



Evaluation of sesame oil cake based floating feed on growth performance and non-specific immune response of *Labeo rohita* (Hamilton, 1822)

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ABSTRACT

A sesame (or til) oil cake based floating feed (TOCF) was formulated and prepared for *Labeo rohita* (Hamilton, 1822) using maize, soybean meal, til oil cake (TOC), groundnut oil cake (GOC) and de-oiled rice bran (DORB) with supplementation of minerals and vitamin mixture and its performance was compared with a commercial floating feed (CFF) purchased from local market. Both the experimental and commercial floating feed contained 28% crude protein and the floating percentage was 100. The weight and feed conversion ratio (FCR) of fish fed on TOCF was significantly higher ($p < 0.01$) than fish fed on commercial floating feed. Non-specific immune parameters like respiratory burst activity, myeloperoxidase, lysozyme, bacterial agglutination, haemagglutination and haemolysin activity of fish fed on TOCF did not show any significant difference ($p > 0.01$). However, the serum enzyme activities like lactate dehydrogenase and alkaline phosphatase of fish fed on TOCF had lower values ($p < 0.01$) compared to fish fed on CFF indicating anti-stress and anti-oxidant effect in developed feed. Sensory evaluation showed that, the fish from TOCF exhibited better ($p < 0.01$) colour, flavour, sweetness and sourness than fish fed on CFF. Results of this experiment showed that, fish fed TOCF had higher growth performance and sensory score compared to fish fed on CFF whereas there was no change in non-specific immune response of fish fed on two types of feeds.

Keywords: Feed quality, Growth performance, Immunity, Rohu, Til oil cake

Introduction

Feed constitutes 50 to 60% of the total expenditure in aquaculture production. Among plant protein sources, soybean meal and groundnut oil cake have been extensively used for the production of compounded aqua feeds (Barman and Karim, 2007; Manomaitis, 2009) which ultimately increases the cost of production. Hence, inclusion of locally available feed ingredients can reduce the cost of fish feed (Oyetayo, 1985; Begum *et al.*, 2008; Mzengereza *et al.*, 2014). Sesame (til, *Sesamum indicum*) oil cake is a good source of plant protein for Indian major carps (IMC) and it is available in plenty in different states of India such as Uttar Pradesh, Andhra Pradesh, Maharashtra, Gujarat, Tamil Nadu and Odisha. It contains about 35% crude protein and is good source of arginine, methionine and calcium (Ramachandran *et al.*, 2007).

In the present day aquaculture, extrusion technology is gaining importance for production of region based floating feed for IMCs. 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 containing til oil

cake could be produced through extrusion technology by maintaining extrusion temperature of 130°C and moisture of 20% (Das *et al.*, 2012). It has been experimented that, the quality of til oil cake based floating feed for *L. rohita* are superior than that of soybean meal based floating feed and also economical for the farmers (Das *et al.*, 2016).

In this experiment, til oil cake based floating feed was produced through extrusion technology and performance evaluation was done in terms of growth, feed efficiency, immunological parameters and organoleptic evaluation by comparing with commercial feed for IMC available in the market.

Materials and methods

Formulation and production of floating feed for rohu

Fish feed was formulated using locally available feed ingredients *i.e.* maize, soybean meal, til oil cake (TOC), groundnut oil cake (GOC), de-oiled rice bran (DORB), minerals (with nano-selenium and nano-zinc) and vitamin mixture as per requirement. The feeds were produced in the feed mill facilities of ICAR-Central Institute of Freshwater Aquaculture (ICAR-CIFA), Bhubaneswar, India with extrusion temperatures of 130°C and moisture of 20% maintaining constant pressure

(10 kg cm²) employing a twin screw extruder (Jinan Saibainuo Machinery, co. Ltd., China) and 3 mm die was used for production of experimental feed. The feed produced was floating feed as sufficient maize (30%) was there during extrusion for proper gelatinisation of starch. Similarly, til oil cake (20%) was used for maintaining proper consistency and floating percentage of feed. Commercial floating feed purchased from the local market was used as control diet and compared its performance with the til oil cake based floating feed prepared. Both experimental and commercial feed were then analysed for physical and chemical characteristics to ascertain the quality of the feed.

