Pakistan J. Zool., pp 1-9, 2023. DOI: https://dx.doi.org/10.17582/journal.pjz/20230514060530

Performance of Dried Distillery Grain Waste on the Growth and Survival of Rohu (*Labeo rohita*) Larvae

Padala Dharmakar[1]*, S. Aanand[1], J. Stephen Sampath Kumar[2], Muralidhar P. Ande[3], P. Padmavathy[4], J. Jaculine Pereira[4] and Ch. Balakrishna[5]

[1]*Erode Bhavanisagar Centre for Aquaculture, Tamil Nadu Dr. J. Jayalalithaa Fisheries University, Bhavanisagar, India*
[2]*Directorate of Sustainable Aquaculture, Tamil Nadu Dr. J. Jayalalithaa Fisheries University, Nagapattinam, India.*
[3]*ICAR-Central Institute of Fisheries Education, Kakinada Center, Kakinada, India*
[4]*Fisheries College and Research Institute, Tamil Nadu Dr. J. Jayalalithaa Fisheries University, Thoothukudi, India.*
[5]*ICAR-Krishi Vigyan Kendra, Acharya N G Ranga Agricultural University, Amadalavalasa, India.*

Article Information
Received 14 May 2023
Revised 05 August 2023
Accepted 24 August 2023
Available online 11 November 2023 (early access)

Authors' Contribution
This study was conducted in cooperation between all authors. PD: Investigation and conducted the feeding trial, Analyzed the samples for proximate and amino acid, writing drafted the original manuscript, SA: Designed the study, Analysed the results, and reviewed the manuscript. Supervision, JSSK: Investigation, Supervision, APM: Technical and Statistical support, and reviewed the manuscript, PP: reviewed the manuscript JJP: reviewed the manuscript and analysed the results and CHB: Data analysis, manuscript review and editing.

Key words
Dried distiller's grains waste, Growth performance, *Labeo rohita*, Digestive enzyme

ABSTRACT

Fish meals followed by oil cakes are the primary protein sources in any aquafeed formulation. The higher-cost of oil cakes and uncertainty in the fish meal (FM) availability led to the search for good quality alternative feed ingredients. By-product protein sources are cheaper and readily available to substitute the expensive ingredients in traditional feed production. The present study was carried out to investigate the performance of dried distiller's grain waste (DDGW) by partial replacement of groundnut oil cake (GNOC) on the growth and survival of *Labeo rohita* in the nursery phase. Three isonitrogenous diets incorporating DDGW at (T1) 30%, (T2) 40% and (T3) 50% kg/feed replacement of GNOC were used as test diets with and diets only GNOC was taken as the control (C). The experimental trial was conducted in duplicate groups of 3000 rohu juvenile (mean initial weight 0.04±0.03 g) and was fed with the diets for 60 days. The growth performance and feed utilization parameters, *viz*., weight gain % (WG), specific growth rate (SGR), protein efficiency ratio (PER) and feed conversion ratio (FCR), were recorded. The fishes' whole body proximate composition and amino acid profiles were not significantly different among experimental diets. The results revealed that dietary DDGW @50% replacement of GNOC had shown the higher final weight and specific growth rate. Feed conversion and protein efficiency rates were similar among the dietary treatments. The digestive enzyme activity remained unaffected except for amylase activity, which increased significantly in the 50% DDGW replacement group. The present study indicates that DDGW can replace GNOC without affecting feed utilization parameters for better growth performance and digestive enzyme activity in the diet of *L. rohita*.

INTRODUCTION

The rapid expansion of aquaculture among food-producing sectors was due to the demand created the increasing population growth and over-exploitation of existing resources. The farm production of fish has continuously grown to sustain the demand gap (Lahsen and Iddya, 2014). The global average per capita fish consumption rose from 17.4 to 20.5 kg annually (FAO, 2022). Further expansion of the aquaculture industry was attributed to advanced technology, improved farming methods, and high-quality fish feed production (Tran and Rodela, 2019). Carp culture forms the backbone of freshwater aquaculture practice in India, contributing to about 87% of total production. The improved feed management practice in the culture of indian major carps can successfully fill the gap between productivity and potential. The nutrient and feed inputs supply will grow equally to maintain its current growth rate. In contrast, feed ingredient availability for aquaculture remains static,

* Corresponding author: dharmabfsc@gmail.com
0030-9923/2023/0001-0001 $ 9.00/0

and other sectors compete for the same feed resources (Tacon and Metian, 2015).

Carp culture largely depends on the quality of feeds, but ensuring the availability of these feed ingredients remains a challenge for the Indian aquaculture industry. The key aquafeed constituents like fish meal, soy meal and oilseed cakes are in a race with native animal husbandry (Hasan, 2007). Plant-based oilseed meals processed in traditional methods cannot be utilized at high levels (Soltan *et al*., 2015). Contaminants like mycotoxins may also limit vegetable feedstuffs in aquafeeds (Hendricks, 2003). Among the oilcake diets, groundnut oil cake (GNOC) is widely used feed ingredient in nursery rearing of carps, but deficient in methionine, tryptophan and tyrosine (Singh *et al*., 1981). Its storage duration and quality are poor as it may develop aflatoxins in the long run (Girma *et al*., 2011). Therefore, partially replacing of these ingredients with alternate feed ingredients like millets, distiller's dried grains waste (DDGW) and other brewery waste ingredients containing low phytate and highly digestible nutrient composition seems promising for aquaculture feed industry. However, the potential of these alternate feed ingredients needs to be explored based on their price, availability, and nutritive value (Ravindran, 2013). The basic knowledge of small-scale farmers on fish feed needs to be enlightened to enhance aquaculture production. In practice, the concept of complete feed has not reached the fish culturist, unlike shrimp farmers. So to find an alternative source of protein with some better characteristics, cost or performance are considered for an expected success. One solution to meeting the protein requirement of cultured species is the utilization of by-products from the brewing industry, which are natural diet additives that have shown better growth on a few fish species (Rumsey *et al*., 1991; Oliva-Teles and Goncalves, 2001).

