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Growth and Non-specific Immunity Parameters of Rohu Fed with a Floating Feed Prepared from Locally Available Ingredients (CIFA-Carp Grower)

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ABSTRACT

A Grower feed carp (CIFA-Carp Grower) was formulated and prepared using locally available ingredients, minerals (with nano-selenium and nano-zinc) and vitamins mixture through extrusion technology and it's performance was compared with an existing feed used by farmers of eastern region. The developed feed contains 28 percent crude protein (CP) and 4 percent crude fat. The feed has been commercialized, IP protected and reached to aquaculture farmers covering about 10-12 states of India. As per the scheduled methodology of demonstration, rohu fingerlings (average 105 g) were stocked randomly into triplicate ponds for both control and treatment following a completely randomised design and stocking density of 7500/hectre was maintained for each pond. The fish of control ponds were fed with a mixture of groundnut oil cake (GNOC) and rice bran (50:50) with minerals and vitamins mixture where as fish of treatment ponds were fed with a grow out feed developed by ICAR-CIFA(CIFA-Carp Grower). During the experimental period of 10 months, growth, survivability, feed conversion ratio (FCR) and non-specific immunity parameters of rohu fed with CIFA-Carp Grower were recorded and compared with rohu fed with an existing feed used by farmers of this region. The average size of fish after feeding with CIFA- Carp Grower were 1088 gm at 10 months of feeding with average feed conversion ratio (FCR) of 1.290 and it was better than existing/traditional method of feeding. The results of the demonstration showed that weight gain, survivability, feed convetrsion ratio (FCR) and nonspecific immunity parameters of grow out rohu fed with CIFA-Carp Grower was better as compared to existing feed practiced by many farmers in this region. So, CIFA- Carp Grower may be popularised among farmers, entrepreneurs and researchers for aquaculture development of the whole country.

Key words: CIFA-Carp Grower, Immunity, Floating feed, Growth performance, Rohu

INTRODUCTION

The extrusion process improves nutrient digestibility, palatability, pellet durability, water stability and pellet storage life (Barrows and Hardy, 2000). It also increases *in vivo* digestibility of dry matter and energy of feed in rainbow trout *Oncorhynchus mykiss* (Cheng and Hardy, 2003). Good quality floating feed on locally available ingredients could be produced through extrusion technology by maintaining extrusion temperature of 130°C and moisture of 20 percentages (Das *et al.,* 2018). It has been experimented that, the quality of til oil cake based floating feed for *Labeo rohita* are superior as that of soybean meal based floating feed and that is also economical for the farmers (Das *et al.,* 2016).

ICAR-CIFA, Bhubaneswar has developed a grower feed for carp (CIFA-Carp Grower) out of locally available ingredients through extrusion technology. This is a floating feed for carp which ensures better growth, improved FCR, higher palatability to fish, disease resistance and production of tasty fish. This is suitable for carp grower to enhance their production and profitability. It contains 28 percent crude protein, 4% fat, and nano zinc and nano selenium. This feed is suitable for carp growers to enhance their production and profitability. This feed has been IP protected and commercialized for increasing aquaculture production and productivity in the country and have been reached to aquaculture farmers covering about 10-12 states of India. However, farmers still use traditional method of practices and a survey revealed that 65.4 percent of Indian major carp farmers in Andhra Pradesh used mash feed as their sole feed source(Ramakrishna *et al.,* 2013). In this experiment,

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pelleted floating feed was produced through extrusion technology and performance evaluation was done in terms of growth, feed efficiency and immunological parameters by comparing with an existing mash feed used by farmers of this region.

MATERIALS AND METHODS

Fish feed was formulated using locally available feed ingredients *i.e.* maize, soyabean meal, til oil cake (TOC), groundnut oil cake (GOC), mahua oil cake, de-oiled rice bran (DORB) and minerals (with nano selenium and nano-zinc) and vitamin mixture as per requirement. The extruded floating feeds were produced in the feed mill facilities of ICAR-CIFA, Bhubaneswar. The ingredients were pulverized, mixed, extruded and then dried (Fig 1). Experimental feed were then analyzed for physical and chemical characteristics to ascertain the quality of the feed (Tables 1 and 2).

Fig 1. CIFA-Carp Grower developed by ICAR-CIFA

CIFA-Carp Grower after production was transported and demonstration was done in grow out ponds of KVK, Ranital, Bhadrak. Odisha. The demonstration was carried out as per scheduled methodology. Rohu fingerlings (average 105 g) were stocked randomly into triplicate ponds for both control

Table 1. Physical characteristics of CIFA-Carp Grower

Parameters	%
Floating percentage	100
Sinking percentage	0
Apparent Density (gram/ cubic cm)	0.96

Table 2. Chemical characteristics of CIFA-Carp Grower

Parameters	% % on DM basis
Crude protein (CP)	28.00
Crude fibre (CF)	5.42
Ether extract (EE)	4.00
Nitrogen free extract (NFE)	52.00
Energy (kcal/g)	3.51

and treatment following a completely randomised design and stocking density of 7500/hectre was maintained for each pond. The fertilizer was applied as per normal schedule. The fish of control ponds were fed with mash feed used by farmers (ground nut oil cake and rice bran mixture, 50:50 supplemented with minerals and vitamin mixture) whereas fish of treatment ponds were fed with CIFA-Carp Grower developed by ICAR-CIFA. The experiment was continued for 10 months period (Fig 2).

