JETIR.ORG ISSN: 2349-5162 | ESTD Year : 2014 | Monthly Issue
JOURNAL OF EMERGING TECHNOLOGIES AND INNOVATIVE RESEARCH (JETIR)
An International Scholarly Open Access, Peer-reviewed, Refereed Journal

EFFECT OF COWDUNG ALONG WITH FREE AND IMMOBILIZED PROBIOTIC BACTERIA ON GROWTH AND FOOD UTILIZATION OF CYPRINUS CARPIO UNDER LABORATORY CONDITION.

[1]Jayalakshmi S. and [2]Ebanasar J.

1. Assistant Professor, Department of Zoology, Nallamuthu GounderMahalingam College, Pollachi, Coimbatore District, Tamil Nadu, India
2. Associate Professor, Department of Zoology, Government Arts College, Ooty, The Nilgiris, Tamil Nadu, India

Abstract

Aim

Aim of the study is to understand the changes in the growth of fishes due to manuring with cowdung and the synergistic effects with immobilized and free probiotic bacteria Lactobacillus sporogenes

Methodology

The fingerlings of commoncarp Cyprinuscarpio of1.21$\pm$0.21g size were used. The treatments include control(C), cowdung manure of .5gm per litre(T1), cowdung manuring with free cells of L. sporogenes $5x10^4$cells/ml(T2) and cowdung manuring with immobilized bacteria at a concentration of bacteria $5x10^4$cells/ml(T3). Each experiment had three replicates. The experiment was conducted for a period of 30 days. The growth and food utilization conversion efficiency were assayed. Final weight was statistically analyzed using MINITAB software and the results were presented.

Results

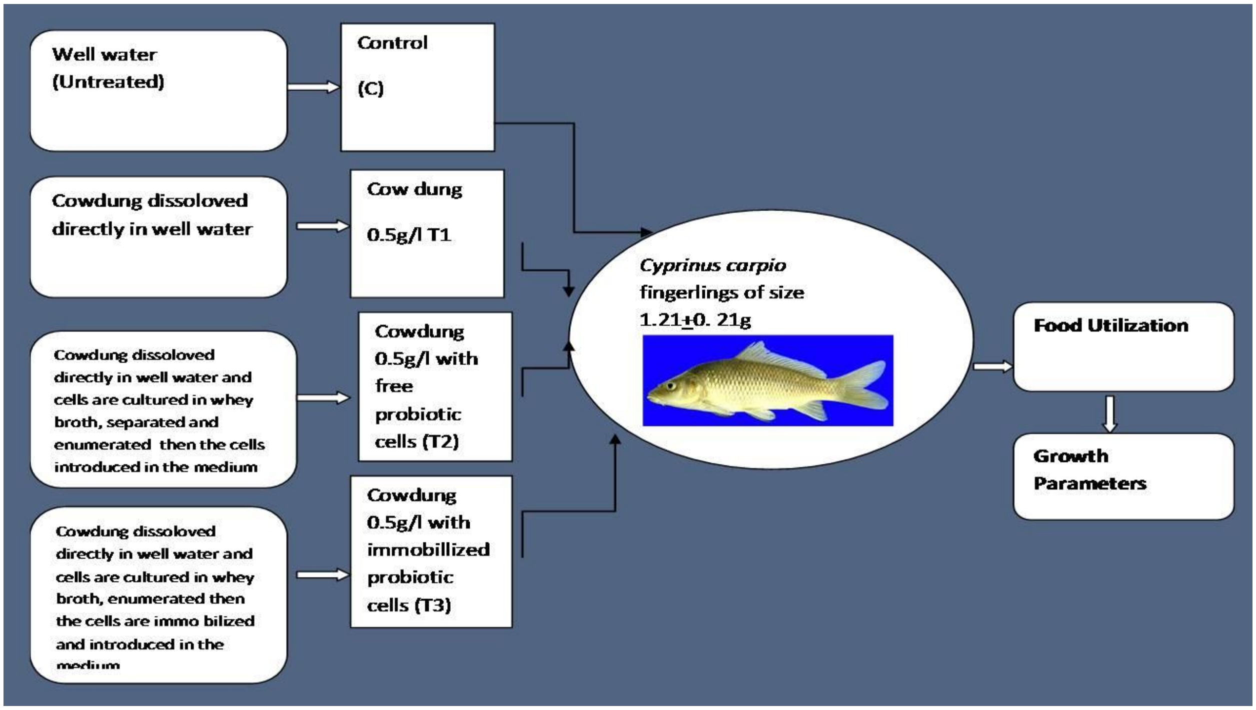
High food consumption and high growth was also found in C.carpio fingerlings treated with cowdung and immobilized bacteria(T3).

