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Survival and Non-specific Immune Parameters of Nursery Carp (Spawn to Fry) Fed with CIFA-Carp Starter

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ABSTRACT

Background: Nursery phase is one of most critical phase in the production of stockable seed materials in carp culture. Generally, it is observed that the recovery of carp during spawn to fry is very low in farmers' practice because of unavailability of nutrition rich feed with essencial macro and micronutrients.

Methods: To address this problem, a nursery feed for carp was formulated and prepared using high-quality feed ingredients *i.e.* maize, soybean meal, groundnut oil cake (GNOC), fish meal, rice bran with supplementation of oil, minerals (with nano selenium and nano zinc) and vitamin mixture and compared the performance of nursery rohu with existing feed used by farmers Odisha, West Bengal and Chhattisgarh. During the demonstration period of two weeks (spawn to fry), fish of treatment ponds were fed with nursery feed (CIFA-Carp Starter) whereas the fish of control ponds were fed with existing feeds used by the farmers. The parameters like survivability, growth and immunity of nursery rohu in treatment ponds were recorded and compared with nursery rohu of control ponds.

Result: The results of demonstration showed that, the weight gain, survival and immunity of nursery rohu was superior when fed with CIFA-Carp Starter compared to existing feed practiced by farmers in three states. Hence, there is need to popularise this feed for improvement of aquaculture sector in the whole country.

Key words: Carp starter, Carp, Growth performance, Immunity, Nursery feed, Survivability.

INTRODUCTION

Nursery phase is one of the most critical phase in the production of stockable seed materials in carp culture. Generally, it is observed that the recovery of spawn to fry and fry to fingerling is low (25-30% and 40-50%, respectively in farmers' practice). To address this problem, ICAR-CIFA, Bhubaneswar has developed a carp nursery feed that ensures higher survival, better growth and uniform-sized fingerling production. This feed is highly nutritious and palatable and also suitable for carp seed growers to enhance their production and profitability. The feed contains moisture 8-10%; crude protein 34-35%; crude fat 5-6% and crude fibre 4-5%. The benefits of using nursery feed are faster growth rate, better survival and uniform growth of nursery carp and production of healthy fingerlings.

In this experiment, carp nursery feed (CIFA-Carp Starter) was produced through extrusion technology and performance evaluation was done in rohu spawn in terms of growth, feed efficiency and immunological parameters by comparing with the traditional feed used by most of the farmers of this region.

MATERIALS AND METHODS

The experiment was conducted during the year of 2018-19 at ICAR-Central Institute of Freshwater Aquaculrure, Kausalyaganga, Bhubaneswar involving farmers of Odisha, West Bengal and Chhattisgarh through one project sponsored by National Fisheries Development Board, Hyderabad.

Formulation and production of nursery feed for IMC

Fish feed for nursery carp was formulated using high-quality locally available feed ingredients *i.e.* maize, soybean meal, groundnut oil cake (GNOC), fish meal and rice bran with supplementation of oil, minerals (with nano selenium and nano zinc) and vitamin mixture (Fig 1). The ingredients were pulverized, mixed, extruded and then dried. The extruded and dried feed was then ground into suitable size, packaged and stored for feeding of fish. The feeds were produced in the feed mill facilities of ICAR-CIFA, Bhubaneswar, India with extrusion temperatures of 130°C and moisture of 20 percentages maintaining constant pressure (10 kg/cm^2). After the production of extruded feed, it was packaged for easy transportation to the experimental site.

Demonstration of nursery feed

Nursery feed after production was transported to different locations of Odisha, West Bengal and Chhattisgarh. Feed was transported either by bus or railways and the experiment was started after receiving the nursery feed.

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The demonstration was conducted in Rambhila, Bhadrak, Odisha, Duba, Ganjam Odisha, Subarnapur, Gop, Odisha, Sarakana, Khordha, Odisha, Boisinga, Balasore, Odisha, Kalna, West Bengal, Tirga, Durg, Chhattisgarh and Kapsi, Kanker, Chhattisgarh and NFDB, Bhubaneswar, Odisha. The fish of control ponds were fed with groundnut oil cake (GNOC) and rice bran and fish of treatment ponds were fed with nursery feed developed by ICAR-CIFA. During the experiment survivability, growth and immunity of nursery carp in the treatment group were compared with nursery carp in the control group.

