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Microbes as an Alternative Aquaculture Feed

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Abstract: *Demand for basic human food and as well as for animal feed proteins from nonconventional sources has increased subsequentially, particularly in fat-developing countries. Microbial protein is one of the such source. It is desirable because it is amenable and can be controlled for intensive cultivation and is also less dependent on variations in climate, weather, and soil. This research paper deals with the potential of microorganism specially bacteria to provide with an enhanced aquaculture feed. 3 betta fish were taken into consideration and were placed in 3 different bowls. One was fed with Vibact capsule which contained Lactic acid bacillus while on the other hand Darolac contained Lactobacillus Lp299v was fed to second fish and market fed was fed to third fish. They were fed twice in a day onetime in daytime and one small sphere in evening and all fish had an initial weight of 2 grams, and their weight was measured after every 5 days for upto1 month, gradual gain in weight was observed in every Siemese fish. Final weight of market fed fish was found to be 3 grams which is similar to Vibact fed fish, but the highest weight gain was found to be in Darolac fed fish of around 4 grams, it means that I has gained weight of over 2 grams from day 1. This Experiment shows that microorganism can too be a great and major source of nutrient for fish and be a potential aquaculture feed.*

Keywords: Nonconvential, subsequent,Darolac, Vibact and Nutrient

I. INTRODUCTION

With the subsequent increasing demand for seafood and the rapid development of culture technology, as well as of the aquaculture industry has been growing rapidly in recent few years. In fact, the growth of aquaculture sector is comparatively faster than any other animal food-producing sectors (1), and it can be quite well predicted that aquatic organisms will also represent the predominant sources of high-quality protein in the upcoming near future (2). In contrast, the fast-developing aquaculture industry has also been facing serious challenges and obstacles as well.Bacteria are usually very rich in proteins and easy to large-scale production as well, making them possible to be an excellent alternative source of protein for feeds. A large percentage of bacteria are beneficial or useful or can be termed probiotic that do not cause any serious harm to host health but show some major beneficial biological activities to facilitate and provide good host growth. Consequently, beneficial bacteria might be used as anti-pathogenic reagents for given a host species with specific disease or also can be used as anti-spoilage reagents during product storage.

Thus, it is safe to say that beneficial bacteria are a promising supplement with multiple or many functions for aquaculture. This will give detailed information about the applications of beneficial bacteria and discuss the possible mechanisms underlying the many benefits of using bacteria in aquaculture, thus lending support for further mechanistic investigations and as well as for novel applications.

So it is important to find or biotechnologically engineer microbes needed as aquaculture feed.

Bacteria-derived nutrients for aquaculture

With the rapid and increasing growth of aquaculture industry, the requirement of aquaculture feeds too hasalsoincreased. The availability of ingredients and resources for aquaculture feeds has toobecome limited in the aquaculture industry (3). Furthermore, the increase or rise in traditional fish meal production may increase economic and environmental concerns subsequently. Thus, alternative feed sources are needed, which has attracted great efforts in both research as well as applications; among this, microorganisms are greatly considered promising for aquaculture feed. In contrast, some beneficial bacteria can tooprovide micronutrients such as vitamins, fatty acids, and essential amino acids in addition to other macronutrients to support the healthy growth of aquatic animals fully. In addition, bacteria may also quite well regulate the host digestion process by producing different extracellular enzymes.

Bacteria as the alternative aquaculture feeds:

Aquatic animals are not only an ideal source of protein for humans but also due to their high protein content, but they also require abundant protein in their feed. Microorganisms, especially bacteria, are usually or commonly found to be rich in proteins which can make up to 65% of their total dry weight. In addition, bacteria contain more nucleotides compared to any other traditional fish meals. Different dietary supplementation with bacteria can too be beneficial for aquatic animals' growth, immunity and stress response, and can also improve diet palatability effectively (4).

