

INFLUENCE OF A PROBIOTIC *PSEUDOMONAS PSEUDOALCALIGENES* FERMENTED FEED ON GROWTH PERFORMANCE OF ROHU (*Labeo rohita*) FINGERLINGS.

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Abstract: This study reports the effect of a probiotic strain *Pseudomonas pseudoalcaligenes* on growth of rohu (*Labeo rohita*) fingerlings. The growth was assessed by morphometric measurements, feed conversion ratio, feed conversion efficiency and protein efficiency. The formulated fish feed was fermented by *P. pseudoalcaligenes*. Which was introduced in fish aquaria @ 3% b.w.t. in two forms; with live bacteria (SSF1) and without live bacteria (SSF2). In 90 days experiment, morphometric measurements were made fortnightly. SSF1 showed significantly better growth performance than those of SSF2 and control groups. At last phase (90 days), SSF1, SSF2 and control groups showed FCR as 1.68, 2.28, 2.20 and FCE% 59.53, 44.75, 45.38 respectively. Both groups showed wet body weight gain than control group at all phases while significantly better weight gain was recorded in SSF1 group. Furthermore, SSF1 showed increased protein efficiency ratio (0.43) than SSF2 and control groups. These results clearly indicate the importance of probiotics and fermentation technology in aquaculture science.

Keywords: Probiotic, bacteria, Fish feed, feed conversion ratio, feed conversion efficiency.

INTRODUCTION

In Asia, on average, almost 30 per cent of the total animal protein intake is derived from fish. Among the Southeast Asian countries, fish protein provides 45 per cent of the total protein consumed (Prein and Ahmed, 2000). Fish is a highly nutritious food, containing high amounts of protein with high biochemical value for humans. Fish is a principal source of animal protein for over half of the global population. The major carps like *Catla catla*, *Labeo rohita* and *Cirrhina mrigala* are the most preferred farmed fish

species in the Punjab (Pakistan), because of their fast growth and higher acceptability to the consumers (Javaid, 1990; Javed *et al.*, 1993). For the present study, *L. rohita* (Rohu) was selected due to its rapid growth, attainment of large size, quality of flesh and consumer preference. It is a fresh water herbivore. Rohu, (*L. rohita*) is known as a water column feeder mainly feeding on plankton (Hasan and Das, 1994; Wahab *et al.*, 1994) and common carp is a bottom feeder mainly feeding on benthic macroinvertebrate and zooplankton (Hepher and Pruginin, 1981; Spataru *et al.*, 1983).

When artificial feed is applied, common carp readily accepts artificial feeds (Schroeder, 1983; Milstein and Hulata, 1993). The dietary protein requirement of *L. rohita* has been reported (De Silva and Gunasekera, 1991; Khan and Jaffri, 1991; Mohanty *et al.*, 1996). The food and feeding habits of rohu and common carp might differ according to the overall food and feed availability. Now a day, use of supplementary feed has become inevitable for the success of contemporary fish culturing. Supplementary feeding is known to increase the carrying capacity of culture systems and can enhance fish production by many folds (Balogu *et al.*, 1993; Mahboob *et al.*, 1997). Different feed supplements such as antibiotics and single cell protein may be used to exploit the maximum growth potential of animals (Ahmad *et al.*, 1995; Salminen *et al.*, 1999).

Probiotics have a direct growth promoting effect on fish either by a direct involvement in nutrient uptake, or by providing nutrients or vitamins (Ringo and Gatesoupe, 1998). It has also been demonstrated experimentally that probiotics indeed may enhance growth of fish (Noh *et al.*, 1994; Bogut *et al.*, 1998). Different forms of probiotics, application have varying effects it has been found that the use of live probiotic cells is more effective. Thus viability of probiotic bacteria is a key factor (De Simone *et al.*, 1986; Panigrahi *et al.*, 2005). Yasuda and Taga (1980) anticipated that bacteria might be useful as food and as biological control agents of fish disease in aquaculture. Probiotics have a long history with humans and livestock, and unknowingly they have been applied in food safety. Probiotics are being increasingly used in human food as well as animal feed (Dunne *et al.*, 1999; Sander and Veld, 1999).

