* on reduction of feed requirement in *Penaeus vannamei*. There was five treatments in the experiment; Control (only feed), T1 (Feed + PPlus), T2 (10 % less Feed + PPlus), T3 (20 % less Feed + PPlus), T4 (30 % less Feed + PPlus).The experiment revealed that Plankton*Plus* supplementation @20 ppm significantly (P<0.01) increased average daily gain (ADG), specific growth rate (SGR), total biomass gain and survival of *P. vannamei* even when 30 % less feed was



Fi 10: Growth and survival of *P vannamei* supplemented with PPlus

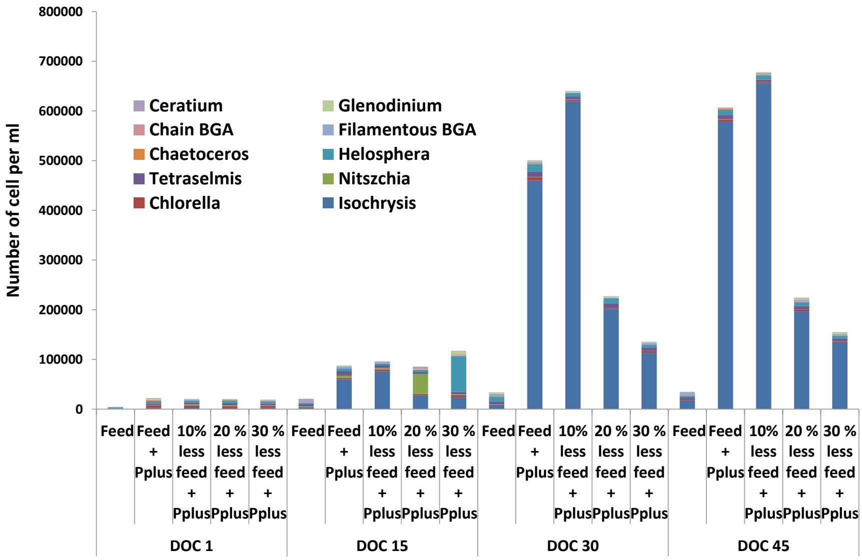


Fig 11: Density of microalgae in PPlus supplemented tanks

offered. FCR was within range of 0.44 to 0.50 in Plankton*Plus* supplemented groups whereas it was 1.41 in control group. Irrespective of dosages, a clear dominance of beneficial microalgae, *Isochrysis galbana* was noticed in all Plankton*Plus* supplemented tanks. Weight gain was highest when Plankton*Plus* @20 ppm was supplemented without any reduction of feed quantity.

Frequency of application of Plankton*Plus*

Plankton*Plus* @ 20 ppm applied at two split doses revealed better growth performances of *Penaeus vannamei* in outdoor tank experiment compared to single application. Plankton abundance was significantly higher with clear dominance of a beneficial microalgae, *Isochrysis galbana* in tanks. Increasing the dose of Plankton*Plus* has no significant (P>0.05) effect on growth, feed intake, feed conversion ratio and survival.