Analysis of feed

The physical parameters *i.e.* floating and sinking percentage were calculated from floating and sinking record of floating feed in glass aquaria (30 x 30 x 30 cm). Apparent densities of the floating feeds were calculated by measuring volume and mass by a digital screw gauge and weighing balance respectively. 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) of til oil cake (TOC), groundnut oil cake (GOC) and floating feed were analysed for quality evaluation (AOAC, 2012). 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 10-12 h. Total ash content was determined by incinerating the sample at 650°C for 6 h and crude fibre by acid digestion (1.25%) followed by alkali digestion (1.25%). Chemical analysis of fish like moisture, crude protein, ether extract (EE), ash and carbohydrate were analysed by same procedure.

Pond preparation

Six earthen ponds of 0.05 ha water-spread area each were used for rearing the experimental fish. Before stocking the fish, all the ponds were dried and bleaching powder (30% active chlorine) was applied at 300 kg ha⁻¹ to eradicate the unwanted predatory and weed fishes. Lime was applied at 1000 kg ha⁻¹. After 7 days of lime application, water was filled up to 1.0 m depth and the ponds were fertilised with raw cowdung, urea and bleaching powder at 3 t ha⁻¹, 40 kg ha⁻¹ and 60 kg ha⁻¹ respectively (ICAR, 2009).

Growth experiment

Twelve hundred rohu fingerlings were procured from ICAR-CIFA farm, Bhubaneswar, India and were acclimatised in a pond for seven days. After

acclimatisation, a group of 200 fishes (average weight 365.7±8.5 g) were stocked randomly into triplicate ponds (each 130 ft x 60 ft) for both control and treatment groups following a completely randomised design. Experimental and commercial floating feeds of 3 mm size were prepared and fed *ad libitum* to fish of treatment and control groups respectively, once daily. A group of fifteen numbers of fishes in each pond were batch weighed randomly once in every month to estimate the average weight and biomass of fish in each pond.

At the end of the experiment, fishes were batch weighed in each pond to know the final weight. Blood was collected from tail vein of five fish samples in each pond (n=5 for control and treatment) to study the immunological parameters. Similarly, five fishes from each pond (n=5 for control and treatment) were taken for chemical and sensory evaluation. Experiment was continued for a period of six months.

Non-specific immune response

Myeloperoxidase activity

For determination of myeloperoxidase activity, 15 µl of fish serum was diluted in 135 µl of Hank's balanced salt solution (Ca²⁺, Mg²⁺ free) and 50 µl of 20 mM of 3, 3', 5, 5'- tetra methyl benzidine and 5 mM of hydrogen peroxide were added. The mixture was incubated for a period of 2 min at room temperature. After incubation period, the reaction was stopped by the addition of 4 M sulphuric acid. Optical density was then read at 450 nm using a UV-VIS spectrophotometer (Thermo Spectronic, UK) (Quade and Roth, 1997).

Respiratory burst activity

The reduction of nitro blue tetrazolium (NBT) by intracellular superoxide radicals (respiratory burst activity) was measured as per Anderson and Siwicki (1994). Briefly, 50 µl of heparinised blood from each experimental group of fish was mixed with 50 µl of 0.2% NBT (Sigma, USA) solution. The mixture was incubated for 30 min at 25°C and 50 µl of the mixture was added to 1 ml of N, N di ethymethyl formamide (Qualigens, India) after incubation. The entire mixture was then centrifuged at 6000 g for 5 min. The optical density of the supernatant was read at 540 nm using UV- VIS spectrophotometer (Thermo Spectronic, UK)

