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Clean valorization of distillery industry co-product for fish cage aquaculture: a waste-to-wealth approach

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Abstract

The present study investigated the use of brewery waste as a sustainable and cost-effective feed ingredient for *Pangasianodon hypophthalmus* farming in floating cages in tropical reservoirs. Three isonitrogenous and isocaloric diets were formulated: brewery waste-based floating (T2), brewery waste-based sinking (T3), and soybean meal-based floating (control). Fish fingerlings were fed each diet for 60 days and their growth performance, feed utilization, nutrient retention, and carcass composition were evaluated. Fish fed brewery waste-based floating feed (T2) had the highest weight gain, specific growth rate, and protein efficiency ratio, and the lowest feed conversion ratio. Their growth performance was statistically significant ($p < 0.05$) compared to fish fed brewery waste-based sinking feed (T3), but not statistically different ($p > 0.05$) from fish fed soybean meal-based floating feed (control). The cost of brewery waste-based floating feed was 31.45% lower than the cost of soybean meal-based floating feed. Overall, the results of this study suggest that brewery waste is a promising feed ingredient for *Pangasianodon hypophthalmus* farming in floating cages in tropical reservoirs. It can be used to replace expensive soybean meal without compromising fish growth performance. The use of brewery waste also reduces feed costs and makes cage farming of *Pangasianodon hypophthalmus* more economically sustainable.

Keywords Brewery waste, *Pangasianodon hypophthalmus*, Floating cage farming, Clean technology

Introduction

India being an agrarian country, a lot of by-products, co-products/wastes are available for useful bio-conversion to fish biomass to overcome protein malnutrition and creating rural livelihood opportunity [1]. A major chunk of cereals produced in the country are used in brewery industry for the production of alcoholic products. After the extraction of alcohol, the dried distillery residues are a major concern of the industry for environmentally safe

disposal. Worldwide, researchers have attempted to use this product as silage in agriculture, protein concentrate, animal feed and human nutrition, flavouring agents' production, beverage clarification, enzymatic production, fermentation medium for microorganism, single cell protein production, substrate for micro algae cultivation, biosorption and precipitation of heavy metals, energy and biogas production. The product was successfully utilised in the development of feed for Indian Major Carp (IMC) and minor carp [2, 3]. Such useful and safe utilization/bioconversion can reduce environmental hazards to a great extent.

As marine capture fish production in India is near stagnation, more emphasis is being given towards harnessing the fish production potential of 3.5 million ha reservoirs

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through cage culture [4–6]. The increase in reservoir fish production in India can be attributed to cage farming of introduced catfish species, *Pangasianodon hypophthalmus* [7]. The Ministry of Agriculture, Government of India, has given a special impetus to cage farming in the country’s vast 3.5 mha reservoir resources in order to supplement the nation’s protein needs [8, 9]. This has created a huge demand in the feed market resulting into competition for prime ingredients such as SOC, FM, cereal products etc. The resultant cost escalation of feed making the venture of catfish culture economically unviable. The researchers in the country are, therefore, looking for alternative cost effective ingredients for use in fish feed industry [10]. The bulk availability of rice based DDGS in the country offers potential for its utility in livestock and fish feed.

The successful use of this ingredient in preparing extrusion pellet has been demonstrated and applied in farming of carp species in cages. If, the catfish with relatively low mRGL (mean Relative Gut Length) is equally effective in utilizing this cereal coproduct needs scientific investigation before their use in feed formulation. As Pangas is a new candidate species for cage culture in the reservoirs of India [11, 12], the information on niche of feeding, cost effectiveness, appropriate form of feed and feeding man-

and T3) with 40% brewery waste, completely replacing the soybean meal. The proportion of maize in the T1, T2, and T3 diets was 44.5%, 49.5%, and 49.5%, respectively, and all three diets contained a constant 5% of fish meal. The ingredients were finely ground, blended according to the formulation (Table 1), and extruded to obtain 2 mm floating pellets. For the sinking pellets, the cooked ingredients were pelletized using a hand pelletizer.

The fish in each cage were fed 5% of their body weight per day in two equal portions at 10:00 and 16:00 during the 60-day feeding trial. The feed ration was adjusted every 15 days based on the fish weight. At the end of the trial, all the fish in cages were collected, and their lengths and weights were noted. A proximate biochemical examination was performed on ten fish from each cage. The ingredients, formulation, and proximate biochemical composition of the three experimental diets are listed in Table 1, and the proximate biochemical composition of the ingredients, gross energy, and limiting amino acids are shown in Table 2.