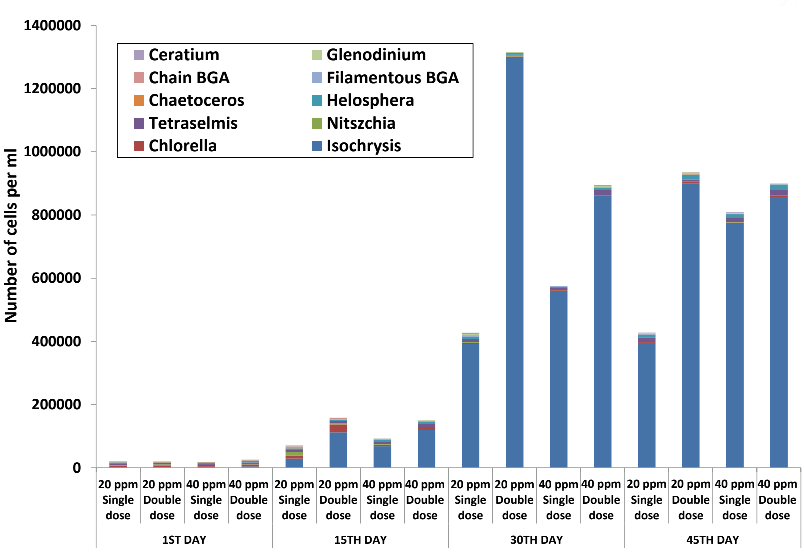


Fig 12: Abundance of microalgae in different frequencies of application of PPlus

Potential of Plankton*Plus* on reduction of feed requirement in fish

A 45 days outdoor experiment was conducted to study the potential of Plankton*Plus* on reduction of feed requirement in milk fish (*Chanos chanos*) culture without any water exchange. The experiment was conducted in 3^2 factorial design where, factor 1 was Plankton*Plus* supplementation (at three levels 0, 20 and 40 ppm) and factor 2 was feed supplementation (at three levels 0, 50 and 100 %). Study revealed that at both 20 and 40 ppm PlanktonPlus supplementation with 50 % feed expressed similar average daily gain (ADG), specific growth rate (SGR), and biomass gain in Chanos chanos when compared with group received 100% feed but without Plankton*Plus* supplementation. Therefore, it can be concluded that Plankton*Plus* supplementation either @ 20 and 40 ppm can save 50 % of feed without affecting growth performance and survival of *Chanos chanos.*

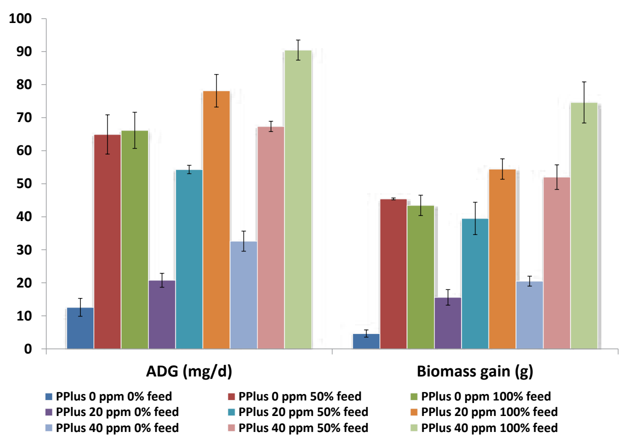


Fig 13: Growth performance of *Chanos chanos* at different level of PPlus and feed supplementation

Influence on water quality parameters

There was no significant difference in water quality parameters like total ammonia nitrogen, nitrite and nitrate due to supplementation of Plankton *Plus* as compared to control at different days of the experiment. The pH was in the range of 8.21 to 8.54 and water temperature was in the range of 27 ^{0}C to 32 ^{0}C during the study. The parameters were in the safe range for aquaculture

Influence on gut and water microbial count

There was no significant difference in total microbial count and vibrio count when Plankton*Plus* (up to 40 ppm) was supplemented with feed when compared with treatment receiving only feed in shrimp (*Penaeus vannamei*) culture system. Total gut microbial load and vibrio population was also not affected when Plankton^{Plus} was supplemented with feed. In milk fish, results indicated that PPlus significantly influenced the gut microbial population, specifically, *Vibrio* ($p<0.05$) and amylolytic bacterial counts ($p<0.01$). The cellulolytic bacterial population was rather influenced by feed level ($p<0.01$). The lowest level of bacterial, Vibrio, amylolytic and cellulolytic bacterial counts were observed in the treatment group receiving no feed or PPlus. Overall, total microbial population, amylolytic and cellulolytic bacterial population showed rising trends with increase in feed levels.