Lysozyme activity assay

A lysozyme assay utilising lyophilised *Micrococcus lysodeikticus* (Sigma, USA) was carried out as described by Ellis (1990). Freshly prepared *M. lysodeikticus* solution (130 µl) at a concentration of 0.6 mg ml⁻¹ (in 0.02 M sodium citrate buffer) was added to a mixture containing

10 µl fish serum samples and 10 µl of 0.02 M sodium citrate buffer. Once the bacterial solution was added, the initial OD was read at 450 nm immediately. OD of the samples was again read at 450 nm after incubation of samples at 24°C for 1 h. A standard curve was prepared using a mixture of 20 µl working standard and 130 µl of *M. lysodeikticus* solution. Lysozyme activity was expressed in units per ml, where one unit is defined as the decrease in absorbance of 0.001 min⁻¹.

Bacterial agglutination activity

Bacterial agglutination test was carried out in 'U' shaped micro titre plates. Fish serum samples (25 µl each) were two fold serially diluted with an equal volume of normal saline solution (NSS) in each well and 25 µl of formalin-killed *A. hydrophila* (10⁷ cells ml⁻¹) suspension was added next to each of the wells. The plates were then kept for incubation overnight at 37°C. The titre was calculated as the reciprocal of the highest dilution of serum showing complete agglutination of the bacterial cells.

Hemagglutination activity

The hemagglutination activity of the fish serum samples was analysed as described by Blazer and Wolke (1984). The assay was done in 'U' shaped microtitre plates. Two-fold serial dilution of 25 µl fish serum samples (inactivated at 45°C for 30 min) was carried out using equal volume of NSS and 25 µl of freshly prepared 1% New Zealand white rabbit red blood cell (RBC) suspension was added to the wells. The plates were incubated at room temperature (28-30°C) for 2 h or at 4°C overnight, in case agglutination was not observed within 2 h. The titre was calculated as the reciprocal of the highest dilution of serum showing complete agglutination of RBC.

Haemolytic activity

The hemolytic assay of serum samples was carried out in a manner similar to one as described for hemagglutination titre (Blazer and Wolke, 1984). Fresh sera of fish were collected from all experimental ponds and were subjected to analysis. In this case, the plates were incubated at room temperature overnight. The titre was expressed as the reciprocal of the highest dilution of serum showing complete hemolysis of rabbit RBCs.

Serum enzyme assays

Acetylcholine esterase activity

The acetylcholine esterase activities in the fish sera were assessed using the Acetylcholine esterase activity kit (Sigma- Aldrich Chemie, USA) as per the manufacturer's instructions. This assay is an optimised version of the Ellman method in which thiocholine, produced by AChE,

reacts with 5, 5'- dithiobis (2-nitrobenzoic acid) to form a colorimetric (412 nm) product, proportional to the AChE activity present.

Alkaline phosphatase activity

The alkaline phosphatase activities in the fish sera were assessed using the Alkaline Phosphatase activity kit (Bio Vision, USA) following the manufacturer's instructions. This kit uses p-nitrophenyl phosphate (pNPP) as a phosphatase substrate, which turns yellow ($\lambda_{\text{max}} = 405 \text{ nm}$) when dephosphorylated by ALP.

Super oxide dismutase (SOD) activity

The SOD activities in the fish sera samples were assessed using the SOD assay kit (Sigma-Aldrich Chemie, USA). The percentage of inhibition, *i.e.*, the SOD activity was computed using the instructions and formula mentioned in the assay kit. The SOD Assay kit uses Dojindo's highly water soluble tetrazolium salt which produces a water soluble formazan dye upon reduction with a superoxide anion.

Lactate dehydrogenase activity

The lactate dehydrogenase activities in the fish serum samples were analysed using the lactate dehydrogenase activity assay kit (Sigma-Aldrich Chemie, USA). In this kit, LDH reduces NAD to NADH, which is specifically detected by colourimetric (450 nm) assay.