Analysis of feed

The chemical parameters *i.e.* dry matter (DM), crude protein (CP), ether extract (EE), crude fibre (CF), nitrogen-free extract (NFE) and total ash (TA) were analyzed (AOAC 2012) for quality evaluation. Dry matter was estimated by oven drying the samples at 105°C till a constant weight and crude protein percent was calculated by estimating nitrogen content by Micro-Kjeldahl method and multiplying with a factor 6.25. Ether extract (EE) was determined by solvent extraction with petroleum ether, boiling point 40-60°C, for 4-5 h. Total ash content was determined by incinerating the sample at 650°C for 6 h and crude fiber by acid digestion (1.25%) followed by alkali digestion (1.25%).

Pond demonstration

Nursery ponds were prepared by standard carp nursery management practices. Ponds were treated with mahua oil cake in recommended dose @2500 kg/ha/m. After three weeks interval, healthy carp spawns were stocked at six nos of ponds (each about 0.1 hectare) with stocking density of 50 lakhs per hectare. Fish in three number of ponds were fed with CIFA-Carp Starter and fish in three nos of ponds were fed with exiting feed (groundnut oil cake, rice bran mixed with minerals and vitamin mixture) following a completely randomized design. The feed was provided @ 300 gm/1 lakh spawn for 7 days followed by 600 gm/1 lakh spawn up to 15 days. The demonstration is being carried out as per the scheduled methodology. A group of fishes in each pond were batch weighed randomly once in every week to estimate the average weight and survival of fish in each pond (Fig 2). After completion of 2 weeks of experiment, samples of fish fry from both control and treatment ponds were taken to laboratory for nonspecific immune parameters.



Fig 1: A picture of carp nursery feed after preparation.

Non-specific immune parameters

Extract of carp fry for nonspecific immune parameters were prepared using the method of Swain *et al.* (2002).

Myeloperoxidase activity

The myeloperoxidase activity was assessed according to the method of Quade and Roth (1997). In brief, 15 µl of fish extract was diluted in 135 µl of Hank's balanced salt solution (Ca^{2+}, Mg^{2+} free) and further 50 µl of 20 mm of 3, 3′, 5, 5′ tetramethylbenzidine and 5 mm of hydrogen peroxide were added to the above mixture. Incubation was done for 2 min at room temperature. To stop the reaction 35 µl of 4M sulphuric acid was added and the optical density (OD) was read at 450 nm in the UV VIS Spectrophotometer, Thermo Spectronic, UK.

Lysozyme activity assay

A total of 130 µl of freshly prepared Lyophilized *Micrococcus lysodeikticus* (Sigma, USA) at a concentration of 0.6 mg/ml (in 0.02 M sodium citrate buffer) was added to a mixture containing 10 µl fish extract and 10 l of 0.02 M sodium citrate buffer (Ellis, 1990). After immediately adding bacterial solution, the initial OD was read at 450 nm. The OD of the samples was assessed at 450 nm after 1 hour of incubation at 24°C. Using a mixture of 20 µl working standard and 130 µl *M. lysodeikticus* solution, a standard curve was created.



Fig 2: Sampling of nursery carp at Sarakana and Subarnapur, Odisha.

Lysozyme activity was measured in units/ml, with one unit equaling a 0.001/min decrease in absorbance.

Bacterial agglutination activity

The bacterial agglutination test was performed using method of Swain *et al.* (2018) in "U" shaped microtitre plates. In a nutshell, 25 µl of fish extract was serially diluted twice and mixed with an equal volume of regular saline solution in each well (NSS). Every well received 25 µl of formalin-killed *Aeromonas hydrophila* suspension (10^7 cells/ml). The titre was measured as the reciprocal of the highest dilution of extract indicating total agglutination of the bacterial cells after overnight incubation at 37°C.

Haemagglutination activity

The haemagglutination activity was carried out as described by Blazer and Wolke (1984). A 25 µl extract was two fold serial diluted (inactivated at 45°C for 30 min) and mixed with equal volume of NSS in "U" shaped microtitre plates. Freshly prepared (25 µl) 1% New Zealand white rabbit red blood cell (RBC) suspension was then added to the wells. After 2 hr of incubation at room temperature, the titre was calculated as the reciprocal of the highest dilution of extract showing complete agglutination of RBCs.

Hemolytic activity

The haemolytic assay was carried out as described for HA titre (Blazer and Wolke, 1984). In this case, the microtitre plates were incubated at room temperature overnight. The titre was expressed as the reciprocal of the highest dilution of extract showing complete haemolysis of rabbit RBCs.

Table 1: Chemical characteristics of nursery feed.