Furthermore, environmentally friendly diets can be produced by developing diets with reduced food conversion ratios (FCR), *e.g.*, by improving palatability and digestibility of raw ingredients (Alvarado, 1997). While considering economical aspects of aquaculture, fermented feeds had been hypothesized to enhance the assimilation efficiency of supplementary feeds (Mukhopadhyay and Ray, 1999; Skrede *et al.*, 2001; Skrede *et al.*, 2003).

The present investigation reports solid-state fermentation (SSF) of a formulated feed employing the bacterial isolate, *Pseudomonas pseudoalcaligenes* AsCh-A4 and its effects on growth of the fish. Feed conversion ratio and efficiencies of feed conversion and protein of control and SSF feeds reported here appear promising in developing the aquaculture.

MATERIALS AND METHODS

Formulation of fish feed

The feed was prepared by mixing thoroughly fish meal 5.0%, rice polishing 34.3%, ground nut oil cake 53.7%, molasses 4.0%, dicalcium phosphate 1.0%, table salt 1.0% and vitamin premix 1.0%.

Test organism

Test organism *P. pseudoalcaligenes* AsCh-A4 elevating different contents of the fermented feed up to 100 % was selected from a previous study. For solid-state fermentation, an apparatus was designed and installed according to Hofrichter *et al.* (1999).

Effect of fermented feed on growth of fish fingerlings.

To investigate the influence of the bacterial isolate *P. pseudoalcaligenes* AsCh-A4 fermented fish feed on the growth of rohu fingerlings, an experiment was designed that comprised of three groups. About five hundred rohu fingerlings ranging from 5-7.5cm in length were obtained from a fish farm in the vicinity of Muridke, a small city about 21 km from Lahore, Pakistan. The rohu fingerlings were acclimatized in laboratory conditions for 15 days. During acclimation period, fish were fed with the control-formulated feed.

Experimental set up

The experiment was carried out in triplicates and each aquarium had 30 fingerlings. The control group was fed with simple autoclaved formulated feed. Fingerlings in the Group 2 (SSF1) were fed with fermented fish feed with live bacteria, while the animals in Group 3 (SSF2) were provided autoclaved fermented fish feed. The fish feed in both SSF1 and SSF2 was fermented by the bacterial isolate *P. pseudoalcaligenes* AsCh-A4. At the time of stocking, wet body weight of the fingerlings in each aquarium was measured and recorded as zero readings. The room temperature was maintained between 24-30°C and the experiment was allowed to proceed in 90days.

Feeding regimes of fish

Fingerlings in each group were fed once daily with the respective feed at a feeding rate of about 3% of total body weight for 90 days. Weight of feed to be administered was calculated fortnightly on the basis of wet body weight of the fingerlings per aquarium. Water of aquaria was changed daily and the feed remains and faeces were siphoned and collected on a muslin cloth. The collected material was re-suspended in water and filtered through a pre-weighed Watman No. 1 filter paper, which was dried till consistent weight and freeze-dried for further analysis. Total feed consumption was calculated by subtracting the weight of dried feed remains plus fecal matter from the feed provided.