Interpretation

Immobilized cells can be effectively used in promoting growth and production characteristics of Cyprinus carpio which is also an ecofriendly biomonitoring tool.

Keywords: Cyprinus carpio, Immobilized cells, Manuring, Probiotics, Aquaculture, Cowdung

Abstract
Experimental protocol for growth responses of Cow dung and Cowdung along with Lactobacillus sporogenes treated Cyprinus carpio fingerlings administrated as three different routes



Introduction

The human need for the consumption of aquatic products and for aquarium operation is strong and the demand for the world development of fisheries is not satisfactorily met. This opens up a wide range of possibilities for the aquaculture industry (FAO, 2004). In several countries, aquaculture has become an important economic practice (Balcazar et al., 2006). However, many problems such as widespread epizootics, feed efficiency and growth performance are involved in its production (Subasinghe, 1997; Fegan, 2001; Gaiotto, 2005).This is primarily caused by large-scale manufacturing facilities, where aquatic animals are subjected to stressful environments, disease-related issues, insufficient nutritional balance in artificial diets and degradation of environmental conditions. It has been noted that physiological stress is one of the major contributing factors to the disease, poor growth and mortality of aquatic species (Balcazar et al., 2004; El-Haroun et al., 2006; Rollo et al., 2006).

The key goals of the aquaculture industry are currently to improve growth or survival efficiency and feed. The productivity and resistance of aquatic species would have a positive impact on the cost of production (Gatlin III, 2002). As growth promoters, and to some degree to avoid disease, hormones, antibiotics, ionophers and some salt compounds have been used. However, their ineffective implementations had a negative impact on the development of aquaculture and the environment (Gongora,1998).

A modern term for functional additives, such as probiotics, is aquaculture (Li and Gatlin III, 2004) where dietary additions of microorganisms have a positive growth impact resulting from the best use of carbohydrates, protein and electricity. (Moriarty, 1998; Skejermo and Vadstein,1999; Chang and Liu, 2002; Irianto and Austin, 2002a, b).It further decreases disease mortality, pathogen antagonism, and environmental change in microbial intestinal equilibrium (Subasinghe, 1997; Moriarty, 1998; Holmström et al., 2003).Lilley and Stillwell (1965) originally used the definition of probiotics to mean a substance (s) that promotes the growth of another microorganism(Chunkeatirote, 2002). The description was changed by Parker in 1974 to "organisms and substances that contribute to intestinal balance."Other concepts in aquaculture indicate that probiotic is a live microbial food supplement that is ingested by contributing to an improved microbial balance within the intestinal microbiota in order to provide health benefit to the host (Gram et al., 1999). These are biologically active components of single or mixed microorganism cultures capable of enhancing the host's health (Salminen et al., 1999; Ochoa-Solano and Olmos-Soto, 2006).

Live micro-organisms are resistant to disease (Tacon, 2002). Live microorganisms administered in sufficient quantities have a health effect on the host (Gomez et al., 2007). The use of microorganisms or their products in tanks and ponds in which animals live is expressed in the components of these concepts. They have been used as biological control of their potential to change the bacterial composition of the stomach, water and soil of aquatic animals, or to use feed as a health supplement and/or biological control. The present work assessed the Synergistic effect of cowdung along with free and immobilized probiotic bacteria on growth and food utilization of Cyprinus carpio.