Several studies have investigated and researched about the possibility of partially or even totally replacing fish meal by bacterial biomass and quit encouraging results were obtained. In a 10-week growth trial in juvenile Florida pompano (*Trachinotuscarolinus*), partially replacing or changing fish meal with up to 12.82% of dried fermented biomass on an isonitrogenous and isolipidic basis did not significantly or majorly change the final weight, survival, percent weight gain, food conversion ratio and thermal-unit growth coefficient (5). Similarly in an other experiment, partially replacing fish meal with bacterial single-cell protein did not influence the growth rates, feed consumption and absorption efficiency of rainbow trout (*Oncorhynchus mykiss*) (6).In addition to any other common bacteria that take complex organics as the carbon source, methanotroph bacteria that usually grow or found on natural gas have also been studied as a substitute for fish meal. In Atlantic salmon (*Salmo salar*) culture, experiments that have partially replace fish diet with methanotroph bacteria, especially *Methylococcuscapsulatus*, demonstrated or yielded result that bacterial protein meal could be an alternative protein source to fish meal. Intake of the bacteria protein meal does not largely change or affects the fish growth rate or induce health problems and it can relieve soybean meal-induced enteritis (7)(8). Furthermore, compared to another culture that is finfish culture, in shellfish culture, the application of bacterial biomass not just only supported animal growth as traditional feeds but it also improved culture efficiency in some aspects. In case of black tiger prawn culture, the application of bacterial biomass offset the growth losses usually result from the absence of fish meal or oil (9), and additional bacterial biomass in feeds further improved shrimp growth (10).

Moreover, the bacteria can further form major flocculated material which not only provides an additional food source but also improves the water quality and thus raises the breeding density (11). In the infamous Pacific white shrimp (*Litopenaeusvannamei*), dry bioflocculated material collected from aquaculture can also be used to partially replace fish meal, and replacing over 20% of fish meal with the biofloc meal may actually improve shrimp growth and ahow significant results(12). Similar studies, researches and applications performed in other aquatic animals, such as flatfish (*Paralichthysolivaceus*) and sea cucumber (*Apostichopus japonicus*), also showed improved and excellent growth performance (13)(14).

However, it should be also noted that some studies also found that the overuse or over feeding of bacterial biomass may be counterproductive, resulting in the low digestibility of nutrition and the reduction in growth, which could be due to the decrease in N absorption and the subsequent increase in urea excretion (15). Moreover, even though the reproductive performance of domesticated broodstock was not influenced at all, a comparative high percentage of bacterial biomass may highly reduce the egg hatching of black tiger prawn (16).

Micronutrients produced by beneficial bacteria

In addition to the basic and essential macronutrients that are usually provided by feeds, aquatic animals also need various other micronutrients such as vitamins, fatty acids and essential amino acids to support their growth and other normal physiological functions, which may be insufficient from some different feeds. A large number of bacteria also produce various kinds of micronutrients; the application of some of the strains of these bacteria has been widely reported in human beings(17) and also the utilization of these basic micronutrients to support the growth of aquatic animal has also been investigated vastly.

Vitamin B-12 is considered one of the important vitamins produced by various probiotic strains, which is a cofactor in DNA synthesis and takes part in both fatty acid and amino acid metabolism. A study or research on isolated intestinal bacteria from carp showed that a large percentage of the commensal bacteria can produce vitamin B-12 in a sufficient quantity(18). Furthermore, these bacteria can also provide the essential vitamin B-12 for supporting fish growth even in the absence of diet vitamin B-12 (19). Similarly, another research showed that dietary supplementation of vitamin B-12 was not at all necessary for the normal growth of channel catfish becauseintestinal bacteria synthesize approximately 1·4 ng of essential vitamin B-12 per gram of body weight per day, which can be absorbed and taken up directly from the digestive tract into the blood. Consequently, the dysbiosis induced by antibiotics could subsequently reduce the rate of intestinal synthesis and liver stores of vitamin B-12 (20). In addition, other kinds of major vitamins, such as other B-group vitamins and vitamin K, which are synthesized by human gut microbiome have also been reported (21); however, relevant studies in aquatic animals are still limited and further and thorough investigations are needed.

The plant protein source is considered as a viable and meaningful alternative to fish meal due to its sustainable availability and reasonable pricing too. However, because of the deficiencies in other major essential amino acids like tryptophan, lysine and sulphur-containing amino acids, the use of plant protein sources may result in the reduction of growth performance and efficiency in shrimp. To overcome the disadvantage, Jannathulla and colleagues at the institution treated plant protein sources with bacterial, fungal and yeast fermentation methods and got to know that the essential amino acid contents were greatly increased after the treatment. Moreover, the anti-nutrients elements such as trypsin inhibitor, phytic acid, saponin, tannin, guar gum and glucosinolatein plant protein sources were also reduced after the fermentation. The application of micro-organism in treating plant protein sources also paves way for a higher ratio of replacement or changing for fish meal in aquaculture feed formulations too(22).