Morphometric measurements of the fingerlings

At every 15th day all the fishes of an aquarium were measured for wet body weight. After obtaining the data, five fish per aquarium were sampled while the remaining released back to their respective aquaria. Wet weight gain was calculated by the following expression;

Wet weight gain (g) = Final weight (g) – Initial weight (g)
Feed conversion ratio (FCR) was calculated as;

$$FCR = \text{Total feed consumption (g)} / \text{weight gain (g)}$$
While the Percent Feed Conversion Efficiency (FCE %) for each aquarium was calculated by the following expression;

$$\text{FCE\%} = \text{Wet weight gain (g)} / \text{feed consumed (g)} \times 100$$

Proximate analysis of feed and faeces

Each category of the feed and faecal matter were dried at 105°C (in an electric oven) till consistent weight. Weighed amounts of the dried feed (0.5 g) and faeces (0.25g) were taken and homogenized. For this purpose a given sample was immersed in 4 ml of 0.89% cold saline solution and homogenized for one minute by employing a motor driven homogenizer at 8000 rpm. The homogenate was centrifuged at 4900 rpm for 45 minutes. The clear supernatant was separated and used for determining total protein (Gornall *et al.*, 1949).

From the difference of total protein (mg/g) of respective feeds and the faeces, protein intake was calculated. Protein Efficiency Ratio (PER) was then calculated as;

$$\text{PER} = (\text{wet weight gain/feed protein intake}) \times 100$$

Statistical analysis

All the experimental data were analyzed using one way analysis of variance (ANOVA) followed by Tukey's multiple range test (SPSS ver.12.0 software, SPSS, Chicago, IL, USA).

RESULTS

Effect of Pseudomonas pseudoalcaligenes AsCh-A4 fermented feed on growth performance of Labeo rohita fingerlings

The control, SSF1 and SSF2 fishes were fed with the sterile formulated feed (@ 3% b.wt.), the SSF feed with live bacteria (SSF1) and the SSF autoclaved (SSF2) for 90 days. The feed inputs and recovery of the fecal matter and unconsumed feeds are shown in Tables I and II. Five fishes from each of the triplicate aquaria for each experiment were sampled at 15 days intervals and accordingly the total feed per aquarium administered was decreased (Table I). The progressive decrease in total amount of feed given paralleled decreasing trends for all the three groups when faeces and unconsumed feeds were recorded (Table II). The fermented feed given to the both experimental groups (3% body weight),

turned out to be significantly different at the last two sampling periods as compared to the respective control values.

Table I: Input of fish feed (g) (3% b.w.) administered to control and experimental groups of *L. rohita* at different phases.

Phase (days)	No. of fish	Control	SSF1	SSF2
I (15)	90	71.35 ± 0.87	71.00 ± 1.69	71.55 ± 2.35
II(30)	75	67.20 ± 1.43	68.35 ± 1.67	68.40 ± 1.65
III (45)	60	56.30 ± 2.15	59.35 ± 1.18	55.98 ± 3.59
IV (60)	45	45.20 ± 1.40	50.20 ± 1.41	48.90 ± 0.84
V(75)	30	32.15 ± 0.73a	38.00 ± 1.15b	36.40 ± 0.61b
VI(90)	15	17.50 ± 0.39a	21.50 ± 0.59b	20.35 ± 0.33b

All values represent means of three replicates ±S.E.M. Two values within respective rows not sharing a common alphabet differ significantly from each other. Values are significantly different at $p \leq 0.5$ at single factor analysis of variance.

Abbreviation used: SSF2, the group received the autoclaved SSF feed.
SSF1, the group received the SSF feed with live *P. pseudoaicaligenes*;

Table II: Recovery of feces and remaining feed in control and experimental groups of *L. rohita* within different phases

Phase (Days)	No of fish/ aquarium	Control	SSF1	SSF2
I(15)	90	18.60±0.49	16.99±0.45	18.02±1.27
II(30)	75	16.51±1.11	14.23±1.81	16.61±0.58
III(45)	60	15.18±1.08	13.67±0.76	15.55±0.48
IV(60)	45	14.70±1.04	12.08±1.04	14.12±0.16
V(75)	30	11.98±0.65	9.48±0.51	10.43±0.59
VI(90)	15	7.94±0.39a	6.06±0.10b	6.84±0.39ab

All values represent means of three replicates ±S.E.M. Two values within respective rows not sharing a common alphabet differ significantly from each other. Values are significantly different at $p \leq 0.5$ at single factor analysis of variance.