The brewing industry tends to be more environmentally friendly (Ishiwaki *et al*., 2000). The dried distiller's grain waste (DDGW) is a by-product of the spirit/brewery industry during bio-ethanol production. Spent grain is the most abundant brewery by-product, contributing to 85% of the total by-products generated (Reinold, 1997). The continuous large-scale production by industries has led to a significant increase in waste generation, causing detrimental environmental pollution. Utilization of these wastes as animal feed is an economical alternative. Brewers spent grain is a high-value product containing hemicelluloses, lignin, proteins, and sugars (Buffington, 2014).

Earlier studies among a few fishes showed that brewery waste-activated sludge (BWAS) could be added to trout diets up to 10% (Windell, 1974). Bays (1977) recommended using the brewery-waste product as an essential source of vitamin B_{12} for *Clarias gariepinus* fingerlings. Tidwell *et al*. (1993) observed remarkable growth in pond-raised *Macrobrachium rosenbergii* at 40% distillers dried grains with solubles (DDGS) diets. Kaur (2004) observed that incorporation of brewery spent grain at 30% in supplementary fish feed, replacing rice bran, improved growth in *Catla catla* (Ham.), *Labeo rohita* (Ham.) and *Cirrhinus mrigala* (Ham.). The present study was there fore designed to investigate, the effect of the diets prepared with DDGW replacing GNOC on the growth performance and digestive enzyme activity without affecting feed utilization parameters of *L. rohita*.

MATERIALS AND METHODS

Diet and experimental design

Dried distiller's grain waste (DDGW) was acquired from a local market and ground to fine powder in a blender, and used it in diet preparation (Table I, Supplementary Fig. 1A). The control diet (0% DDGW) was prepared using groundnut oil cake, wheat flour, rice bran, vitamins and minerals. Three iso-nitrogenous experimental diets [30% crude protein (CP)], were formulated with DDGW, by replacing groundnut oil cake (GNOC) at 30%, 40%, and 50% (T1, T2, and T3), respectively. The proximate analysis of basal and experimental diets was performed by the method outlined in Association of Official Analytical Chemists (Horwitz and Latimer, 2005) (Table I).

Table I. Composition of dried distillery grain waste of feed ingredients for the experimental diets (T1 – T3).

Ingredients	C	T1 (30%)	T2 (40%)	T3 (50%)
DDGW	-	22.5	30	37.5
GNOC	75	52.5	45	37.5
Wheat flour	4	4	4	4
DORB	20	20	20	20
Vitamin premix	0.5	0.5	0.5	0.5
Mineral premix	0.5	0.5	0.5	0.5
Crude protein (%)	29.57	31.48	30.57	32.10
Ether extract (%)	5.28	5.04	5.34	5.28
Total ash (%)	5.04	3.97	3.98	4.01
Moisture (%)	6.52	7.21	7.20	7.21
Energy (K cal g^{-1})	469.4	475.4	475.6	477.4

DDGW, Dried distiller's grain waste; GNOC, Groundnut oil cake; DORB, De-oiled rice bran.

Feeding trials were conducted in hapas measuring 10m*3m*1m in size and installed in the earthen ponds covered with bird fencing nets (Supplementary Fig. 1B). All the treatment and control groups were conducted in duplicates. The experimental animals were stocked @ 100 per m^3 each happa of size 0.04±0.03 g and were procured from the fish farm, Erode Bhavanisagar Centre for Sustainable Aquaculture, Bhavanisagar (Supplementary Fig. 1C). Until the beginning of the trials, rohu juvenile was fed with a basal diet. The feeding trial was conducted for 60 days. Fishes were fed @10% of body weight (BW) for the first 30 days, followed by 5% BW for another 30 days, twice a day at 10:00 h in the morning and at 16:00 h in the evening (Supplementary Fig. 1D) (Vinod and Basavaraja, 2010; Mmanda *et al*., 2020).

Water quality measurements

In all experimental groups, the nutrient quality of pond water was recorded every fortnight during the trial period. The water quality parameters i.e., water temperature, pH, dissolved oxygen (DO), ammonia (NH_3), nitrite (NO_2), nitrate (NO_3), inorganic phosphate (PO_4), free CO_2, total hardness, total alkalinity, total suspended solids (TSS), total dissolved solids (TDS) and electrical conductivity (EC) were analyzed using standard procedures (APHA, 2005).

Bio-growth analysis

Growth parameters such as weight gain, weight gain percentage (WG%), specific growth rate (SGR), feed conversion ratio (FCR), feed efficient ratio (FER), protein efficient ratio (PER) and survival rate were determined using the following standard formula.

$$Weight\ gain\ (WG) = Final\ fish\ weight\ (g) - Initial\ fish\ weight\ (g)$$

$$Weight\ gain\ percentage\ (WG\ \%) = \frac{\text{Final weight (g)} - \text{Initial weight (g)}}{\text{Initial weight (g)}} \times 100$$

$$Specific\ growth\ rate\ (SGR) = \frac{\ln \text{final weight (g)} - \ln \text{Initial weight (g)}}{\text{Number of days}} \times 100$$

$$Feed\ conversion\ ratio\ (FCR) = \frac{\text{Total feed consumed by fish}}{\text{Total weight gain by fish}}$$

$$Feed\ efficient\ ratio\ (FER) = \frac{1}{\text{Feed conversion ratio}}$$

$$\text{Protein efficient ratio (PER)} = \frac{\text{Net weight gain}}{\text{Protein fed}}$$

$$\text{Survival rate (\%)} = \frac{\text{Final number of fish}}{\text{Initial number of fish}} \times 100$$

Proximate and amino acid analysis

At the end of the feeding trial, the fish from each replicate hapa were collected (n=8) to determine the whole-body composition and amino acid profile. The body composition was estimated following standard protocols (AOAC, 2010). Precisely 5g dry powder form of control and DDGW incorporated feed and fish samples were analysed for amino acid analysis at ATOZ Pharmaceuticals PVT.LTD., Chennai, Tamil Nadu. Using HPLC, LACHROM L-7000 and a ChromNAV software system from JASCO-HPLC analysis.