Treatments	**Total bacterial count/gram gut × 10⁵ CFU**	**Total *Vibrio* count/ gram gut × 10⁴ CFU**	**Total amylolytic bacterial count/gram gut × 10⁴ CFU**	**Total cellulolytic bacterial count/gram gut × 10⁴ CFU**
A (Feed 0%+ Pplus 0ppm)	$0.17±0.06^{b}$	$0.23±0.23^{c}$	$2.5±2.5^{d}$	$0.40±0.21^{a}$
B (Feed 50%+ Pplus 0ppm)	$72.23±66.14^{ab}$	$90.29±85.81^{abc}$	$58.84±38.65^{bcd}$	$18.46±11.73^{a}$
C (Feed 100%+ Pplus 0ppm)	$56.59±32.3^{ab}$	$262.54±80.32^{ab}$	$128.89±4.44^{abc}$	$24.57±0.76^{a}$
D (Feed 0%+ Pplus 20 ppm)	$125.63±81.92^{ab}$	$285.65±28.82^{a}$	$96.22±16.99^{abcd}$	$8.62±2.07^{a}$
E (Feed 50%+ Pplus 20 ppm)	$128.29±61.63^{ab}$	$170±3.33^{abc}$	$37.48±25.85^{bcd}$	$5.77±0.43^{a}$
F (Feed 100%+ Pplus 20 ppm)	$115.69±17.65^{ab}$	$105.77±57.63^{abc}$	$32.96±22.59^{bcd}$	$9.40±3.19^{a}$
G (Feed 0%+ Pplus 40 ppm)	$0.86±0.22^{b}$	$1.98±0.96^{c}$	$5.38±4.89^{d}$	$0.73±0.45^{a}$
H (Feed 50%+ Pplus 40 ppm)	$71.52±8.03^{ab}$	$4.75±0.94^{bc}$	$166.18±4.27^{ab}$	$7.23±3.64^{a}$
I (Feed 100%+ Pplus 40 ppm)	$309.38±89.93^{a}$	$145.49±37.85^{abc}$	$201.74±23.96^{a}$	$19.38±2.15^{a}$

Table 1: Total bacterial and vibrio counts on PPlus supplemented milkfish

Influence on immunological parameter

Population of total haemocyte count (THC) significantly (P<0.05) increased in Plankton Plus treated groups. The population of small nongranular haemocyte (SNGH) and large nongranular haemocyte (LNGH) significantly (P<0.01) decreased, but small granular haemocyte (SGH) and large granular haemocyte (LGH) population significantly (P<0.01) increased in Plankton Plus treated groups compared to control. Among the different treatment groups, application of Plankton Plus @ 20 ppm was beneficial in terms of enhanced innate immunity by increase in THC, SGH and LGH counts.

Demonstration of Plankton^Plus as cost effective plankton booster in farmer's pond

A 77 days trial was conducted in shrimp (*Penaeus vannamei*) ponds at Bapatla, Andhra Pradesh, India to study the effect of Plankton Plus on augmenting plankton in shrimp culture pond. PPlus was supplemented @ 10 ppm in split doses. Control ponds were supplemented with rice bran-yeast-mollases juice and dolomite as and when required to maintain the bloom and water quality. At the end of the culture period, average weight of shrimp was higher (11.82 g) in pond supplemented with PPlus compared to control (9. 93 g). There was a significant increase (12.31%) in survival, and enhancement of yield to the tune of 1.71 t/ha in pond supplemented with Plankton Plus compared to control. Plankton Plus supplemented ponds were dominated by beneficial microalgae (Bacillariophyceae, Prasinophyceae and Dinophyceae etc.) and zooplankton. Enhanced growth performance of *Penaeus vannamei* might be due to the augmented density and diversity of phytoplankton and zooplankton in the Plankton Plus supplemented ponds.