Feed offered was recorded on regular basis and then calculated at the end of the experiment to record feed intake. A group of fifteen numbers of fishes in each pond were batch weighed randomly once in every month till completion of experiment to estimate the average weight and biomass of fish in each pond. The survivability was checked after harvesting the fish after experiment. The water quality parameters were checked regularly and found to be optimum. Blood was collected from tail vein of five fish samples in each pond n=5 for control and treatment to study the immunological parameters.

Non-specific immune parameters like lysozyme, myeloperoxidase, bacterial agglutination, haemagglutination and haemolytic activity were estimated in rohu fed on existing and developed fish feed (CIFA-Carp Grower). For lysozyme assay, a 130 ìl 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 ìl fish serum samples and 10 ìl 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 measured at 450 nm

Fig 2. Sampling of fish in Bhadrak, Odisha during demonstration of CIFA-Carp Grower

after 1 hour of incubation at 24°C. Using a mixture of 20 ìl working standard and 130 ìl *M. lysodeikticus* solution, a standard curve was created. Lysozyme activity was measured in units/ml, with one unit equaling a 0.001/min decrease in absorbance. Myeloperoxidase activity was performed as described by Quade and Roth (1997). In brief, fish serum (15 ìl) was diluted in 135 ìl of Hank's balanced salt solution (Ca^{2+}, Mg^{2+} free) and further, 50 ìl of 20 mM of 3, 32, 5, 52 tetramethylbenzidine and 5 mM of hydrogen peroxide were added in the same well. The mixture was incubated for 2 min at room temperature. The final reaction was stopped by the addition of 4 M sulphuric acid and the optical density (OD) was read at 450 nm using the UV VIS Spectrophotometer, Thermo Spectronic, UK. Bacterial agglutination activity was carried out in a "U" shaped microtitre plate as described by Swain *et al.* (2018). In brief, two-fold serial dilution of 25 ìl serum sample was done in the titre plate with an equal volume of normal saline solution (NSS) in each well. A 25 ìl of formalin killed *Aeromonas hydrophila* (10^7 cells/ml) suspension was then added to each of the wells. After an overnight incubation at 37°C, the titre was calculated as the reciprocal of the highest dilution of serum showing complete agglutination of the bacterial cells. The haemagglutination activity was carried out according to procedures of Blazer and Wolke's (1984). A two-fold serially diluted 25 ìl fish serum sample was inactivated at 45°C for 30 minutes and combined with an equivalent amount of NSS in a "U" shaped microtitre plate. The wells were then filled with a freshly manufactured (25 ìl) 1 percent New Zealand white rabbit red blood cell (RBC) suspension. The titre was estimated as the reciprocal of the greatest dilution of serum demonstrating complete agglutination of RBCs after 2 hours of incubation at room temperature. The hemolytic activity was carried out as described for HA titre (Blazer and Wolke, 1984). But here, plates were kept in room temperature for overnight and the HA titre was presented as the reciprocal of the highest dilution of serum showing complete hemolysis of rabbit RBCs.

The physico-chemical parameters of the water were routinely monitored. The temperature, pH, total alkalinity and total hardness of water were 25-30 ºC, 7.5-8.5, 100-140 ppm, and 100-130 ppm, respectively in both control nad treatment ponds. All the data of the experiment were statistically analyzed by using statistical software (Prism, version 4.0, Graph Pad Software, San Diego, CA, U.S.A). Values were expressed as standard error of the mean (SEM) and P values of <0.05 were considered significant.

RESULTS AND DISCUSSION

The Extrusion technology is the primary technique now-a-days used in aquatic feed industry for fish feed production to maintain high physical and nutritional quality of the feed (Hilton *et al.,* 1981). It is a high-temperature-short-time heating process that minimizes the degradation of food nutrients while improving the digestibility of protein and starches. It has

been reported that, aquatic animals cannot digest starch effectively resulting in excessive excrement which causes physiological problems such as excessive gas, bloating diarrhea and these apart from affecting the growth of the fish also lead to water pollution. Starch in the feed is gelatinized and is effectively utilised by this technology for production of floating fish feed. Floating fish feed comes with a host of advantages in terms of digestion, growth, water protection, zero water pollution, optimized labor usage and zero wastage of raw materials (Hashimoto *et al.,* 2003). The farmer can directly observe the feeding requirements and adjust feeding rates accordingly. It has been experimented that, floating feed could be prepared successfully by inclusion of locally available ingredients. However, maintenance of appropriate temperature, pressure and moisture during extrusion is the key step in production of quality fish feed from locally available feed resources in spite of the fact that, it varies on the type of ingredients and extruder used for floating feed production. 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 aqua feed production (FMT, 2005). The feed is floated for 2 to 3 hours which indirectly reduced the wastage of feed as compared to mash or sinking feed. Higher feed utilization as reported for extruded feed was responsible for improved growth performance in growing carp (Das *et al.,* 2018). CIFA-Carp Grower was produced through extrusion technology as sufficient starch was there for gelatinization of starch.