Determination of digestive enzyme activities

At the end of the experiment, ten fishes were collected from each replicate, and using a normal homogenizer, prepared a 5% tissue homogenate. The whole procedure was carried out in ice-cold condition. Homogenized samples were centrifuged at 5000 rpm for 10 min at 4°C. The supernatant was collected in a 5 ml tube and stored at -20°C for enzyme assay. Suitable dilution of the samples were done depending on the requirement. The Bradford method was used to estimate the total protein content of each tissue sample in enzyme assays (Bradford, 1976).

Amylase activity was estimated as the reducing sugars produced due to the action of gluco-amylase, and amylase on carbohydrates was determined using the di-nitro salicylic acid (DNS) method (Rick and Stegbauer, 1974). Protease activity was estimated by the casein digestion method (Drapeau, 1976). The lipase activity was determined by the titrimetric method according to the procedure described by Cherry and Crandell (1932). Aspartate amino transferase (AST) and alanine aminotransferase (ALT), lactate dehydrogenase (LDH), and malate dehydrogenase (MDH) activity were assayed with muscle tissue homogenates as described by Wooten (1964).

Statistical analysis

Statistical Package for Social Sciences (SPSS) version 25.0 (IBM Corp.) was used to test the differences between various treatments by one-way analysis of variance (ANOVA). Duncan's multiple range test ($p<0.05$) was used to find the significant difference between treatments.

RESULTS AND DISCUSSION

Growth performances and nutrient utilization of rohu juvenile

Water quality parameters recorded during the experimental period were in the acceptable range for fish. Water quality parameters play an essential role in the biology and physiology of fish (Cho and Kaushik, 1990), as fishes are sensitive to change in water quality parameters. Optimum water quality assures maximum survival rate, nutrient utilization, and fish growth. In the present study, the selected ponds for the installation of hapa were free from sewage discharge or other anthropogenic activities. All the physicochemical parameters of water, such as water temperature (26.3-28.8°C), pH (7.2-7.8), dissolved oxygen (DO, 4.0-4.1 mgL^{-1}), ammonia (NH_3, 0.02-0.03

mg L^{-1}), nitrite (NO_2, 0.8-0.9 mg L^{-1}), nitrate (NO_3, 2.9-3.4 mg L^{-1}), inorganic phosphate (PO_4, 1.77-1.89 mg L^{-1}), free (CO_2, <0.01 mg L^{-1}), total hardness (78-82 mg L^{-1}), total alkalinity (77.2-81.0 mg L^{-1}), total suspended solids (TSS, 312-322 mg L^{-1}), total dissolved solids (TDS, 229-238 mg L^{-1}) and electrical conductivity (EC, 0.28-0.33µ mhos cm^{-1}) were within the optimum range of requirements for fish during the experimental period. Temperature is the main factor determining the growth and wellbeing of the fish. Das *et al*. (2005) found that the temperature ranges of 26–36 °C are not fatal to *L. rohita* juvenile. The optimum temperature range for growth was 25–28°C, temperature range during the feeding trial was within this recommended level. The water pH in the experimental pond ranged from 7.2-7.8, which is well within the acceptable range as suggested by Banerjea (1967). The ammonia values were in the range of 0.02-0.03 mg L^{-1}. Jhingran (1991) suggested that the ammonia concentration of water must be in the range of 0.05-0.1 mg L^{-1}. The present experimental study's water quality parameters were in the optimal range (Harshavardhan *et al*., 2021).

Growth performance and nutrient utilization of rohu juvenile fed after 60 days was presented in Table II. An apparent increase in weight gain and improved feed utilization during the experimental period was observed. The nutritional quality of DDGW in rohu utilization from the feed was determined in terms of body weight gain (WG %), specific growth rate (SGR), feed conversion ratio (FCR), and protein efficiency ratio (PER). The growth and nutrient utilization parameters *viz*., WG (%), SGR, FCR, and PER are presented in Table II. The weight gain percentage, SGR, FCR, and PER vary significantly ($p<0.05$) among the various treatment groups. The results showed that the final weight and weight gain were significantly higher in fish fed at 50% DDGW diet group than in the other diet groups ($p<0.05$). Similarly, FCR was superior in the treatment group compared to the control group. The survival rates among the experimental groups did not vary significantly ($p>0.05$) (Table II). Maximum and minimum survival rates were found in T3 (54.02±0.21) and control (C) group (31.12±0.03), respectively among the different treatments.

Table II. Growth performance of rohu juvenile fed with DDGW during the experiment.

Treatments	0th day	15th day	30th day	45th day	60th day	Survival %
C	0.04 ± 0.02	0.29 ± 0.13	0.42 ± 0.02	0.84 ± 0.16^{c}	1.53 ± 0.04^{c}	31.12 ± 0.03
DDGW						
T1 (30%)	0.04 ± 0.02	0.32 ± 0.19	0.46 ± 0.02	0.86 ± 0.32^{c}	1.84 ± 0.07^{c}	30.24 ± 0.02
T2 (40%)	0.04 ± 0.02	0.42 ± 0.10	0.73 ± 0.02	1.25 ± 0.06^{b}	2.91 ± 0.02^{b}	46.24 ± 0.15
T3 (50%)	0.04 ± 0.02	0.59 ± 0.13	0.87 ± 0.04	2.58 ± 0.16^{a}	4.12 ± 0.02^{a}	54.02 ± 0.21

Data expressed as mean ± standard error (M±SE); (n=15, r=2); Mean values in the same column with different superscript differ significantly ($p<0.05$).
C, Control; T, Treatment; DDGW, Dried distiller's grain waste.