Sensory and chemical evaluation

Quality of fish was evaluated by a 20 member sensory panel comprising experienced scientists and research scholars, on a 9 point hedonic scale after cooking the fish as per the method of Keeton (1983); where 9 = excellent and 1=extremely poor. The panelists were explained about the nature of experiments without disclosing the identity of samples and were asked to rate their preference on 9 point scale on sensory evaluation proforma for different traits. Samples were warmed in a microwave oven for 1 min and served to the panelists. Water was provided to rinse mouth between the samples. The panelists judged the samples for general appearance, colour, flavour, texture, juiciness, sweetness, sourness, saltiness and overall acceptability. Chemical analysis of fish like moisture, crude protein, ether extract (EE), ash and carbohydrate were analysed following standard procedures (AOAC 2012).

Statistical analysis

The data of the experiment were statistically analysed using GraphPad Prism software (GraphPad Prism. ver. 5. San Diego California USA).

Results

Physical and chemical characteristics of floating feed

The physical and chemical characteristics of til oil cake based floating feed (TOCF) and commercial floating feed (CFF) used in the experiment is presented in Table 1. Both feeds contained 28% protein. The size of two types of floating feed was also same. The comparative analysis showed that, there was little difference in chemical composition and physical characteristics of both floating feeds.

Til oil cake used for floating feed production (TOCF) contained 35% crude protein, 6.10% ether extract, 4.4% crude fibre, 11.60% total ash and 42.50% nitrogen free

Table 1. Physical characteristics and chemical composition (% on DM basis) of commercial floating feed (CFF) and til oil cake based floating feed (TOCF)

Parameters	CFF	TOCF
Floating percentage	100	100
Apparent density (g cm ⁻³)	0.86	0.96
Size of the pellets (mm)	3	3
Dry matter (DM)	93.5	94.0
Crude protein (CP)	28.0	28.0
Crude fibre (CF)	7.96	5.42
Ether extract (EE)	2.80	3.00

extract on DM basis. Similarly, GOC contained 42% crude protein, 6.5% ether extract, 5.5% crude fibre, 6.5% total ash and 39% nitrogen free extract. However, TOC is more economical for aqua feed production, as cost of GOC is almost double.

Growth performance

Growth performance and feed utilisation of rohu fed TOCF and CFF is presented in Table 2. The weight gain of fish fed TOCF was significantly higher ($p < 0.01$) than CFF and is also indicated graphically in Fig. 1. Similarly, feed conversion ratio (FCR) of fish fed TOCF was significantly superior ($p < 0.01$) compared to CFF. The results showed that, TOCF had better growth performance as compared to CFF.

Non-specific immune response

Studies on non-specific immune response and serum enzyme of rohu fed on TOCF and CFF is presented in Table 3. The parameters like respiratory burst, lysozyme, bacterial agglutination, haemagglutination, myeloperoxidase and haemolysin did not show any significant difference ($p > 0.01$). The results indicated that, TOCF exhibited similar immune response in fish compared to CFF used in this experiment.

Table 2. Growth performance of rohu fed commercial floating feed (CFF) and til oil cake based floating feed (TOCF)

Parameters	CFF	TOCF	SEM	p value
Initial weight (g fish ⁻¹)	365.7±6.17	365.7±11.55	5.858	>0.9999
Final weight (g fish ⁻¹)	736.3±6.64	825.3 ^b ±1.45	20.13	0.0002
Weight gain (g fish ⁻¹)	370.7±12.77	459.7 ^b ±12.99	0.2956	0.0081
Feed intake (g fish ⁻¹)	781.0 ^b ±15.63	688.0 ^a ±9.86	22.38	0.0073
Feed conversion ratio	2.11 ^b ±0.072	1.70 ^a ±0.048	0.0993	0.0093

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

Table 3. Non-specific immune response and serum enzyme of *L. rohita* fed on commercial floating feed (CFF) and til oil cake based floating feed (TOCF)