Recent studies have also revealed that commensal and probiotic bacteria can too produce short-chain fatty acids (SCFAs) by fermentation of certain fibres in their intestine, which also participate in host energy metabolism and immunity (23). A feeding trial using dietary probiotic like *Clostridium butyricum* in the kuruma shrimp (*Marsupenaeus japonicas*) demonstrated that the administration of the strain subsequently increased the content of intestine short-chain fatty acids (SCFAs) including elements like propionic acid and butyric acid, which together with several other beneficial effects promoted the *M. japonicus* growth and elevated body crude protein content too(24). Also, in grass carp (*Ctenopharyngodonidellus*), the diet type can also modify the hindgut microbiota structure, which is tightly correlated with short-chain fatty acids (SCFAs) concentration (25).

Nitrogen as a Pollutant:

Nitrogen present in wastewater from aquaculture effluents is often considered a pollutant. In freshwater systems, nitrogen is sometimes also a limiting nutrient, so adding it stimulates unwanted plant and algal growth. A majority of the excess nitrogen in either tank or pond culture systems originates in form of ammonia which is excreted by fish respectively. The ammonia, as a waste product, is formed during the subsequent breakdown of proteins and excess amino acids which were not incorporated into tissue by the fish (27).

Probiotic Containing Capsule:

1) Vibact Capsule:

It is basically a dietary supplement which is formulated with probiotics to support immune and digestive health. Probiotics support healthy digestion, essential nutrient absorption, and immune function too. Probiotics may also help to prevent the growth of harmful bacteria present in the gut. It may also help to relieve occasional gas and bloating as well.

Key Ingredient: *Lactic acid bacillus*

2) Darolac Sachet

Darolac sachets are basically fortified with Probiotic composite containing of lactobacillus which is used to treat and also prevent diarrhoea and also the problems associated with it. It is generally used to restore the normal microbial flora of the intestine and also to relieve symptoms of digestive disorders.

Key Ingredients: *Lactobacillus Lp299v*

3) Fighter fish (Bettas):

Bettas generally or majorly originate in the shallow waters of Thailand (formerly called Siam, hence their name), Indonesia, Malaysia, Vietnam, and other different parts of China. These areas are basically home to rice paddies, ponds, slow-moving streams or water bodies, and swamps, all of which are home to bettas or the fighting fish. Today bettas have been introduced into many other locations too, giving rise to non-native populations in a number of different countries.

The brilliant coloration and long flowing beautiful fins of the male betta make it one of the most well known of aquarium fish which can be domesticated as well. Females are usually not as highly colored or vivid and have much shorter fins in comparison to males. In nature, this species is not usually that brightly colored.

However, captive breeding programs and domestication have resulted in a wide variety of colors, including white, yellow, orange, red, pink, blue, green, turquoise, brown, and black and different other shades too. A myriad of combinations can also be seen, from solid colors to those with different fin and body colors too, to different patterned colors. Fin types have also changed due to their selective breeding. Veil tails have also been joined by crown tails, deltas, fans, half moons, lyre, and split tails, to name a few and many more.

Bettas are considered one of the most recognized, most colorful, and often most controversial fish too in the freshwater aquarium hobby. To fully understand their needs it is very important to become familiar with their native habitat too, where they live normally in large rice paddies, shallow ponds, and even in very slow-moving streams or water bodies. Although many fish keepers are also aware that bettas come from shallow waters, the water temperature is often overlooked or ignored or taken not too much into consideration.

The home countries of the betta are basically tropical, which means that the water temperature is quite warm, often into the 80s respectively. Bettas basically thrive on heat and will become increasingly listless when the water temperature falls usually below 75 F. Water temperature is also perhaps the biggest argument against keeping a betta in a tiny bowl or small container, which cannot be heat controlled.

Even though bettas do well or survive in waters low in dissolved oxygen, that does not mean they require less oxygen than any other fish. Bettas also have a special respiratory organ that allows or through which they manage to breathe air directly from the surface. In fact, they inherently must do so to survive. In experiments where their labyrinth organ was removed, the fish suffered and died from suffocation even though the water was saturated with sufficient oxygen. For this particular reason, bettas must have access to the water surface to breathe or take in air directly from the atmosphere (30).

Diet and Feeding:

In nature, bettas subsist or survive almost exclusively on insects and insect's larvae. They are built or have an upturned mouth which is well suited to snatching any hapless insect that might fall into the water accidently. Internally their digestive system or body is geared for meat, having a much shorter and small alimentary track than those of vegetarian fish. For this very reason, live foods are an ideal diet for the betta, however, they will or have adapted to eating flake foods and frozen or freeze-dried foods.