Table III. Growth parameters of rohu juvenile fed with DDGW at different levels by replacement of GNOC.

Param-eters	30th day				60th Day			
	C	T1	T2	T3	C	T1	T2	T3
MIW	0.04±0.02	0.04±0.02	0.04± 0.02	0.04±0.02	0.04±0.02	0.04±0.02	0.04 ± 0.02	0.04 ±0.02
MFW	0.42±0.02	0.46±0.02	0.73±0.02	0.87±0.04	1.53^{c}±0.04	1.84^{c}±0.07	2.91^{b}±0.02	4.12^{a}±0.02
MWG	0.38±0.01	0.42±0.02	0.69±0.02	0.83±0.04	1.49^{c}±0.02	1.80^{c}±0.03	2.87^{b}±0.02	4.08^{a}±0.03
WG%	950^{c}±138.05	1050^{c}±166.37	1725^{b}±166.37	2087^{a}±291.16	3725^{c}±291.16	4500^{c}±415.94	7175^{b}±166.37	10200^{a}±124.78
SGR	3.92^{b}±0.10	4.07^{b}±0.10	4.84^{ab}±0.06	5.14^{a}±0.09	6.07^{b}±0.05	6.38^{b}±0.06	7.15^{a}±0.06	7.72^{a}±0.08
FCR	1.33^{a}±0.01	1.26^{a}±0.06	1.13^{b}±0.01	1.08^{b}±0.09	0.97^{a}±0.01	0.87^{b}±0.05	0.85^{b}±0.01	0.79^{c}±0.01
FER	0.75^{b}±0.07	0.79^{b}±0.16	0.87^{a}±0.31	0.92^{a}±0.19	1.03^{c}±0.08	1.15^{b}±0.04	1.17^{b}±0.15	1.26^{a}±0.08
PER	1.27^{b}±0.65	1.29^{b}±0.29	1.37^{ab}±0.57	1.62^{a}±0.58	0.87^{b}±0.05	0.89^{b}±0.12	0.97^{a}±0.18	1.07^{a}±0.11
p value	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05

Data expressed as mean ± standard error (M ± SE); n=2. Means in the same row having different superscripts are significantly different ($p<0.05$).
C, Control; T, Treatment; MIW, Mean Initial Weight; MFW, Mean Final Weight; MWG, Mean Weight Gain; SGR, Specific Growth Rate; FCR, Feed Conversion Ratio; FER, Feed Efficiency Ratio; PER, Protein Efficiency Ratio.

In the present study, there was a significant difference ($p<0.05$) in weight gain of *L. rohita* juvenile-fed diets that contained the graded levels of dried distiller grain waste (DDGW) with that of the control (C) diet and other experimental diets. Feeding dried brewery waste to livestock has been common practice since the initiation of beer production (Westendorf and Wohlt, 2002). The surplus availability of dried brewery waste makes it an excellent alternative to plant protein sources like soybean meal and GNOC in aqua-feeds. In the present investigation, inclusion at 50% DDGW diet in the T3 had the highest weight gain percentage (10200±124.78), SGR (7.72±0.08), FCR (0.79±0.01), and PER (1.07±0.11). Oliva-Teles and Goncalves (2001) reported that 50% of the brewery waste could replace fish meal with no adverse effect on the growth in sea bass. Similar results were reported in striped catfish fingerlings (Jayant *et al*., 2018). Zerai *et al*. (2008) reported that 50% brewery waste replacing fish meal promotes better growth and feed conversion in tilapia.

Table IV. Carcass composition of whole body of early rohu fingerlings of different experimental groups (% Wet weight basis).

Treat-ments	Moisture %	DM %	CP %	Lipid %	Ash %
C	76.22 ± 0.20	23.78 ± 0.20	14.12 ± 0.11	2.73 ± 0.13	3.51 ± 0.01
T1	75.78 ± 0.59	24.22 ± 0.59	15.88 ± 0.06	2.53 ± 0.05	3.43 ± 0.07
T2	75.00 ± 0.82	25.00 ± 0.82	16.56 ± 0.42	2.84 ± 0.21	3.56 ± 0.07
T3	73.49 ± 0.17	26.51 ± 0.17	17.05 ± 0.10	3.38 ± 0.12	3.61 ± 0.04

Data are expressed as mean ± standard error (M ± SE), n=2. C, Control; T, Treatment; DM, Dry matter; CP, Crude protein.

In channel catfish (*Ictalurus punctatus*), DDGW was used to replace fish and soybean meals, up to 40% of the diet, without lysine supplementation (Webster *et al*. 1991, 1992, 1993; Lim and Lee, 2009) and no negative repercussions on growth performance. Higher dietary replacement levels might be achieved with the adequate restoration of the dietary essential amino acid profile by using amino acid supplements or a combination among different protein sources (Webster *et al*., 1991; Cheng and Hardy, 2004). In channel catfish (*Ictalurus punctatus*), the dietary lysine supplementation allowed increased DDGW in the diet up to 70% (Webster *et al*., 1991; Robinson and Li, 2008). In blue catfish (*Ictalurus furcatus*), Webster *et al*. (1992a) stated that a combination of DDGW with soybean meal (35% DDGW and 49% soybean meal) could replace fish meal in the diet with or without lysine supplementation and methionine.

Carcass composition

The body composition of fish fed with different experimental diets is shown in Table IV. The highest body CP% was observed for fishes fed with the T3 diet, which was not significantly different than fishes fed with the other diets. The lowest body CP% was observed in fishes fed with the control diet. The highest tissue lipid accumulation was also recorded in the fish fed diet T3. The whole body moisture content (%) was lowest in the carcass of fish fed diet T3, and it was highest for the control diet and T1. The ash % did not vary significantly ($p>0.05$) among the groups fed different experimental diets.