Parameters	% on DM basis
Moisture	7.5
Crude protein (CP)	35.58
Crude fibre (CF)	4.62
Total ash	10.74
Ether extract (EE)	6.10
Nitrogen free extract (NFE)	42.96
Energy (kcal/gm)	4.00

Table 2: Growth and survival performance of *L. rohita* spawn fed with different diets.

Parameters	Control	Carp starter	P value	SEM
Duba, Ganjam				
2 weeks weight (g/fish)	0.06^a	0.12^b	0.0018	0.0139
Survival (%)	35^a	65^b	0.0002	6.7770
Bhadrak, Odisha				
2 weeks weight (g/fish)	0.04^a	0.08^b	0.0363	0.0106
Survival (%)	45^a	65^b	0.0028	4.6760
Gop, Puri, Odisha				
2 weeks weight (g/fish)	0.09^a	0.15^b	0.0499	0.0165
Survival (%)	40^a	60^b	0.0086	4.8440
Boisinga, Odisha				
2 weeks weight (g/fish)	0.05^a	0.10^b	0.0241	0.0128
Survival (%)	45^a	65^b	0.0049	4.7470
Sarakana, Odisha				
2 weeks weight (g/fish)	0.02^a	0.04^b	0.0257	0.0516
Survival (%)	30^a	45^b	0.0148	3.7310
Kalna, West Bengal				
2 weeks weight (g/fish)	0.05^a	0.09^b	0.0080	0.0097
Survival (%)	30^a	45^b	0.0407	4.0390
Purana, Khordha				
2 weeks weight (g/fish)	0.05^a	0.08^b	0.0213	0.0076
Survival (%)	30^a	50^b	0.0030	4.6830
Kapsi, Chhatisgarh				
2 weeks weight (g/fish)	0.08^a	0.16^b	0.0283	0.0208
Survival (%)	30^a	55^b	0.0136	6.1900
Tirga, Chhatisgarh				
2 weeks weight (g/fish)	0.07^a	0.10^b	0.0376	0.0133
Survival (%)	35^a	50^b	0.0101	3.658
NFDB, Bhubsaneswar				
2 weeks weight (g/fish)	0.25^a	0.30^b	0.0123	1.2320
Survival (%)	48^a	63^b	0.0023	3.4900

Means bearing the different superscripts differ significantly ($P<0.05$).

Statistical analysis

All the data of the experiment were statistically analysed by using statistical software (Prism, version 4.0, GraphPad Software, San Diego, CA, U.S.A.). Values were expressed as mean, SEM and p values of <0.05 were considered significant.

RESULTS AND DISCUSSION

Growth and survival of *L. rohita* spawn

The chemical characteristics of nursery feed (CIFA-Carp Starter) used in the experiment is presented in Table 1. The feed contained 35.58 percent protein, 6.10% fat and 4.62% crude fibre and size of feed particles varied from 0.4 to 0.5 mm. The chemical characteristics showed that it is a very good feed for nursery carp. The performance of nursery feed during 1st phase (spawn to fry) was successfully demonstrated in three states involving private farms of Odisha, West Bengal and Chhattisgarh and is presented in Table 2. The average survival percentage of fish fed on control feed during demonstration was 37 percentages (varied from 30 to 48%) while survival percentage in fish fed on nursery feed was 57 percentages (varied from 45 to 65%). Similarly, initial average weight of fish on control feed was 0.09 gm (varied from 0.04 to 0.25 gm) whereas average weight after feeding with CIFA-Carp Starter was 0.16 (varied from 0.08 to 0.3). The experiment showed that the survival percentage of fish was 20 percent higher and growth rate was 1.5-2.0 times more than existing method of feeding.

Survivability of nursery carp is an important parameters as generally, it is observed in the farmers practice that recovery of spawn to fry and fry to fingerling is around 25-30% and 40-50%, respectively. Though planktons are available in natural rearing process, it is not sufficient to meet nutritional requirement of all nursery carp. Availability of nutrition-rich carp starter along with natural planktons is prime factors for improving survival of carp spawn. Moreover, rich source of vitamins including vitamin A and essential fatty acids are responsible for improved performance in carp spawn. Another factor that is also responsible for survival of nursery carp is stocking density. Many farmers maintain stocking density of 20-30 lakhs of spawn per hectare to get higher survivability. However, when carp starter is used, higher survivability can be achieved when stocking density is maintained over 50 lakhs per hectare. However, in this experiment, stocking density of 50 lakhs of spawns per hectre was maintained.