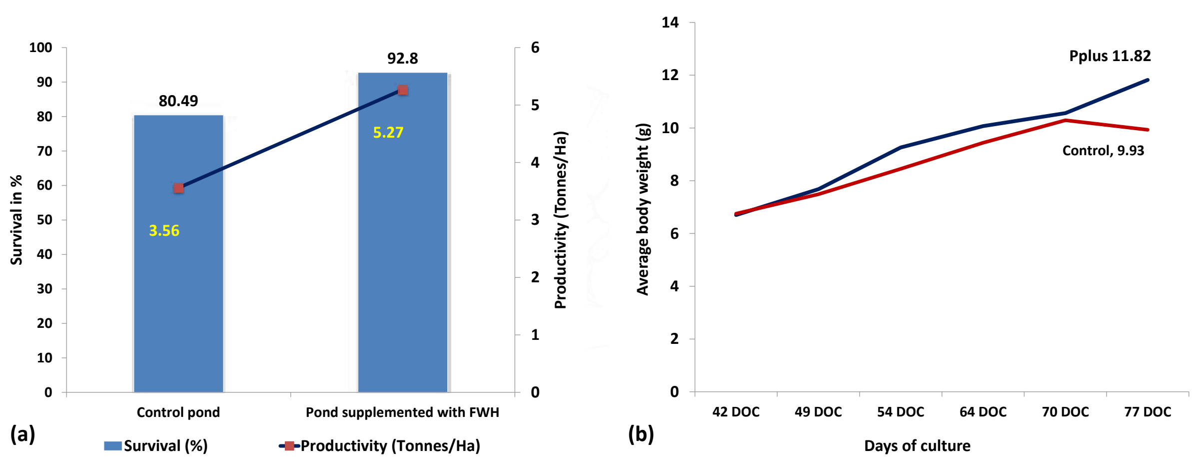


Fig 14: Productivity and survival of *P vannamei* supplemented with PPlus

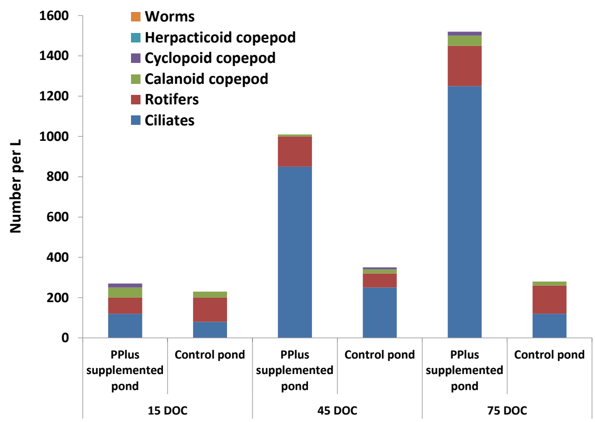
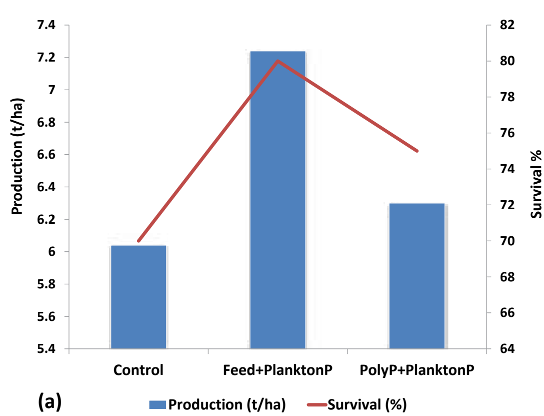


Fig 15: Abundance of zooplankton in shrimp ponds supplemented with PPlus

Pond trial in West Bengal

Plankton *plus* demonstration was conducted in farmer's ponds at Lalpool, Namkhana, West Bengal. Three ponds were used for demonstration. One pond used as control (Control) without PPlus application (Feed used: Commercial shrimp feed). Another pond (T1) was supplemented with PPlus (Feed used: Commercial shrimp feed). The third pond (T2) was supplemted with PPlus and the feed used was a low cost feed, Poly *plus* developed by ICAR-CIBA. Plankton plus was used at 30 ppm. A significant increase of phytoplankton count was noticed in T1 and T2. Highest phytoplankton cell count (2.1 X 10^6 cells/ml) and zooplankton cell count (12991 no/ml) was achieved in treatment with Poly *plus* + Plankton *plus*. Highest production of 7.24 t/ha was achieved when Plankton plus supplemented with commercial feed followed by Poly *plus* feed with plankton plus 6.30 t/ha and control 6.04 t/ha.



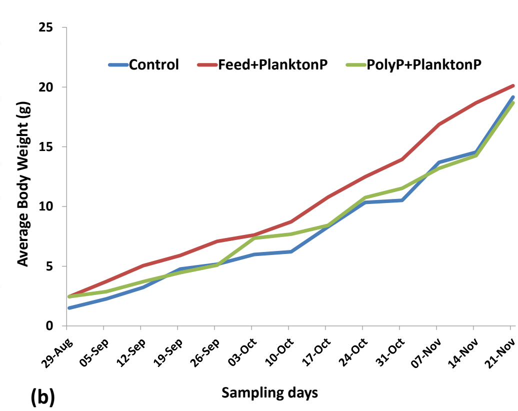


Fig 16: Production and ABW of P vannamei supplemented with Plankton *plus*

Training of farmers on Plankton^{Plus} production

Several training cum demonstration programmes on Plankton ^{Plus} production were conducted to train Self Help Groups, coastal and tribal communities of four villages viz., Srinivasapuram, Nambikkai Nagar, Mullikuppam and Mullimanagar located near CIBA headquarters in Chennai on the theme of 'Waste to Wealth: Recycling of fish waste" under Swachh Bharat Mission since 2017.