Digestive and metabolic enzymes

Digestive and metabolic enzyme activities of *L. rohita* fed with different experimental diets were carried out after 60 days of the feeding trial. The activity of digestive enzymes like protease and amylase *L. rohita* juveniles' intestine was found significantly ($p<0.05$) higher in the fish-fed diets containing among different experimental groups than the control and T1 fed fish (Table V). The maximum protease and amylase activities were noticed in the fish-fed diet T3, though it was not significantly ($p<0.05$) different from other diets of T1, T2 and control. However, the activities of the lipase enzyme were similar ($p>0.05$) among different experimental groups.

Table V. Specific enzyme activities in the digestive tract of early rohu fingerling fed with DDGW with different experimental group.

Treatments	Amylase	Protease	Lipase
C	3.65^{b} ± 0.12	0.30^{b} ± 0.57	0.68 ± 0.33
T1	3.96^{ab} ± 0.09	0.34^{ab} ± 0.37	0.67 ± 0.19
T2	4.13^{a} ± 0.10	0.34^{ab} ± 0.25	0.70 ± 0.21
T3	4.69^{a} ± 1.17	0.39^{a} ± 0.21	0.69 ± 0.20
p value	<0.05	<0.05	>0.05

Data are expressed as mean ± standard error (M ± SE). (n=15, r=3); Mean values in the same column having different superscripts differ significantly ($p<0.05$). C, Control; T, Treatment. Protease as micromol of tyrosine released/ min/mg protein. Amylase as micromol of maltose released/min/mg protein. Lipase as units/mg protein.

The metabolic enzyme activities of *L. rohita* fed with different experimental diets were shown in Table V. The Aspartate amino transaminase (AST) and alanine amino transaminase (ALT) activity in the muscle T3 fed groups was statistically higher ($p<0.05$) than that of the control and other groups. The lactate dehydrogenase (LDH) and malate dehydrogenase (MDH) activity in the muscle of the fish fed different experimental diets did not show any significant variation (p>0.05). The activities of digestive enzymes (protease, lipase, and amylase) were higher in T3 (50% DDGW), followed by T2 and T1 experimental groups. In contrast, the lowest activity of these enzymes was observed in the control (C) group. There was no significant difference between the experimental groups included with DDGW. Similar findings by Lim and Yildirim (2008) was suggested that lysine supplementation at 40% of DDGW in the diet has shown better growth performance, feed utilization, and survival of juvenile channel catfish. In the present study, metabolic enzyme activities like AST, ALT, LDH, and MDH have shown no significant difference between the treatments indicating low stress due to the experimental feed (Chatterjee *et al*., 2003).

Table VI. Metabolic enzyme activities of muscle of *L. rohita* fed with different replacement level of DDGW with the GNOC.

Treat-ments	AST Muscle	ALT Muscle	LDH Muscle	MDH Muscle
C	$26.62^{c}\pm0.13$	$14.84^{c}\pm0.70$	4.09±0.22	3.01±0.01
T1	$27.31^{bc}\pm0.46$	$15.14^{c}\pm0.41$	3.97±0.04	2.71±0.02
T2	$28.09^{b}\pm0.13$	$16.86^{b}\pm0.26$	3.98±0.03	2.48±0.03
T3	$29.99^{a}\pm0.96$	$17.43^{a}\pm0.41$	3.62±0.02	2.34±0.02

Data expressed as mean ± Standard Error (M±SE) (n=15, r=2); Mean values in the same column with different superscript differ significantly (p<0.05). C, Control; T, Treatment. ALT, specific activities expressed as Nano moles of sodium pyruvate formed/mg protein/minute at 37⁰C. AST, specific activities expressed as Nano moles of oxaloacetate released/min/mg protein at 37⁰C. LDH, specific activities expressed as units/mg protein/ min at 37°C; MDH, specific activities expressed as units/mg protein/ min at 37°C.

Amino acid analysis

The amino acids profile of DDGW of feed and fish is presented in Tables VII and VIII. Essential amino acids like histidine, threonine and methionine were higher in the T3 fish group supplemented with the 50% DDGW than control feed. The essential aminoacids were found higher in DDGW incorporated diets than that of control diet. Webster *et al*. (1992) revealed that growth rate and feed conversion indices were not affected by the complete substitution of soybean meal with distiller's grain soluble (DGS) in the diet of channel catfish. Similarly, Jayant *et al*. (2018) also reported that protein intake values were significantly (p<0.05) higher in fish fed at 25% and 50% brewery spent grain (BSG) levels in striped catfish (*Pangasianodon hypophthalmus*) fingerlings. No adversegrowth effect reportd in yellow perch (*Perca flavescens*), fed with

Table VII. Amino acid profile of DDGW of feed.

Amino acid (mg/5g DM)	C Feed	T1 Feed	T2 Feed	T3 Feed
Essential amino acids				
Arginine	18.08	19.01	19.6	20.0
Histidine	8.9	9.4	9.6	10.2
Isoleucine	13.8	13.5	13.7	13.9
Leucine	26.8	25.2	25.6	26.0
Lysine	17.4	16.8	16.4	15.8
Phenylalanine	14.6	14.4	14.3	14.4
Methionine	9.6	9.3	9.1	8.9
Threonine	11.1	11.3	11.6	11.7
Tryptophan	2.9	2.9	2.8	2.6
Valine	14.3	14.5	14.6	14.7
Non-essential amino acids				
Alanine	18.3	16.1	15.8	15.2
Glycine	22.1	21.6	21.0	20.8
Aspartic acid	33.4	32.8	32.1	31.3
Glutamic acid	62.4	64.3	64.9	65.4
Serine	13.7	14.6	15.4	15.9
Tyrosine	10.4	11.5	11.9	13.01

DM, Dry Matter; C, Control; T, Treatment.

Table VIII. Amino acid Profile of DDGW of fish.