Parameters	CFF	TOCF	SEM	p value
Immune response				
Myeloperoxidase*	0.1285±0.026	0.1173±0.003	0.0128	0.6747
Respiratory burst*	0.3106±0.025	0.2876±0.017	0.0160	0.0986
Lysozyme (U ml ⁻¹)	57.4±2.984	54.87±0.559	1.43	0.3918
Bacterial agglutination ¹	4.8±0.467	4.8±0.388	0.2956	>0.9999
Haemagglutination ¹	3.7±0.213	3.8±0.133	0.123	0.6958
Haemolysis ¹	5.9±0.458	5.6±0.305	0.2702	0.5926
Serum enzyme assay				
Acetyl choline esterase (U l ⁻¹)	465±47.48	460.2±54.0	32.17	0.9505
Alkaline phosphatase (U ml ⁻¹)	0.002933 ^b ±0.0002	0.001633 ^a ±0.0001	0.00036	0.0057
Superoxide dismutase (%)	152.3 ^b ±4.2	150.3±1.35	18.99	0.4505
Lactate dehydrogenase (mU ml ⁻¹)	350 ^b ±43.3	230.9 ^a ±2.64	52.69	0.0072

*Absorbance at 450 nm; ¹Titer value in log₂

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

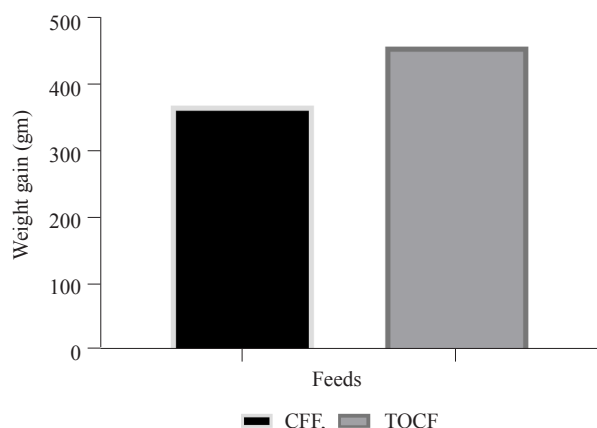


Fig. 1. Weight gain in *L. rohita* fed commercial floating feed (CFF) and til oil cake based floating feed (TOCF)

Sensory and chemical evaluation

The results of sensory evaluation are presented in Table 4. Chemical composition of rohu fed on CFF and TOCF is presented in Table 5. The results showed that, the fish fed TOCF exhibited better result in parameters like colour, flavour, sweetness and sourness compared to CFF. Similarly, chemical evaluation of fish showed non-significant difference between CFF and TOCF.

Discussion

Extrusion technology is being used in the feed industry for almost one century (Hardy and Barrows, 2000) and it has become popular for aqua feed

production (FMT, 2005) as extruded pelleted feed reduces feed wastage and improves the overall growth performance of fish. Incorporation of locally available cheap ingredients like TOC along with other ingredients produces cost-effective floating feed for carp. It has been experimented that, floating feed prepared by replacement of soybean meal by TOC are superior to floating feed prepared by inclusion of only soybean meal (Das *et al.*, 2016).

In this experiment, til oil cake based floating feeds were prepared and compared with a commercial floating feed. Both the feeds exhibited 100% floating indicating proper gelatinisation of starch. The 100% floating percentage was also found in both feeds even after 3 h which is in agreement with earlier findings of other researchers (Sorensen *et al.*, 2005; Barrows *et al.*, 2007; De Cruz *et al.*, 2015). Other physical parameters like apparent density (g cm^{-3}) was comparable between the two types of feeds used in this experiment.