Survival rate

The survival rate of *Pangasianodon hypophthalmus* fingerlings was calculated by counting the number of fish in each cage at the end of the experiment and dividing it by the total number of fingerlings stocked, multiplied by 100. This formula can be expressed mathematically as follows:

$$\text{Survival rate (\%)} = (\text{Total number of fish harvested} / \text{Total number of fingerlings stocked}) \times 100$$

agement are scanty for cage culture. Thus, an attempt has been made to evaluate the replacement of soybean meal by brewery waste in the feed of Pangas to develop cost effective feed. Also, the bioconversion efficiency of brewery waste and economic viability was tested by using different forms of feed (floating and sinking feed).

Materials and methods

Experimental set-up and design

A 60-day feeding experiment was conducted in cages set up at the Maithon reservoir in Jharkhand, India. The cages were stocked with *Pangasianodon hypophthalmus* fingerlings obtained from a commercial hatchery. After a 15-day acclimation period, the trial began. A total of 29,997 fingerlings (mean body weight: 3.8 ± 0.2 g) were evenly distributed (@ 3333 individuals per cage) in nine 5 m x 5 m x 3 m square cages. The fish were fed one of three diets: reference diets (T1: Soybean-based floating) and test diets (T2: Brewery waste-based floating; T3: Brewery waste-based sinking).

Diet preparation and feeding trial

Three floating and sinking diets were prepared: a reference diet (T1) with 44.5% soybean meal and two test diets (T2

Table 1 Formulation and proximate composition of the experimental diets (%)

Ingredients	Percentage inclusion		
	T1	T2	T3
Soybean meal ^a	44.5	0	0
Brewery waste ^b	0	40	40
Maize meal	44.5	49.5	49.5
Fish meal	5	5	5
Vegetable oil	4	3.5	3.5
Vitamin-mineral mix ^c	2	2	2
Proximate composition (calculated on % dry matter basis)			
Crude protein (CP)	28.45	29.02	29.02
Crude lipid	6.00	6.04	6.04
Crude fibre	6.60	8.00	8.00
Ash	4.60	3.93	3.93
Nitrogen-free extract (NFE)	54.30	53.01	53.01
Moisture	6.21	6.00	6.00
Gross energy (KJ/g)	16.66	17.30	17.30
Protein energy ratio (P:E ratio)	17.07	16.90	16.90

^a Crude protein, 44.4%

^b Crude protein, 49.2%

^c Vitamin-mineral mix (Agrimin Forte, Virbac, India)

Table 2 Proximate composition (g/100 g dry matter), gross energy (kJ/g) and limiting amino acid content (g/100 g protein) of ingredients

Proximate composition		
	BW	SM
Crude protein (% DM)	49.2	44.4
Crude lipid (% DM)	3.12	1.7
Crude fibre (% DM)	14.8	11.1
NFE (% DM)	24.1	32.2
Ash (% DM)	7.3	8.5
Gross energy (kJg ⁻¹) ^a	19.6	18.4
Limiting amino acids (g/100 g protein)		
Methionine	1.8	1.4
Cystine	2.1	1.6
Lysine	5.5	6.1

^a Gross energy was calculated using conversion factors of 17.2, 23.6, and 39.5 kJ/g for carbohydrate, protein, and lipid, respectively [14]

Growth parameters and feed utilization

Growth parameters of *Pangasianodon hypophthalmus* fingerlings were measured every 15 days by weighing the fish after an overnight fast. The following formulas were used to calculate weight gain percentage, specific growth rate (SGR), feed conversion ratio (FCR), and protein efficiency ratio (PER):

Weight gain percentage = (Final weight – Initial weight)/ Initial weight * 100%

Specific growth rate (SGR)

SGR = (Ln(Final weight) – Ln(Initial weight))/ Time period in days * 100%

Feed conversion ratio (FCR)

FCR = Feed intake / Weight gain

Protein efficiency ratio (PER)

PER = Weight gain / Protein intake

Proximate analysis and amino acid analysis

Drying to a constant weight means drying the samples until their weight does not change significantly after further drying. This ensures that all of the moisture has been removed. Crude protein is a measure of the total nitrogen content of a sample. It is calculated by multiplying the nitrogen content by 6.25, which is a conversion factor that accounts for the non-protein nitrogen content of most samples. Crude lipid is a measure of the total fat content of a sample. It is

determined by extracting the fat from the sample with a solvent. Ash content is a measure of the inorganic material remaining in a sample after incineration. It is determined by burning the sample at a high temperature in a muffle furnace. Limiting amino acids are the amino acids that are present in the smallest quantities in a sample. They are the most important amino acids for growth and development, and they must be obtained from the diet. Gross energy is the total amount of energy that can be released from a sample when it is completely combusted. It is estimated using conversion factors that are based on the chemical composition of the sample. Moisture content, protein, lipid, ash, limiting amino acids, and gross energy were determined in whole fish and feed samples using the following methods:

- Moisture content: Samples were dried to a constant weight at 105 °C.
- Protein content: Nitrogen was measured in a micro-Kjeldahl apparatus and multiplied by 6.25 to obtain crude protein content.
- Lipid content: Crude lipid content was determined using a Soxhlet apparatus.
- Ash content: Samples were incinerated in a muffle furnace at 550 °C for 6 h [13].
- Limiting amino acids: Samples were acid hydrolyzed with 6 M HCl and analysed using HPLC.