Amino Acid (mg/5g DM)	C Fish	T1 Fish	T2 Fish	T3 Fish
Essential amino acids				
Arginine	11.1	12.5	12.4	12.7
Histidine	5.9	6.7	6.6	6.4
Isoleucine	8.9	9.3	9.4	9.4
Leucine	10.9	13.1	13.4	13.7
Lysine	11.8	12.2	12.6	12.9
Phenylalanine	6.8	7.8	7.6	7.1
Methionine	4.9	5.8	5.9	6.1
Threonine	8.3	9.4	9.6	9.6
Tryptophan	2.5	2.6	2.7	2.7
Valine	8.3	9.2	9.3	9.3
Non-essential amino acids				
Alanine	15.1	15.5	15.3	15.2
Glycine	13.4	13.5	13.6	13.7
Aspartic acid	25.9	26.1	26.0	26.2
Glutamic acid	27.9	27.8	28.3	28.4
Serine	4.2	6.2	6.4	6.7
Tyrosine	3.1	3.7	3.7	3.4

DM, Dry Mass; C, Control; T, Treatment.

DDGW and soybean meal mixture up to 49.5% (Schaeffer *et al*., 2011). The improved growth performance of rohu juvenile in the present study suggests that protein and nucleic acid content in DDGW improved the nutrient utilization and growth performance of *L. rohita* juvenile. In the present study, the amino acid concentrations like lysine, leucine, threonine, and methionine contents in DDGW fishes showed higher, in fish fed with 50% DDGW than the T2, T3, and control group. Zerai *et al*. (2008) had seen similar increases of essential amino acids in the 25% to 50% inclusion levels of brewery waste with partial replacement of fish meal in Nile tilapia diet. There are some hindrances in using GNOC as fish feed ingredient. Although it is an excellent source of the amino acid arginine, it is deficient in sulphur containing amino acids like lysine, cysteine and methionine (Green *et al*., 1988).

CONCLUSION

The rising cost and the limited supply of conventional fish feed ingredients have necessitated focusing research efforts towards the potential utilisation of energy and proteins from several agribyproducts that are cheaper with high nutritive value. During the study period, traditionally local farmers generally use a 50:50 mixture of rice bran (@ 20/kg) and GNOC (@ 45/kg) which would cost Rs. 32 per kg, while when DDGW (@ 27/kg) when used the cost of the feed would be around Rs. 28/- per kg. The present study showed improved growth performance, nutrient utilization, and survival rate of juvenile *L. rohita* fed with a diet of 50% DDGW. Hence, it can be conluded that utilization of DDGW upto 50% replacement of GNOC can be scientifically and economically better than the traditional practice of utilizing GNOC and rice bran alone in the rearing of *L. rohita* in the nursery phase.

ACKNOWLEDGEMENT

The present study was an outcome of the student research carried out for the award of Ph.D. The study was supported by Tamil Nadu Dr. J. Jayalalithaa Fisheries University, Nagapattinam, Tamil Nadu, India, in the form of a fellowship. We also thank the Director, Directorate of Sustainable Aquaculture, TNJFU, for necessary infrastructure facilities as well as scientific support for carrying out the research work.

Funding

The study received no external funds.

IRB approval

The research committee of the University has approved the work.

Ethical statement

The experiment was conducted following the procedures of CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals), Ministry of Environment and Forests (Animal Welfare Division), Govt. of India on care and use of animals in scientific research. The procedural handling and rearing of the fish were performed according to the animal ethics committee, Tamil Nadu Dr. J. Jayalalithaa Fisheries University, Nagapattinam.

Data availability statement

The data that support the findings of this study are available within the article.

Supplementary material

There is supplementary material associated with this article. Access the material online at: https://dx.doi.org/10.17582/journal.pjz/20230514060530

Statement of conflict of interest

The authors have declared no conflict of interest.

REFERENCES

AOAC, 2010. *Association of official analytical chemists Official methods of analysis* (18th ed.). AOAC.

APHA, 2005. *Standard methods of water and wastewater*. 21st Edn., American Public Health Association, Washington, DC., pp. 2-61.

Banerjea, S.M., 1967. Water quality and soil condition of fish ponds in some states of India in relation to fish production. *Indian J. Fish*., **14**: 115-144.

Bays, J.D., 1977. *Waste activated sludge a new brewery by-product (Handling and disposal)*. Technical Quarterly Master Brewers Association of America.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem*., **72**: 248–254. https://doi.org/10.1016/0003-2697(76)90527-3

Buffington, J., 2014. The economic potential of Brewer's Spent Grain (BSG) as a biomass feedstock. *Adv. Chem. Eng. Sci*., **4**: 308–318. https://doi.org/10.4236/aces.2014.43034

Chatterjee, P.N., Kaur, H. and Panda, N., 2003. Effect of vitamin-E supplementation on plasma antioxidant vitamins and immunity status of crossbred cows. *Asian-Austral. J. Anim. Sci*., **16**: 1614–1618. https://doi.org/10.5713/ajas.2003.1614

Cheng, Z.J. and Hardy, R.W., 2004. Nutritional value of diets containing distiller's dried grain with solubles for rainbow trout, *Oncorhynchus mykiss*. *J. appl. Aquacult.*, **15**: 101–113. https://doi.org/10.1300/J028v15n03_08

Cherry, I.S. and Crandall, L.A., 1932. The specificity of pancreatic lipase: Its appearance in the blood after pancreatic injury. *Am. J. Physiol. Legacy Content*, **100**: 266–273. https://doi.org/10.1152/ajplegacy.1932.100.2.266

Cho, C.Y. and Kaushik, S.J., 1990. Nutritional energetics in fish: Energy and protein utilization in rainbow trout (*Salmo gairdneri*). *Aspects Fd. Prod. Consump. Energy Values*, **61**: 132-172. https://doi.org/10.1159/000417529

Das, T., Pal, A., Chakraborty, S., Manush, S., Sahu, N. and Mukherjee, S., 2005. Thermal tolerance, growth and oxygen consumption of *Labeo rohita* fry (Hamilton, 1822) acclimated to four temperatures. *J. Therm. Biol.*, **30**: 378–383. https://doi.org/10.1016/j.jtherbio.2005.03.001

Drapeau, G.R., 1976. Protease from *Staphyloccus aureus*. In: *Methods in enzymology*. **45**: 469-475. https://doi.org/10.1016/S0076-6879(76)45041-3

FAO, 2022. *Aquaculture division*. Rome: Food and Agriculture Organization of the United Nations.