Materials and Methods

Fingerlings of Cyprinus carpio were procured in healthy condition from Aliyar and transported to the laboratory in polythene bags filled with oxygen. In the laboratory, the fishes were reared in circular plastic containers for two weeks. During the period of acclimatization, the fishes were fed with a pelleted diet of 35% protein content ad libitum daily. Excess of food was removed and regular aeration and water filtration was provided to keep the experimental tanks clean and the experimental fishes healthy.

Experimental Setup

Twelve Circular plastic containers of 25litres capacity size were used for the present experiments. 5 fingerlings of uniform size 1.21$\pm$0.21gm were transferred to each circular plastic container. The tanks were filled with twenty litres of chlorine free tap water and aerated well. Three replicates were maintained for each treatment.

Preparation of whey broth.

500ml of milk was taken in a borosil beaker. The beaker fixed in the water bath at 80ºC after an hour the milk was cooled to room temperature. Two or three drops of 0.9% HCI was added in the milk. The precipitate appeared was filtered and the supernatant was collected and used as a whey broth.

Preparation of the Experimental medium and Culture of L.sporogenes.

100ml of whey broth was taken. It was sterilized in an autoclave. The sterilized whey broth was kept into the cleaned Laminar flow. One tablet of Sporolac was added into the whey broth. Then the broth was set in the shaker. The control broth was also set into the shaker. After 16hours the L. sporogenes culture of bacteria appeared. The broth is then centrifuged at 3000 rpm/10minutes. The supernatant was discarded. Then the precipitate was separate and then precipitate was dissolved in 100ml of PBS. The diluted solution was taken in a pipette. Sample of solution was taken in the Neubaeur counting chamber from this the number of bacteria cells/ml of the broth was calculated. Control was maintained without any treatment (i.e) neither cowdung nor probiotics.Treatment1 was maintained in which cowdung was mixed with medium(0.5g/l). The treatment 2 was studied in which (0.5g) of cowdung and Lactobacillus sporogenes ($5x10^4$Cells/ml) cultured cells were introduced directly in water. Treatment4 was maintained Immobilized L. sporogenes in $5x10^4$cells/ml concentration and cowdung at a concentration of 0.5g/l were added to treatment 2. All the 4 experimental setups were maintained carefully without contamination and mixing with each other by covering the mouth of the tank with mosquito net.

Preparation of feed for the experimental fishes

The proportion of the various components used in the preparation of the pelleted feed is given in table1.The ingredients were finely ground in a mixer. They were weighed and mixed well. Then water was added insufficient quantity so that the mixture was made into dough. Then the dough was steamed in pressure-cooker for 5minutes.Then the dough was allowed to cool and then it was pelletized in a pelletizer. The pellets were dried in electric oven at 60ºc.So that the proteins in the feed are not denatured. Then the feed is stored in air tight containers.

Table1. Components used in preparation of the feed and its composition.

Sl.No	Ingredient	Proportion(%)
1	Soya Powder	40
2	Groundnu toilcake	25
3	Wheat bran	20
4	Wheat Powder	4
5	Vitamins and mineral mix(AgroMix)	1
6	Tapioca flour	10

Feeding experimental fishes

The experimental fish were fed on formulated feed twice in a day for an hour between 9am to10am and 4pm to 5pm.The unfed was collected and dried in a hot air oven and weighed. Immediately after feeding the food remains in the aquarium jar was removed using a siphon with least disturbance to the fish. The unfed was also dried to constant weight and weighed. From these values the feed consumed was calculated.

Feed Consumed= Feed Given-Unfed collected

Faeces Collection

Faecal matter in the experimental tanks was removed daily. The faeces were collected carefully using a Siphon with least disturbance to the experimental fish. The faeces was also dried in hot air oven at 60ºC and weighed.

Termination of Experiments

The food utilization experiments were continued for 30 days.Live weights of the experimental fishes were also recorded in 10th, 20th and 30th days. Based on this data the growth and food utilization parameters were calculated.**(** Pandian, 1967; Petursewiez and Macfutyen., 1970**)**. The experiment was conducted for 30 days. The length and weight of the fishes were recorded once in 10 days.