This indicated more growth of fishes fed with fermented feed (Table I). Interestingly at the last sampling period, recovered faeces and unconsumed feeds showed significantly decreased values for both the experimental groups as compared to the control values (Table II).

Feed conversion ratio/efficiency

When feed conversion ratio (FCR) and percent feed conversion efficiency (FCE%) were worked out the fishes of SSF1 showed significantly high body weight gain at third phase, while total feed input for both the experimental groups showed significant increases over the control values at accomplishment of the experiment. (Phase VI).

At this phase again the group SSF 1 showed significantly higher wet body weight gain as compared to control as well as the SSF2. Significantly higher and lower FCE% and FCR, respectively, for the SSF1 as compared to both control as well as SSF2 at phase V of experiment apparently showed rapid growth of the fishes fed with fermented feed containing live bacteria. Similarly, significantly higher body weight and FCE% of SSF1 than the other group at last phase of the experiment are indicative of growth promoting effects of probiotic fermented feed (Table 3, Figure 1).

Protein efficiency ratio

Protein efficiency Ratios (PER) were found significantly less for SSF1 and SSF2 as compared to control at phase I of the experiment. However, at third and fourth phases, the experimental groups had significantly higher PER as compared to the respective controls. The SSF1 at last phase showed significantly higher PER as compared to control value (Table IV, Figure 2).

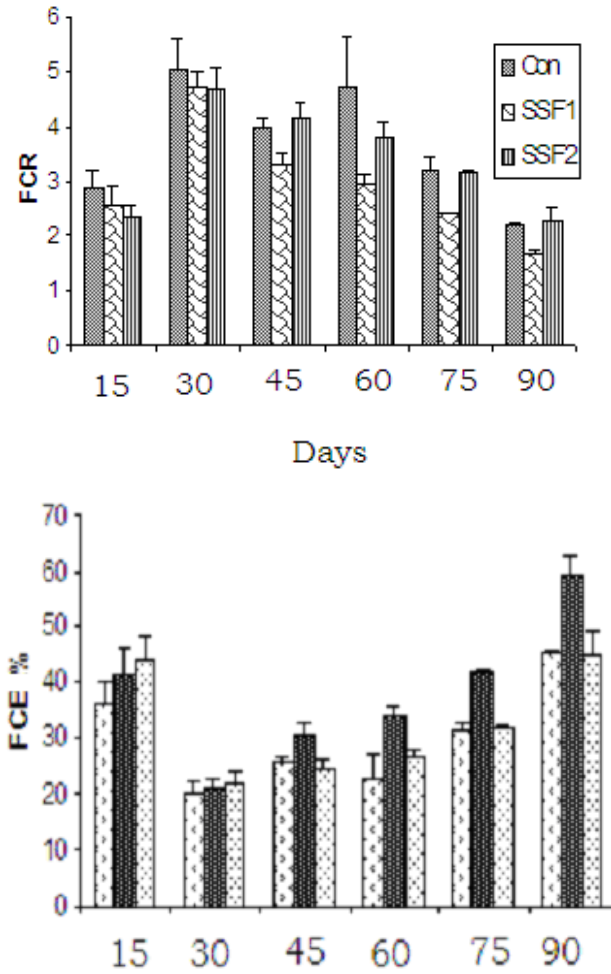


Figure 1: Feed conversion ratio and percent feed conversion efficiency (FCE%) of *L. rohita* fingerlings in control and experimental groups at different time intervals. Bars represents mean \pm SEM.

Table III: Feed conversion ratio (FCR) and Percent feed conversion efficiency (FCE) of *L. rohita* control and experimental groups at different phases.