Daphnia, plankton, tubifex, glass worms, and beef heart, are all excellent options that may be found frozen or even freeze-dried. If flake food is being fed to betta, it should be supplemented with frozen and freeze-dried foods, and if possible, best is the live foods (31).

II. MATERIAL AND METHODS

Choice of the Fish under Investigation with Proper Assigned Feed

The fish chosen was the Siamese fighting fish or the also known as the Betta fish. It would be kept under investigation with taking weight (in grams) after every 5 days for up to 1 month.

Total 3 Betta fish are taken into consideration and for research purpose withplaced in 3 different bowls, with first one fed with market available feed, second one with Vibact and third one with Darolac.

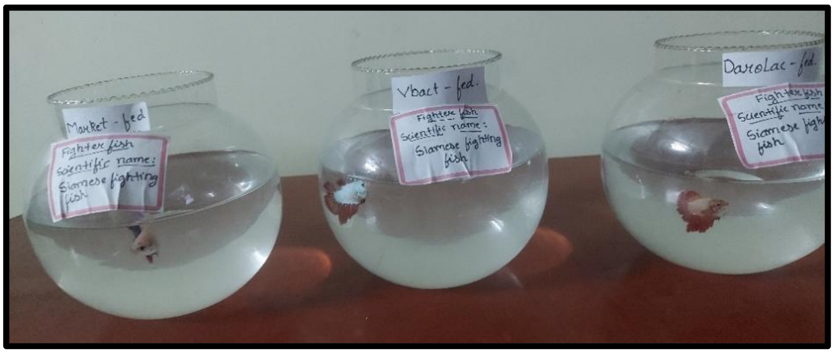

Figure: All 3 fishes in 3 different separate glass containers

Preparation of Vibact and Darolac fed: vibact capsule was made open with its powdery substance mixed in equal proportion with wheat and then small sphere were made and then directly fed to fishes similarly Darolac fed was also made, while the market fed was given directly to fishes.

Other factors affecting the growth of fish were too taken in to consideration like pH was 7, temperature was same as that of room temperature as betta fish normally resides at shallow water in water bodies, glass bowl was washed everyday and kept clean in order to provide clean environment.

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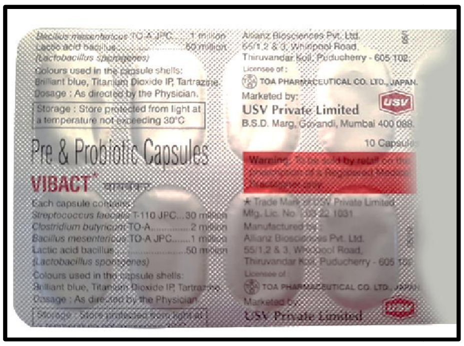

Figure: ViBact Capsules

Figure: Darolac Sachets

Figure: Market fed

Taking Weight Reading

Weight was taken after every 5 days over a span of 1 months, observation and respective readings are as follow:

While measuring and taking reading, weight of glass and water constituted for a totalof 250 grams, then fish was added.

Readings of Day 1:

Sr. no	**Aquaculture feed**	**Weight of glass container and water (in grams) (y)**	**Weight reading after addition of fish (in grams) (x)**	**Final weight of Fish (in grams) (x-y)**
1.	Market – fed	250	252	2
2.	ViBact	250	252	2
3.	Darolac	250	252	2

Initial Weight of all fishes was found to be 2 grams respectively

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Figure: Market Fed Fish

Figure: ViBact Fed Fish

Figure: Darolac Fed Fish

Readings of Day 5:

Sr. no	Aquaculture feed	Weight of glass container and water (in grams) (y)	Weight reading after addition of fish (x)	Final weight of Fish (x-y)
1.	Market – fed	250	252	2
2.	ViBact	250	252	2
3.	Darolac	250	252	2

Figure: Market Fed Fish

Figure: ViBact Fed Fish

Figure: Darolac Fed Fish

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Readings of Day 10:

Sr. no	Aquaculture feed	Weight of glass container and water (in grams) (y)	Weight reading after addition of fish (in grams) (x)	Final weight of Fish (in grams) (x-y)
1.	Market – fed	250	252	2
2.	ViBact	250	252	2
3.	Darolac	250	253	3