Table 2 Growth Parameters of Cyprinus carpio in different treatments

Parameters	C	T1	T2	T3
Initial weight W1 (g)	1.21 ± 0.21^{a}	1.21 ± 0.21^{a}	1.21 ± 0.21^{a}	1.21 ± 0.21^{a}
Final weight W2 (g)*	2.23 ± 0.30^{a}	2.87 ± 0.35^{b}	3.32 ± 0.39^{c}	3.87 ± 0.57^{d}

Growth W2-W1) (g)	1.02± 0.03 [a]	1.66±0.02 [b]	2.12±0.08 [c]	2.66± 0.08 [d]
Growth rate (g/d)	28.18 ±1.3 [a]	45.76± 2.6 [b]	58.36 ±3.9 [c]	73.32±4.8 [d]
Specific growth rate (%)	2.04 ±0.02 [a]	2.88±0.03 [b]	3.37±0.02 [c]	3.88±0.12 [d]
Percentage of increase in body weight (%)	84.54	137.28	175.09	219.95
Food consumed (g/d)	0.06±0.001 [a]	0.10±0.007 [b]	0.11±0.008 [c]	0.12±0.002 [d]
Fecal output (g/d)	0.01 ±0.002 [a]	0.03±0.003 [b]	0.04±0.01 [c]	0.03±0.004 [b]
Food Absorbed (g/d)	0.05	0.07	0.07	0.08
Absorption rate (g/d)	41.32	59.34	59.34	73.55
Absorption efficiency (%)	82.64	70.39	63.53	70.07
Feeding rate (g/d)	1.66	2.81	3.11	3.49
Growth rate (g/ d)	28.18	45.76	58.36	73.31
Metabolic rate (%)	13.14	13.58	0.98	0.23
Gross conversion efficiency (%)	16.90	16.28	18.75	20.95
Net conversion efficiency (%)	0.68	0.77	0.98	0.99

* -Highly significant (P<0.01)

[a,b,c,d] Same superscript do not differ (Duncan's multiple range test)

Results and Discussion

Results of the present investigation revealed that the probiotic bacteria L. sporogens in both immobilized form as well as free in the medium have a significant infunece on the growth and food utilization of C. carpio According to Yassine Gameredinn et al. (2018) manuring promoted the growth of Cyprinus carpio in addition to the different types of manure recorded in the environment. The results of the present study also reveals that the growth of the Cyprinus carpio reared in cowdung manuring water showed better growth characteristics than the control. It reveals that the cowdung manuring has a direct relationship with growth and production of fishes. Table (2) reveals the influence of various treatment on growth parameters like weight of fish growth, growth rate and percentage increase in body weight. Least weight augmentation of 1.023g was recorded in control. Highest weight increment of 2.66g was recorded in treatment 3. Highest specific growth rate of 3.87% was recorded in T2. Least specific growth rate of 2.04% was recorded in Control. Analysis of variance reveals that the difference in the final weight of the fishes is highly significant (P<.05).

Various food utilization parameters like food consumed, food absorbed, feeding rate, absorption rate, absorption efficiency, gross conversion efficiency and net conversion efficiency during 30^{th} day are presented in table2. This food utilization parameters of Cyprinus carpio fingerlings exposed to different treatment. All the food utilization parameters were high in Treatment 3 which was treated with L .sporogenes immobilized forms cells introduced in water which was treated along with manuring. The food utilization characteristics in treatment1, 2 &3 are better than control.

Daily food intake of Cyprinus carpio did not deviate significantly in different treatments. There is no significant difference in daily food intake among the different treatments. The food consumed in different treatments ranged for 0.0605 to 0.127 (g/fish/d). The highest faecal output was recorded in T2, which was treated with L. sporogenes cultured cells directly introduced in water with manuring. The least faecal output was found in control. Food utilization Characteristics are significantly influenced by the use of the probiotic bacteria Lactobacillus. The least feeding rate of 1.666 g/d was absorbed in control. The highest feeding rate is absorbed of 3.498 g/d was absorbed in treatment 3. The least food absorption was found in control. The highest food absorption of 0.0822 g/d as recorded in treatment 3 treated with L. sporogenes.