Girma, M., Tamir, B. and Dessie, T., 2011. Effects of replacing peanut seed cake with brewery dried yeast on laying performance, egg quality and carcass characteristics of rhode island red chicken. *Int. J. Poult. Sci.*, **11**: 65–72. https://doi.org/10.3923/ijps.2012.65.72

Green, S., Bertrand, S.L., Diron, M.J.C. and Maillard, R., 1988. Digestibility of amino acids in soyabean, sunflower and groundnut meal, measured in pigs with ileo-rectal anastomosis and isolation of the large intestine. *J. Sci. Fd. Agric.*, **42**: 119–128. https://doi.org/10.1002/jsfa.2740420204

Harshavardhan, M.A., Aanand, S., Kumar, J.S.S. and Senthilkumar, V., 2021. Comparative evaluation of commercial vegetable oil, fish oil, palm oil and groundnut oil as a lipid source in maturation and reproductive performance of fancy koi, *Cyprinus carpio* var. koi. *Aquaculture*, **545**: 737248. https://doi.org/10.1016/j.aquaculture.2021.737248

Hasan, M.R., 2007. *Study and analysis of feeds and fertilizers for sustainable aquaculture development*. Food and Agriculture Organization of the United Nations. 531.

Hendricks, J.D., 2003. Adventitious toxins. In: *Fish nutrition.* Academic Press. Academic Press, San Diego, CA. pp. 601-649. https://doi.org/10.1016/B978-012319652-1/50011-2

Horwitz, W. and Latimer, G., 2005. *AOAC official methods of analysis of AOAC international*. AOAC International, Gaithersburg, MD, USA.

Ishiwaki, N., Murayama, H., Awayama, H., Kanauchi, O. and Sato, T., 2000. Development of high value uses of spent grain by fractionation technology. *Tech. Quart. Master Brewers Assoc. Am.*, **37**: 261-265.

Jayant, M., Hassan, M., Srivastava, P., Meena, D., Kumar, P., Kumar, A. and Wagde, M., 2018. Brewer's spent grains (BSGs) as feedstuff for striped catfish, *Pangasianodon hypophthalmus* fingerlings: An approach to transform waste into wealth. *J. Cleaner Prod.*, **199**: 716–722. https://doi.org/10.1016/j.jclepro.2018.07.213

Jhingran, V.G., 1991. Fish culture in fresh water pond. In: *Fish and fisheries of India, III edn* (ed. V.G. Jhingran). Hindustan Publishing Corporation, New Delhi. pp. 276.

Kaur, V., 2004. Incorporation of brewery waste in supplementary feed and its impact on growth in some carps. *Bioresour. Technol.*, **91**: 101–104. https://doi.org/10.1016/S0960-8524(03)00073-7

Lahsen, A. and Iddya, K., 2014. The state of world fisheries and aquaculture: Opportunities and challenges. *State World Fish. Aquacult.*, **4**: 40-41.

Lim, C. and Yildirim-Aksoy, M., 2008. *Distillers dried grains with solubles as an alternative protein source in fish feeds*. Proceedings of the 8th International Symposium on Tilapia in Aquaculture. Cairo, Egypt: Agriculture Press Unit, Agriculture Research Center. pp. 12-14.

Lim, S.J., and Lee, K.J., 2009. Partial replacement of fish meal by cottonseed meal and soybean meal with iron and phytase supplementation for parrot fish *Oplegnathus fasciatus*. *Aquaculture*, **290**: 283–289. https://doi.org/10.1016/j.aquaculture.2009.02.018

Mmanda, F.P, Lundh, T., Haldén, A.N., Mtolera, M.S., Kitula, P.R. and Lindberg, J.E., 2020. Replacing fish meal with locally available feed ingredients to reduce feed costs in cultured Nile tilapia (*Oreochromis niloticus*). *Livest. Res. Rural Dev.*, **32**: 178. https://doi.org/10.3390/fishes5040032

Oliva-Teles, A. and Goncalves, P., 2001. Partial replacement of fishmeal by brewers yeast (*Saccaromyces cerevisae*) in diets for sea bass (*Dicentrarchus labrax*) juveniles. *Aquaculture*, **202**: 269–278. https://doi.org/10.1016/S0044-8486(01)00777-3

Ravindran, V., 2013. Feed enzymes: The science, practice, and metabolic realities. *J. appl. Poult. Res.*, **22**: 628–636. https://doi.org/10.3382/