Phase (Days)	No. of fish/aquarium	Parameters	Control	SSF1	SSF2
I(15)	90	Total feed intake	52.62 ± 0.35	54.01 ± 1.43	53.53 ± 1.53
		Wet body weight gain	18.96 ± 2.24	22.11 ± 3.15	23.48 ± 1.68
		FCR	2.86 ± 0.36	2.54 ± 0.37	2.32 ± 0.24
		FCE%	35.97 ± 4.06	40.94 ± 5.59	44.09 ± 4.25
II(30)	75	Total feed intake	50.69 ± 0.33	51.46 ± 2.03	51.42 ± 0.93
		Wet body weight gain	10.30 ± 1.03	10.97 ± 0.26	11.41 ± 1.34
		FCR	5.03 ± 0.54	4.70 ± 0.28	.64 ± 0.45
		FCE%	20.42 ± 2.11	21.40 ± 1.19	21.96 ± 2.17
III(45)	60	Total feed intake	41.21 ± 1.34	45.68 ± 1.94	45.45 ± 0.91
		Wet body weight gain	10.49 ± 0.88 a	13.96 ± 0.91b	10.57 ± 0.38 a
		FCR	3.95 ± 0.21	3.29 ± 0.23	4.13 ± 0.30
		FCE%	5.43 ± 1.32	30.69 ± 2.20	24.45 ± 1.72
IV(60)	45	Total feed intake	30.50 ± 0.79 a	38.12 ± 2.44b	34.78 ± 0.98a
		Wet body weight gain	7.06 ± 1.34a	12.85 ± 0.35b	9.16 ± 0.30a
		FCR	4.67 ± 0.96	2.96 ± 0.15	3.81 ± 0.24
		FCE%	22.93 ± 3.89	33.91 ± 1.61	26.42 ± 1.59
V(75)	30	Total feed intake	20.17 ± 0.66a	28.52 ± 1.66b	25.98 ± 1.14b
		Wet body weight gain	6.28 ± 0.43a	11.91 ± 0.55b	8.31 ± 0.33a
		FCR	3.23 ± 0.19a	2.39 ± 0.03b	3.12 ± 0.05c
		FCE%	31.16 ± 1.85a	41.80 ± 0.48b	32.03 ± 0.53a
VI(90)	15	Total feed intake	12.90 ± 3.34	15.44 ± 0.50	13.51 ± 0.25
		Wet body weight gain	4.34 ± 0.06a	9.26 ± 0.68b	6.03 ± 0.56a
		FCR	2.20 ± 0.03	1.68 ± 0.08	2.28 ± 0.23
		FCE%	45.38±0.56	59.53±3.20a	44.75±4.67b

All values represent means of three replicates ±SEM two values within respective rows not sharing a common alphabet differ significantly from each other at p≤ 0.5 (Single factor analysis of variance).

Abbreviation used: FCR, feed conversion ratio; FCE, feed conversion efficacy.

Table IV: Protein efficiency ratio (PER) based on feed intake of *L. rohita* in control and experimental groups at different phases.

Phase (Days)	No. of fish	Control	SSF1	SSF2
I(15)	90	4.21±0.68a	1.69±0.23b	1.39±0.12b
II(30)	75	0.81±3.56	0.48±0.01	0.70±0.17
III(45)	60	-9.88±3.86a	0.831±0.12b	1.40±0.04b
IV(60)	45	-3.75±1.55a	0.64±0.06b	0.63±0.00b
V(75)	30	-0.124±1.053	0.54±0.05	0.30±0.01
VI(90)	15	-2.67±1.20a	0.43±0.04b	0.26±0.01ab

All values represent means of three replicates ±SEM two values within respective rows not sharing a common alphabet differ significantly from each other at $p \leq 0.5$ (single factor analysis of variance).