Cultured cells in the form of immobilized cells introduced in water with manuring. The highest absorption rate was absorbed in treatment 3. The least absorption rate was recorded in the control. The highest absorption efficiency of 82.644% was recorded in Control. In this treatment the probiotic bacteria increased the absorption efficiency of Cyprinus carpio. Least absorption efficiency of 70.078%was found in treatment 3.The Gross conversion efficiency was found to increase in fish treated with probiotics. Least gross conversion efficiency of 16. 284% was recorded in treatment 1. The highest gross conversion efficiency of 20.955% was found in treatment 3.The lowest net conversion efficiency of 0.682 % was recorded in control. The highest net conversion efficiency of 0.996% was recorded in treatment 3

The faecal output was higher in fishes treated with manuring. The feeding rate and absorption rate showed a decline by manuring. However, the gross conversion efficiency and net conversion efficiency increased from that of the control by the process of manuring. This reveals that the manure added to the medium may have also been ingested by the fish dry filter feeding mechanism and which is also expressed in faecal output. However, the high conversion efficiency reals the economy in feed cost and increased production of C. carpio by the manuring process. Further, the manuring Process also increased the protein level in muscle.This reveals that the manuring process is beneficial for the growth as well as physiological process of the fish as well as its nutritive value.

According Sivagami (1988) the fertilizer applied is not of immediate effect on production. However, the nutrients in fertilizers especially the nitrogen and phosphorous is made available to microbes by the action of microbes in water.The results of the present study reveal that the application of probiotic enhances the growth characteristic of C.carpio. The addition of L. sporogenes by both the routes leads to increased growth rate of C.carpio .The probiotics have numerous roles. Probiotics are applied through two routes. Porubcan (1991) used floating biofilter with nitrifying bacteria to improve water quality. In the present study the application of bacteria both as suspended cells as well as water additive improved growth and food utilization. The percentage of increase in body weight of Cyprinus carpio in the treatment with immobilized cells was 219.95%and that of treatment with suspended cells is 175.09 % against that of manuring alone

137.27% .This reveals that the uses of immobilized cells as well as cells in suspension are beneficial in promoting growth and health of fish. Further, even though probiotics are used as water additive and it is more successful in promoting growth of fish, the environmental safety is taken into account while introducing any microbe into the environment with this view it is recommended that the probiotic L. sporogenes can be used in carp culture preferably in the immobilized form along with manuring as it enhances nutrient cycling process as well as it enhances the growth rate. Survival is another important index in addition to growth and food utilization of fishes. Probiotics are known to enhance survival fishes (Sheeja et al., 2003). The results of the present study also reveal that the probiotic enhances survival of Cyprinus carpio among the different treatments. The use of immobilized bacteria is found to produce more survival than other treatments. As survival is more related to fish production, the results reveal that the application of probiotic bacteria in the form of immobilized cells is more beneficial in getting more survival, better growth rate and high production. Besides these it is a safe way of applying probiotics in the culture of Cyprinus carpio.

Acknowledgements

I wish to express my deepest gratitude to Dr. J. Ebanasar for his valuable guidance, constant encouragements, and innovative suggestions for Publish this paper . I am grateful to Dr. P. M. Palanisamy Principal and and the Management of Nallamuthu Gounder Mahalingam College for providing facilities and their constant encouragement.

Reference

[1] Abraham, S., T.J. Ramesha, B. Ganadhara and T.J. Varghese: Growth responses of common carp Cyprinus carpio (Linn) to varied levels of livol a non hormonal growth promoter. Indian. J. Fish. 48(4), 397-401 (2001)

[2] Arul, V.: Effect of ethylsternol on the growth and food utilization in Channa striatus Proc. Indian Acad.Sci. (Animal Sciences) 95 (1), 51-57 (1986)

[3] Dalmin, G.K. Kathiresan and A. Purusothaman: Effect of probiotic on the bacterial population and health status in shrimp in culture pond ecosystem. Indian. J. Exp. Biol.39 (9), 939-942. (2001)

[4] Ebanasar, J. and V. Jayaprakas : Evaluation of Different diets for the cage culture of Channa striatus J. Inland, Fish Soc. India. 26(1), 59-66. (1994)