japr.2013-00739
Reinold, M.R., 1997. *Manual prático de cervejaria*. Aden, São Paulo. pp. 214.
Rick, W. and Stegbauer, H.P., 1974. α-Amylase measurement of reducing groups. In: *Methods of enzymatic analysis*, Academic Press. pp. 885-890. https://doi.org/10.1016/B978-0-12-091302-2.50074-8
Robinson, E.H., and Li, M.H., 2008. Replacement of soybean meal in channel catfish, *Ictalurus punctatus*, diets with cottonseed meal and distiller's dried grains with solubles. *J. World Aquacult. Soc.*, **39**: 521–527. https://doi.org/10.1111/j.1749-7345.2008.00190.x
Rumsey, G., Kinsella, J., Shetty, K. and Hughes, S., 1991. Effect of high dietary concentrations of brewer's dried yeast on growth performance and liver uricase in rainbow trout (*Oncorhynchus mykiss*). *Anim. Feed Sci. Technol.*, **33**: 177–183. https://doi.org/10.1016/0377-8401(91)90058-Z
Schaeffer, T.W., Brown, M.L. and Rosentrater, K.A., 2011. Effects of dietary distillers dried grains with solubles and soybean meal on extruded pellet characteristics and growth responses of juvenile yellow perch. *N. Am. J. Aquacult.*, **73**: 270–278. https://doi.org/10.1080/15222055.2011.593461
Singh, K., Prasad, C. and Brahmakshatriva, R., 1981. Feeding value of sunflower and groundnut-cakes for laying hens. *Anim. Feed Sci. Technol.*, **6**: 63–71. https://doi.org/10.1016/0377-8401(81)90031-6
Soltan, M., Abdella, M., Hassaan, M., El-Ashry, M. and El-Syaad, G., 2015. Yeast fermented sunflower meal as a replacer for fish meal in diets of the Nile tilapia, *Oreochromis niloticus*. *Egypt J. aquat. Biol. Fish.*, **19:** 65–72. https://doi.org/10.21608/ejabf.2015.2258
Tacon, A.G.J. and Metian, M., 2015. Feed matters: Satisfying the feed demand of aquaculture. *Rev. Fish. Sci.*, **23**: 1–10. https://doi.org/10.1080/23308249.2014.987209
Tidwell, J.H., Webster, C.D., Clark, J.A. and D'Abramo, L.R., 1993. Evaluation of distillers dried grains with solubles as an ingredient in diets for pond culture of the freshwater prawn *Macrobrachium rosenbergii*. *J. World aquacult. Soc.*, **24**: 66-70. https://doi.org/10.1111/j.1749-7345.1993.tb00151.x
Tran, T.A. and Rodela, R., 2019. Integrating farmers adaptive knowledge into flood management and adaptation policies in the Vietnamese Mekong Delta: A social learning perspective. *Glob. Environ. Change*, **55**: 84–96. https://doi.org/10.1016/j.gloenvcha.2019.02.004
Vinod, B.H. and Basavaraja, N., 2010. Effect of diethylstilbestrol and norethindrone on sex ratio and body indices of common carp, *Cyprinus carpio* (Linn.). *Asian Fish. Sci.*, **23**: 100-115. https://doi.org/10.33997/j.afs.2010.23.1.009
Webster, C.D., Tidwell, J.H. and Yancey, D.H., 1991. Evaluation of distillers' grains with solubles as a protein source in diets for channel catfish. *Aquaculture*, **96**: 179–190. https://doi.org/10.1016/0044-8486(91)90148-Z
Webster, C.D., Tidwell, J.H., Goodgame, L.S. and Johnsen, P.B., 1993. Growth, body composition, and organoleptic evaluation of channel catfish fed diets containing different percentages of distillers grains with solubles. *Progr. Fish Cult.*, **55**: 95-100. https://doi.org/10.1577/1548-8640(1993)055<0095:GBCAOE>2.3.CO;2
Webster, C.D., Yancey, D.H. and Tidwell, J.H., 1992a. Effect of partially or totally replacing fish meal with soybean meal on growth of blue catfish (*Ictalurus furcatus*). *Aquaculture*, **103**: 141–152. https://doi.org/10.1016/0044-8486(92)90408-D
Webster, C.D., Tidwell, J.H., Goodgame, L.S., Yancey, D.H. and Mackey, L., 1992. Use of soybean meal and distillers grains with solubles as partial or total replacement of fish meal in diets for channel catfish *Ictalurus punctatus*. *Aquaculture*, **106**: 301–309. https://doi.org/10.1016/0044-8486(92)90262-J
Westendorf, M.L. and Wohlt, J.E., 2002. Brewing by-products: Their use as animal feeds. *Vet. Clin. North Am. Large Anim. Pract.*, **18**: 233–252. https://doi.org/10.1016/S0749-0720(02)00016-6
Windell, J.T., 1974. Substitution of brewer's single cell protein into pelleted fish feed. *Feedstuffs*, **46**: 22-23.
Wooten, I.D.P., 1964. Microanalysis. In: *Medical biochemistry*. 4th edn, J. and A. Churchill, London, pp. 101-107.
Zerai, D.B., Fitzsimmons, K.M., Collier, R.J. and Duff, G.C., 2008. Evaluation of brewer's waste as partial replacement of fish meal protein in Nile tilapia, *Oreochromis niloticus*, diets. *J. World Aquacult. Soc.*, **39**: 556–564. https://doi.org/10.1111/j.1749-7345.2008.00186.x

Pakistan J. Zool., pp 1-9, 2023. DOI: https://dx.doi.org/10.17582/journal.pjz/20230514060530

Supplementary Material

Performance of Dried Distillery Grain Waste on the Growth and Survival of Rohu (*Labeo rohita*) Larvae

Padala Dharmakar[1]*, S. Aanand[1], J. Stephen Sampath Kumar[2], Muralidhar P. Ande[3], P. Padmavathy[4], J. Jaculine Pereira[4] and Ch. Balakrishna[5]

[1]Erode Bhavanisagar Centre for Aquaculture, Tamil Nadu Dr. J. Jayalalithaa Fisheries University, Bhavanisagar, India
[2]Directorate of Sustainable Aquaculture, Tamil Nadu Dr. J. Jayalalithaa Fisheries University, Nagapattinam, India.
[3]ICAR-Central Institute of Fisheries Education, Kakinada Center, Kakinada, India
[4]Fisheries College and Research Institute, Tamil Nadu Dr. J. Jayalalithaa Fisheries University, Thoothukudi, India.
[5]ICAR-Krishi Vigyan Kendra, Acharya N G Ranga Agricultural University, Amadalavalasa, India.

Supplementary Fig. 1. Experimental diet and experimental design for studies on rohu fry. A, Experimental diet ingredients; B, Happas installed in the earthen ponds covered with bird fencing nets; C, experimental rohu fry before stocking; D, experimental diets for the rohu fry; E, Sampling of the experimental rohu fish; F, experimental rohu fish after 60 days of period.

* Corresponding author: dharmabfsc@gmail.com
0030-9923/2023/0001-0001 $ 9.00/0