Figure: Market Fed Fish

Figure: ViBact Fed Fish

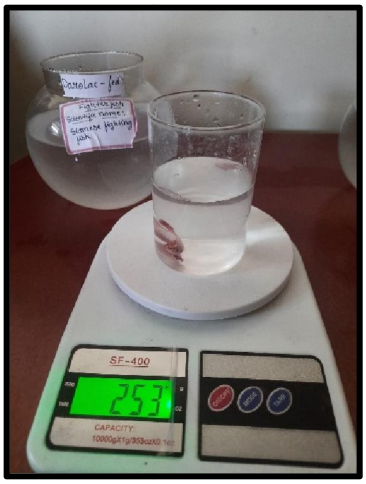

Figure: Darolac Fed Fish

Readings of Day 15:

Sr. no	Aquaculture feed	Weight of glass container and water (in grams) (y)	Weight reading after addition of fish (in grams)(x)	Final weight of Fish (in grams) (x-y)
1.	Market - fed	250	253	3
2.	ViBact	250	252	2
3.	Darolac	250	253	3

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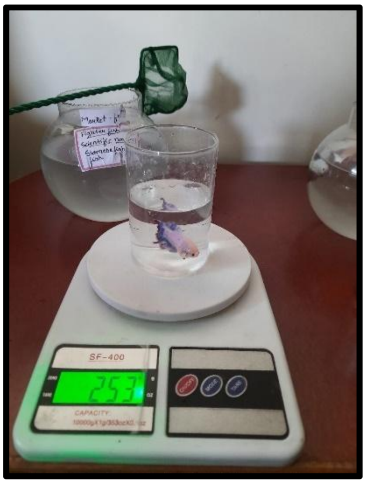

Figure: ViBact Fed Fish

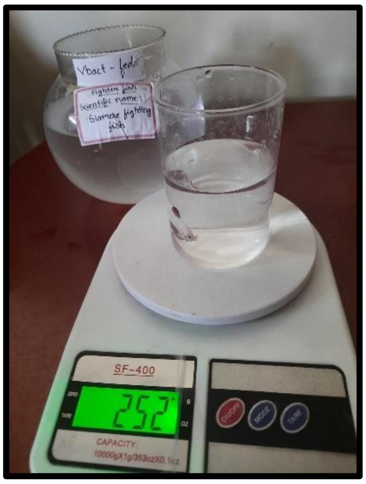

Figure: Darolac Fed Fish

Figure: Market Fed Fish

Readings of Day 20:

Sr. no	Aquaculture feed	Weight of glass container and water (in grams) (y)	Weight reading after addition of fish (in grams) (x)	Final weight of Fish (in grams) (x-y)
1.	Market - fed	250	253	3
2.	ViBact	250	253	3
3.	Darolac	250	253	3

Figure: Market Fed Fish

Figure: ViBact Fed Fish

Figure: Darolac Fed Fish

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Readings of Day 25:

Sr. no	Aquaculture feed	Weight of glass container and water (in grams) (y)	Weight reading after addition of fish (x)	Final weight of Fish (x-y)
1.	Market - fed	250	253	3
2.	ViBact	250	253	3
3.	Darolac	250	254	4

Figure: Darolac Fed Fish

Figure: Market Fed Fish

Figure: ViBact Fed Fish

Readings of Day 30:

Sr. no	Aquaculture feed	Weight of glass container and water (in grams) (y)	Weight reading after addition of fish (x)	Final weight of Fish (x-y)
1.	Market - fed	250	253	3
2.	ViBact	250	253	3
3.	Darolac	250	254	4

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Figure: Market Fed Fish

Figure: ViBact Fed Fish

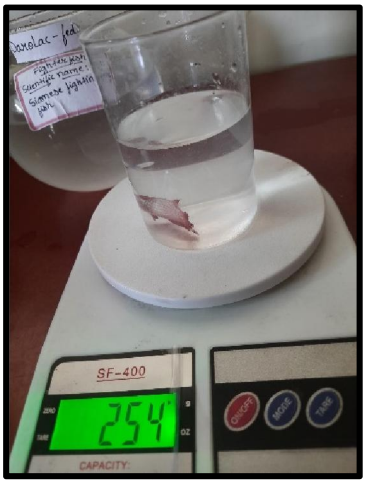

Figure: Darolac Fed Fish

III. RESULT AND DISCUSSION

Initial Weight of Fishes:

Sr. no	**Weight reading initial (In grams)**
1. Market – fed	2
2. ViBact – fed	2
3. Darolac – fed	2

Weight reading over a span of 30 day:

Table: Gain in weight over a 30 day span with an interval of 5 days.