[5] Ebanasar, J.: Studies on some aspects of culture of murrels Channa micropeltes, Channa marulius and Channa striatus. PhD thesis, University of Kerala, Trivandrum (1995)

[6] Ebanasar, J. and V. Jayaprakas : Food utilization of Channa micropeltes (Channidae: Pisces) fed three diets of animal origin Indian. J. Exp. Biol.34, 1262-1264.(1996)

[7] Felix, K. A. Kuebutornye ,Emmanuel Delwin Abarike and Yishan Lu: A review on the application of Bacillus as probiotics in aquaculture ,Fish and Shellfish Immunology 87, 820-828.(2019)

[8] Gerald, V. M.: Effect of size of fish on the consumption absorption and conversion of food in Ophiocephalus punctatus (Bloch.) Hydrobiologia 49 (1),77-85 (1976)

[9] Gupta, A. G. Verma, and P.Gupta: Growth performance, feed utilization, digestive enzyme activity, innate immunity and Protection against Vibrio harvei of freshwater prawn, Macrobacterium rosenbergii fed diets supplemented with bacillus coagulans ,Aquaculture Int.24, 1379-1392 (2016)

[10] Jayaprakas, V. and C. Sambhu: Effect of dietary hormone on the feed utilization, growth and body composition of the pearl spot, Etroplus suratensis(Bloch)in a brakish water pond.Indian J. Mar.Sci. 24, 32-36 (1994)

[11]Jayaprakas, V. and B. S. Sindhu: Effect of hormones on food utilization of the Indian major carp Cirrhinus mrigala. Fishery Technology 33(1), 21-27 (1994)

[12]Jobling, M.: Some effects of temperature, feeding and body weight on nitrogenous excretion in young plaice Pleuronectesplatessa. J. Fish Biol. 17, 325-334.(1981)

[13] Morairity, D. J. W.: The role of microorganism in aquaculture ponds. Aquaculture 151, 333-349 (1997)

[14] Nirmala, A. R. C. and T. J. Pandian: Effect of steroid injection on food utilization in Channa striatus. Proc. Indian Acad. Sci. (Animal Sciences)92, 1-10(1983)

[15] Porubcan, R.S.: Reduction in Chemical oxygen demand and improvement in penaeus monodon yield in ponds inoculated with aerobic Bacillus bacteria. Program and Abstracts of the 22nd Annual Conference and Exposition, 16-20 June 1991, san Juan, Puerto Rico. World Aquacuture society.(1991)

[16] Sambhu C. and V. Jayaprakas: Livol(IHF-1000) a new herbal growth promoter in white prawn, Penaeus indicus (Crustacea) Indian J. Mar. sci.30, 38-43 (2001)

[17] Sheeja, B.D., R. Narayanan and J. Ebanasar: Digestibility of hydrophytes and survival of grass carp Ctenopharyngodon idella exposed to microbial probiotic Lactobacillus. Indian J. Comp. Anim. Physiol. 21, 5-10.(2003)

[18] Sivagami, S.: Observations on the culture of the air- breathing fish Channa striata (Bloch) in farm ponds. Indian J.Fish.,35, 18-25 (1988)

[19] Venkatalakshmi, S. J. Ebanasar and Dinakaran Michael: A novel and ecosafe method for probiotic application in aquaculture. J. Basic and Applied Biology 6,60-68 (2012)

[20]Verschuere, L. G. Rombaut, P. Sorgeloos, W. Verstraete: probiotic bacteria as biological control agents in aquaculture, Microbiol. Mol. Biol. Rev. 64, 655-671(2000)

[21] Vivekanandan, E. and T. J. Pandian: Surfacing activity and food utilization in a tropical air-breathing fish exposed to different temperature. Hydrobiologia. 54 (2), 145-160.(1977)

[22] Vivekanandan, E.: Effects of feeding on the swimming activity and growth of Ophiocephalus striatus J. Fish. Biol. 8, 321-330.(1976)

[23] Yassine Gameredinn* , N. Saharan, G. Prakash and V. K. Tiwari: Growth performance and survival of common carp (Cyprinus carpio, Linnaeus 1758) fingerlings under different fertilizers application. International J. of Livestock Research 8, 235-243 (2018)