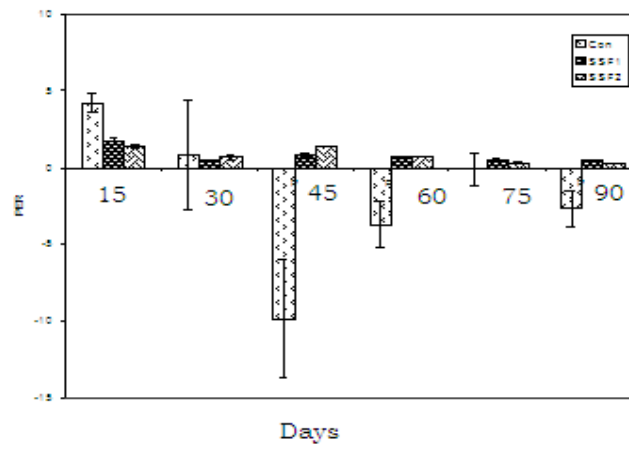


Figure 2: Protein efficiency ratio (PER) based on protein intake of *L. rohita* in control and experimental groups at different phases. Bars represent mean \pm SEM

DISCUSSION

Aquaculture development has been considered a very rich source of high biologic value protein diets to ever-growing human population. Consequently the sector has developed strategies in various countries to improve the fish health and fish growth. Among these strategies, the more promising is the use of probiotics and solid state fermentation. In this investigation, the solid state fermented feeds having viable (SSF1) as well as dead bacterial mass (SSF2), were analyzed for their potential growth promoting effects on rohu (*L. rohita*) fingerlings. Regarding the growth effects of solid state fermented feed containing live and dead probiotic *P. pseudoalcaligenes* cells, significantly higher levels of growth assessing parameters found for the fishes fed the experimental feeds as compared to the control group clearly demonstrate the potential of the reported bacterium. Conclusively, 9.26g and 6.03g higher body weight gain, 1.68 and 2.28 FCR, 59.53 and 44.75 FCE% were recorded for SSF1 and SSF2 respectively at the last phase of experiment. These results suffice to advocate the beneficial role of the probiotic *P. pseudoalcaligenes*.

However, the fermented feed with live probiotics promoted higher growth of the fish as compared to both the control and fishes fed with SSF2 feeds. This claim for the present study has emerged from the foundations laid down by majority of the growth assessing parameter levels. Many authors have commented on the usefulness of administration of live probiotics (Gatesoupe, 1999; Gomez-Gil *et al.*, 2000; Robertson *et al.*, 2000; Nikoskelainen *et al.*, 2001; Siuta Cruce and Goulet, 2001).

Several workers have described benefits of probiotics to the host that include the improvement in nutrition by the detoxification of potentially harmful compounds in feeds, the hydrolysis of potentially indigestible components in the diet by hydrolytic enzymes including amylases and proteases resulting into increased protein and sugar and decreased fiber levels, the production of vitamins, such as biotin and vitamin B₁₂ (Sugita *et al.*, 1991, 1992; Fuller, 1992; Smoragiewicz *et al.*, 1993; Balagopalan, 1996; Sugita *et al.*, 1996; Hoshino *et al.*, 1997). It appears pertinent here to refer that SSF process is simpler and consequently

(potentially) less expensive (Solis-Pereira *et al.*, 1993; Maldonado *et al.*, 1998; Diaz-Godinez, 2001).

Regarding the unsafe disposal of effluents of domestic and industrial origins to the material surface water system in Pakistan it must be noticed that the aquatic systems have not only been disturbed at microorganisms level but microbial communities both at the habitats and host associated levels probably have been disturbed to the level which requires supplementation with live useful microorganisms. Another aspect of fermentative up gradation of feeds is related to the identification of inexpensive substrates. In this regard, Pakistan, being agricultural industry has a great potential to provide not only inexpensive, but otherwise agro industrial wastes whose disposal is another problem. Such negative cost causing substrate can be employed for SSF processes rendering them nutritive to the level not only compensating the negative cost of the respective industry but even may yields benefits by providing growth promoting/supporting feed containing cellular proteins.

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