Establishment of Plankton^{Plus} Production unit

ICAR-CIBA has established a "Fish Waste Processing Unit" on 18th February, 2019 at Nambikkai Nagar, Pattinapakkam, Chennai, Tamil Nadu, which is being operated by Nambikkai Fish Farmers group, a Self Help Group (SHG) of Nambikkai Nagar for recycling of fish waste to value added products, Plankton ^{Plus} and Horti ^{Plus}. After the agreement, till date, the group has produced 6600 Kg of Plankton ^{Plus} and 2500 Horti ^{Plus} and received Rs.4,53,942/-. The group is processing the fish waste persistently and producing Planton ^{plus} and Horti ^{plus} as an alternative livelihoods activity. This initiative is not only cleaning and hygienic disposal of fish market waste which is abundantly available in the village cluster but also will help to produce wealth from waste as a concept of circular economy. This user-friendly technology will help in improving the environment in addition to enhancing their livelihood.

Start-up 'Fish Waste Processing' unit at ICAR-CIBA, Chennai

ICAR-CIBA has developed two quality cost effective indigenous eco-friendly products, branded as CIBA-Plankton^{Plus} and Horti^{Plus}, from fish-waste of fish markets and Fish trimming from fish processing units, to boost and maintain the healthy plankton bloom in aquaculture systems and as an organic manure for agriculture/horticulture. Plankton^{Plus} has proven its efficiency in various aquaculture systems for different species. This technology has been transferred to 'Nambikkai

Fish Farmers Group', Nambikkai Nagar, Chennai, through start-up India programme, Agri-business incubator. CIBA provides the necessary hand-holding and training to the Nambikkai Fish Farmers Group on production and marketing of Plankton^{Plus} and Horti^{Plus}.

The institute has 'Fish Waste Processing' unit at Chennai for the production of Plankton^{Plus} and Horti^{Plus} from fish waste sourced from fish markets. Production capacity of the small scale Fish-Waste to Wealth unit is 2000 L/month. Annual turnover of one unit is Rs.16.80 lakhs with net profit of Rs.4.56 lakhs. Nambikkai Fish Farmers Group, Nambikkai Nagar, Chennai is producing Plankton^{Plus} and Horti^{Plus} using Fish-waste sourced from locals, and Processed at the Start-up Unit established by CIBA.

Dr. Trilochan Mohapatra, Secretary, DARE and Director General, ICAR along with Director and other dignitaries from the Institute visited the 'Fish-Waste to Wealth' start-up unit on 7th August, 2019. Shri.T. Kennit Raj, representative of Nambikkai Fish Farmers Group has expressed their

happiness about the upliftment of their socio-economic status through this technology. They appreciated the genuine efforts for promoting "waste to wealth" concept as an alternative livelihood activity for the fishers in their village. This technology has the potential in cleaning the fish markets across the country and also in providing alternative livelihood to produce wealth from waste as a concept of the circular economy.

Commercialization of Plankton*Plus* Technology

- The technology for production of Plankton^{Plus} from fish waste has been transferred to aqua-entrepreneurs, Sri.Nagakishore Mudedla and Sri Syamala Rao Maradani, from Gudivada, Krishna district, Andhra Pradesh, through an MoU on 16th May 2019, at CIBA Headquarters, Chennai.

- Memorandum of Agreement for partnership has been signed on 5th April, 2019 in establishing and operating Fish-Waste Processing Unit to recycle fish waste with Nambikkai Fish Farmers Group, Nambikkai Nagar, Patinapakkam, Chennai, 600 028 Tamil Nadu.

- Tripartite MoU has been signed with Coastra Biosolutions Pvt Ltd., 175, Ground Floor, Parvathipuram, Part-I, Vadaperumbakkam, Chennai-600 060 and Nambikkai Fish Farmers Group at Nambikkai Nagar, Pattinapakkam, Chennai-600 028, for production and marketing of Plankton booster, Plankton^{Plus} and manure for horticulture, Horti^{Plus} from fish waste on 5th April, 2019.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“Brachishwater aquaculture for food, employment and prosperity”

Headquarters

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