Sr. no	**Day 1 Weight (in grams)**	**Day 5 Weight (in grams)**	**Day 10 Weight (in grams)**	**Day 15 Weight (in grams)**	**Day 20 Weight (in grams)**	**Day 25 Weight (in grams)**	**Day 30 Weight (in grams)**
Market – fed	2	2	2	3	3	3	3
ViBact – fed	2	2	2	2	3	3	3
Darolac – fed	2	2	3	3	3	4	4

Comparative study of gain in weight between different fed fishes via chart:

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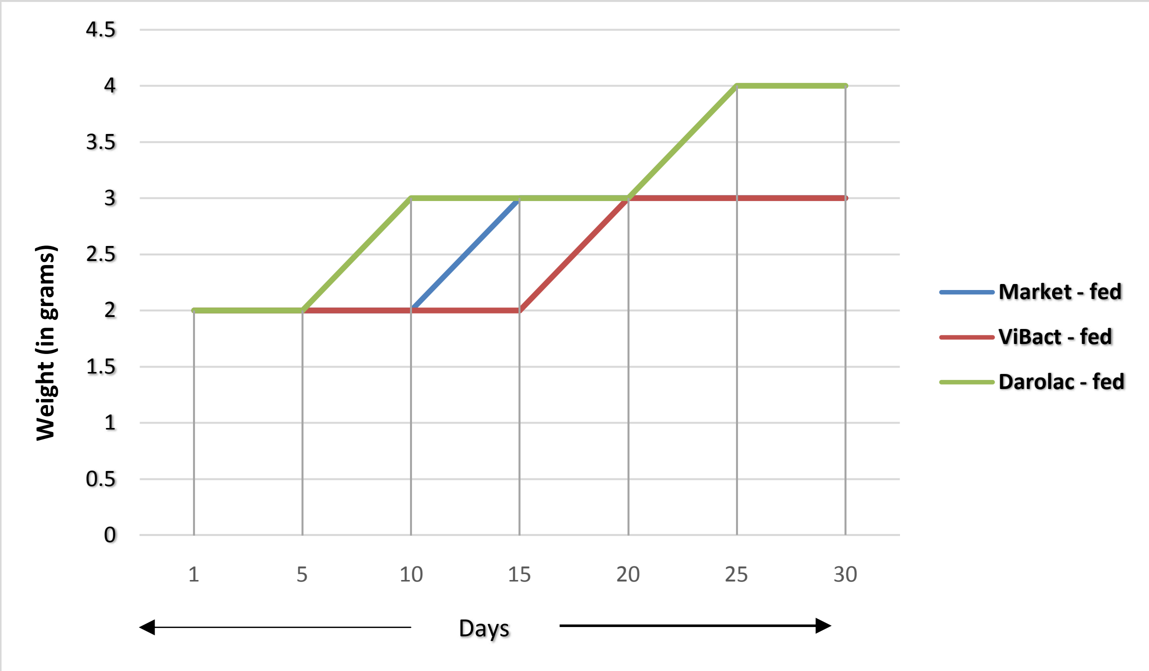

IV. DISCUSSION

Even according to an organization fish farming expert, which specializes in fish related commodities, stated that microorganisms specifically bacteria can too become a potential feed for aquatic creatures, not only just providing it with nutrients but with enhancement in this feature too like colors variation and saturation of fish.A new study by this organization shows that a type of a single-celled protein which is contained in bacteria could even potentially replace wild-caught fish and also agricultural products as an major ingredient in salmon fish feed (32).Sea food organization demonstrated that feed for shrimps can be enhanced by the mere application of microorganism (33).

V. CONCLUSION

Final Weight of **Market- fed** fish was found to be 3 grams.
Final Weight of **ViBact- fed** fish was found to be 3 grams.
Final Weight of **Darolac- fed** fish was found to be 4 grams.
From above observations and finding we can conclude that highest weight gainobserved in **Darolac fed**Betta fish whose weight was found to be 4 grams.
Microorganism found or used in Darolac is*Lactobacillus Lp299v.*

Precaution to be taken while keeping a fish:

- One should properly condition the water.
- Acclimate the fish to the water.
- Float fish in their bag before putting in the glass container.
- Maintain pH balance and other chemical levels too.

- Make sure water temperature is right for the fish.
- One should change water regularly.
- Clean tank glass and other structures also.
- Choose the right size for your tank for optimum growth.
- Avoid overfeeding the fish (33).

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