Chemical composition like DM, CF and EE did not change significantly in both TOCF and CFF. However, growth performance indicated that, the weight gain of fish fed TOCF was higher ($p < 0.01$) than commercial floating feed. This might be due to better utilisation of til oil cake based floating feed compared to commercial floating feed. It has been reported that the feed prepared using more than one protein sources always resulted in better growth in fish due to proper balancing of amino acids (Djissou *et al.*, 2016; Gaylord *et al.*, 2017). In addition, the availability of more methionine and lysine contents in TOCF (Das *et al.*,

Table 4. Organoleptic evaluation of *L. rohita* fed on commercial floating feed (CFF) and til oil cake based floating feed (TOCF) on 9 point hedonic scale

Parameters	CFF	TOCF	SEM	p Value
Colour	6.156 ^a ±0.4075	8 ^b ±0.2887	0.2962	0.0009
Flavour	6.094 ^a ±0.443	7.75 ^b ±0.3594	0.3176	0.0069
Texture	6.594 ^a ±0.4454	7.938 ^b ±0.3091	0.2927	0.0190
Juiciness	6.313±0.4254	8.000±0.2887	0.2948	0.1444
Sweetness	5.719 ^a ±0.2737	7.813 ^b ±0.2772	0.2685	<0.0001
Sourness	5.094 ^a ±0.3298	6.938 ^b ±0.4327	0.3147	0.0020
Saltiness	6.444±0.3451	7.556±0.3256	0.252	0.252
Overall acceptability	6.406 ^a ±0.4164	7.781±0.3505	0.2948	0.0170

Means with different superscripts in a row differ significantly ($p < 0.01$).

Table 5. Chemical composition of *L. rohita* fed on commercial floating feed (CFF) and til oil cake based floating feed (TOCF)

Parameters	CFF	TOCF	SEM	P value
Moisture	75.45±0.5508	76.21±0.5204	0.3832	0.3506
Crude protein	17.29±0.3464	17.70±0.4041	0.2551	0.4841
Ether extract	2.49±0.0577	2.11±0.1367	0.1049	0.0705
Total ash	1.98±0.1155	2.00±0.2021	0.1042	0.9356
Total carbohydrate	2.79±0.2627	1.98±0.2646	0.2462	0.0956

2016) as compared to control feed might have resulted in better growth performance in fish.

Innate immunity involves macrophages, granulocytes and natural cytotoxic cells. Phagocytosis of macrophages and granulocytes is an important line of defense against invading microorganisms and foreign bodies (Secombes, 1996). In this study, no significant difference was recorded on non-specific immune response between CFF and TOCF.

The serum enzyme activity like alkaline phosphatase and lactate dehydrogenase (LDH) decreased with feeding TOCF. LDH concentration is elevated in certain pathological conditions such as cytotoxicity and the decreased LDH release indicates that there was negligible effect on induced cell membrane damage. Low level of serum alkaline phosphatase (ALP) in fish fed TOCF suggests no adverse effect such as liver impairment, kidney dysfunction or bone disease. In our experiment, there was no effect of AChE in fish fed on the two types of feeds. However, monitoring AChE inhibition has been widely used as biomarker in terrestrial and freshwater aquatic systems as an indicator of organo-phosphorus pesticides (Fulton and Key, 2001; Rickwood and Galloway 2004; Jindal and Kaur, 2014). Results showed that TOCF exhibited better serum enzyme activity compared to CFF. The anti-stress and anti-oxidant property of TOCF could be attributed to the availability of all essential nutrients for growth and survival (Oliva-Teles, 2012; Singh, 2016) of fish compared to CFF.

The sensory evaluation showed that colour, flavour, sweetness and sourness of fish fed on TOCF were higher compared to commercial floating feed. This indicated that, fish fed TOCF availed all types of nutrients resulting in higher muscle quality compared to CFF (Sahoo *et al.*, 2000; Abbas *et al.*, 2006; Pawar *et al.*, 2012). The results of the present study indicated that, fish fed TOCF had higher growth performance and sensory score with superior serum enzyme activities compared to CFF whereas there was no change in non-specific immune response between the two feeds.

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