Carp starter as used in this experiment contained many feed ingredients like maize, soybean meal, groundnut oil cake, fish meal and rice bran. Supplementation of many feed ingredients in the production of fish feed improve the keeping quality of feed compared to two or three feed ingredients as has been observed by Das *et al.* (2016). There may be balancing of amino acids like methionine, lysine and arginine in carp starter compared to feed of control group. It has been reported that the feed prepared by using more than one protein sources always resulted better growth in fish due to proper balancing of amino acids (Djissou *et al.,* 2016; Gaylord *et al.,* 2017). CIFA-Carp Starter contained oil which are rich source of vitamin A, D, E and K and are also highly essencial for improving survivability of nursery rohu. Increased palatability of feed as recorded in this experiment was because of oil. Again, higher feed utilization as reported for extruded feed may be responsible for improved growth of nursery carp (Das *et al.,* 2018). The basic concept of extrusion process is high temperature, short time, whereby the high temperature is a direct result of friction or pre-conditioning and steam injection or a combination of both (Levic, 2010) and it has become popular for aquafeed production (FMT, 2005). Extrusion technology can be used to produce a wide variety of food products at lower manufacturing rates, with continuous production, high throughputs and increased product quality, all while improving energy efficiency (Grasso, 2020).

Non-specific immunity parameters of fish fed with nursery feed

After completion of 2 weeks of experiment, samples of fish fry from both control and treatment ponds were collected and pooled for nonspecific immune parameters. The non-specific immune parameter of fish after 2 weeks of feeding with CIFA-Carp Starter is presented in Table 3. In this present study, significant increase in lysozyme, myeloperoxidase, haemagglutination, bacterial agglutination and haemolytic activity was recorded in treatment fish compared to control fish. Increased nonspecific immune parameters in treatment fish indicates improved immunity responses in fish fed with nursery feed compared to traditional feed used by farmers.

Non-specific immune parameters as reported in this experiment, showed that, lysozyme, myeloperoxidase, haem-agglutination and haemolytic activities are main indicators of non-specific immune functions (Swain *et al.,* 2007). Lysozyme is an important defense molecule of the

Table 3: Non-specific immune parameters of rohu fed with different diets.

Parameters	Control	Treatment	P value	SEM
Bacterial agglutination (Log 2)	2.20^{a}	2.45^{b}	0.0293	0.0652
Lysozyme (U/ml)	71.66^{a}	75.82^{b}	<0.0001	0.9316
Myeloperoxidase (OD)	0.253^{a}	0.298^{b}	0.0333	0.0694
Haem-agglutination (log 2)	2.033^{a}	2.330^{b}	0.0010	0. 0681
Haemolytic activity (log 2)	2.783^{a}	3.050^{b}	0.0072	0.0641

Means bearing the different superscripts differ significantly (P<0.05).

innate immune system, which is important in mediating protection against microbial invasion (Saurabh and Sahoo, 2008). Fish lysozyme is responsible for defense, especially in the first line of defense mechanisms (Lie *et al.,* 1989). Increased lysozyme activity indicates improved immunity responses in fish (Panigrahi *et al.,* 2004; Kim and Austin, 2006). Similarly, myeloperoxidase (MPO) is a key element of the innate immune system and is released primarily by neutrophils to provide defense against invading pathogens. So, it is a measure of neutrophil antimicrobial activity. Higher level of myeloperoxidase helps in the destruction and elimination of invading pathogens from the host body (Yano, 1992; Dalmo *et al.,* 1997) and signifies its well developed immune status. Invertebrates' innate immunity is thought to be supported by natural HLYs. In the sera of rohu, there was also significant haemolytic activity (Sahoo *et al.,* 2005). The strong lytic activity of the serum can play an important role in natural resistance to diseases. Major carps are usually cultured in a warm climate, which is conducive to the growth of many pathogens. Natural factors found in normal, healthy fish, such as lysins, agglutinins and precipitins, can help to overcome diseases much faster than the time it takes to develop specific immunity. Here, increased immunity shows due to high quality nutriets present in the feed.

CONCLUSION

The current study indicated that, nursery rohu showed higher growth performance, survivability and immune response when fed with CIFA-Carp Starter compared to nursery rohu fed on existing/traditional feed. So CIFA-Carp Starter may be popularised among farmers and entrepreneurs for improving the growth and survivability of nursery carp.

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Conflict of interest

All authors declared that there is no conflict of interest.

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