The growth performance of rohu fed with existing feed and CIFA-Carp Grower are presented in table 3. The weight gain of rohu fed with CIFA-carp Grower (treatment) was significantly higher ($P<0.05$) as compared to rohu fed with existing feed (control). The feed conversion ratio (FCR) of the fishes was calculated from feed intake and weight gained (g/day) during the experimental period, and the result indicated the superior FCR ($P<0.05$) of CIFA-Carp Grower as compared to existing mash feed. The survival percentage of fish fed with CIFA-Carp grower was higher ($P<0.05$) compared to fish fed with existing mash feed. The yield and net return was higher by feeding with CIFA-Carp Grower compared to other feed indicating higher profits to farmers adopting the technology. Use of locally available ingredients provides a variety of nutrients for fish in addition to growth promoting effects of some local ingredients. Local ingredients are not only economical but also suitable in Indian agro climatic conditions. Many locally available ingredients were included for production of CIFA-Carp Grower along with conventional ingredients (Das *et al.,* 2016 ;Das *et al.,* 2021). The feed was also supplemented with mineral mixture containing nano selenium and nano zinc which was responsible for higher growth performance, immunity and muscle quality (Swain *et al.,* 2019). There may be balancing of amino

Table 3. Growth and production performance of rohu fed with existing feed (control) and CIFA-Carp Grower (treatment)

Parameters	Control	Treatment	SEM	P value
Initial weight (g/fish)	105	105	5.858	>0.9999
Final weight (g/fish)	890[a]	1088[b]	43.49	0.0061
Weight gain (g/fish)	785[a]	983[b]	38.1	0.0001
Feed intake (g/fish)	2261[a]	1268[b]	191.2	0.0001
FCR	2.879[b]	1.290[a]	0.303	0.0001
Survival (%)	78[a]	82[b]	0.982	0.0255
Yield(q/ha)	52.10[a]	67.00[b]	2.984	0.0004
Net Return (₹/ha)	2,74000[a]	3,70000[b]	21733	0.0002

Means with different superscripts in a row differ significantly ($P<0.05$)

Table 4. Non-specific immunity parameters of fish fed with existing feed (control) and CIFA-Carp Grower

Parameters	Control	Treatment	P value	SEM
Bacterial agglutination(log 2)	2.56[a]	3.10[b]	0.0012	0.1240
Lysozyme (ì g/ml)	9.36[a]	10.30[b]	0.0003	0.1966
Myeloperoxidase (OD)	0.656[a]	0.950[b]	0.0039	0.0694
Haem-agglutination (log 2)	2.70[a]	3.12[b]	0.0075	0.1012
Haemolytic activity (log 2)	2.58[a]	2.81[b]	<0.001	0.0497

Means with different superscripts in a row differ significantly (P<0.01)

acids like methionine, lysine and arginine in carp grower compared to feed of control group. It has also been reported that the feed prepared by using more than one protein sources always resulted in better growth of fish due to proper balancing of amino acids (Djissou *et al.,* 2016, Gaylord *et al.,* 2017).

Non-specific immunity parameters of fish like bacterial agglutination, lysozyme, myeloperoxidase, haem-agglutination and haemolytic activity fed on existing feed (control) and CIFA-Carp Grower (treatment) is presented in Table 4. Fish fed on CIFA-Carp Grower had significantly higher levels of lysozyme, myeloperoxidase, haemaglutination, bacterial agglutination, and haemolytic activity than fish fed on existing feed. Increased non-specific immune measures in treatment group of fish indicate that fish fed on CIFA-Carp Grower had higher immunity responses than fish fed on existing feed used by farmers. In this experiment, fish fed on CIFA-Carp Grower had significantly higher levels of lysozyme, myeloperoxidase, haemaglutination, bacterial agglutination, and haemolytic activity than fish fed on existing feed. Increased immune parameters like lysozyme, myeloperoxidase and hem-agglutination are indication of improved immunity responses in fish (Panigrahi *et al.,* 2004; Kim and Austin, 2006). Fish use lysozyme as one of their main defence mechanisms against pathogenic infection (Feng *et al.,* 2011). Myeloperoxidase activity is used to assess neutrophil antimicrobial activity. Increased non-specific immune parameters in fish fed with CIFA-Carp Grower proved that, CIFA-Carp Grower resulted improved disease resistance in fish compared to existing feed used by farmers.

CONCLUSIONS

The results of the demonstration showed that, the weight gain, survivability, feed conversion ratio (FCR) and immunity parameters of grow out rohu fed with CIFA-Carp Grower was higher compared to existing feed practiced by many farmers of eastern region. This is suitable for carp grower to enhance their production and profitability. This feed may be popularized for aquaculture development of the country.

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