- Gross energy: Gross energy was estimated using conversion factors of 17.2, 23.6, and 39.5 kJ/g for carbohydrate, protein, and lipid, respectively [14].

Cage monitoring and water quality parameters

To ensure the health and well-being of the fish and maintain optimal water quality, the following cage management practices were implemented:

- Cage cleaning: Cages were cleaned regularly to remove algae, biofouling, and debris. This helped to prevent net clogging and ensure proper water exchange.
- Feed intake and health monitoring: The fish's feed intake and health status were assessed on a routine

basis. This allowed for early detection and treatment of any problems.

- Removal of dead fish and leftover feed: Dead fish and leftover feed were regularly removed from the cages to prevent the spread of disease and water quality deterioration.
- Preventative disease treatment: As a preventative measure, fish were given a chemical treatment with potassium permanganate (KMnO₄) and copper sulfate (CuSO₄).
- Water quality monitoring: Water quality parameters such as temperature, pH, dissolved oxygen, transparency, and conductivity were monitored every 15 days using standard methods. This allowed for the identification and correction of any water quality problems [15].

Cost estimation of feed

The cost of producing fish per kilogram was estimated by considering the following factors:

- The cost of feed per kilogram: This was calculated by multiplying the cost of each feed ingredient by its proportion in the diet, and then summing these values.
- The feed conversion ratio (FCR): This is a measure of how efficiently the fish convert feed into body weight. A lower FCR indicates more efficient feed utilization. The cost of feed per kilogram of fish production was calculated using the following formula:

$$\text{Cost of feed per kilogram of fish production} = \text{Cost of feed per kilogram} * \text{FCR}$$

This formula considers both the cost of the feed and the efficiency with which it is used by the fish.

Statistical analysis

The effect of completely replacing soybean meal with brewery waste, as well as the form of feed (floating or sinking), on the estimated parameters of *Pangasianodon hypophthalmus* fingerlings was analyzed using a one-way analysis of variance (ANOVA) at a 5% level of significance using the Statistical Package for the Social Sciences (SPSS) software, version 14.

Results

Cage monitoring and water quality parameters

To ensure the health and well-being of the fish and maintain optimal water quality, a battery of nine cages was monitored on a regular schedule. This included

monitoring the temperature, pH, conductivity, and dissolved oxygen (DO) of the water, as well as the depth and transparency of the experimental sites. During the experimental period, the average water temperature was 28.5 ± 0.67 °C, the average pH was 8.1 ± 0.08 , the average conductivity was 201.4 ± 0.65 µS/cm, and the average DO was 6.95 ± 0.2 mg/L. The average depth of the experimental sites was 19.3 ± 0.52 m, and the average transparency was 84 ± 2.5 cm. This data indicates that the water quality in the cages was within the optimal range for *Pangasianodon hypophthalmus* fingerlings. The temperature was slightly warm, but still within the acceptable range. The pH was also slightly high, but this was likely due to the presence of algae in the water. The conductivity was within the acceptable range, and the DO was slightly above the minimum recommended level. The depth and transparency of the water were also within the acceptable range. Overall, the cage monitoring and water quality data suggest that the fish were kept in a healthy and supportive environment during the experimental period. This is important for ensuring accurate and reliable results from the experiment.

Survival and growth parameters

At the end of the 60-day feeding trial, the survival rate (Fig. 1) of fish fed brewery waste-based floating feed was significantly lower than that of fish fed brewery waste-based sinking feed or soybean-based floating feed ($p < 0.05$). There were also significant differences in growth parameters between fish fed different test diets ($p < 0.05$). Fish fed the brewery waste-based sinking diet (T3) showed the lowest growth in terms of weight gain,

specific growth rate (SGR), protein efficiency ratio (PER), and the highest feed conversion ratio (FCR). Fish fed the brewery waste-based floating feed (T2) showed the highest weight gain, SGR, and PER, and the lowest FCR (Fig. 2A-D)). These differences were statistically significant ($p < 0.05$). However, there was no significant difference in growth parameters or feed utilization between fish fed the soybean-based floating feed and the brewery waste-based floating feed after 60 days of the feeding trial. In other words, the brewery waste-based floating feed was just as effective as the soybean-based floating feed in promoting growth and feed utilization in *Pangasianodon hypophthalmus* fingerlings.

Carcass composition

The initial moisture content, crude protein, ether extract, and ash content of *Pangasianodon hypophthalmus* were

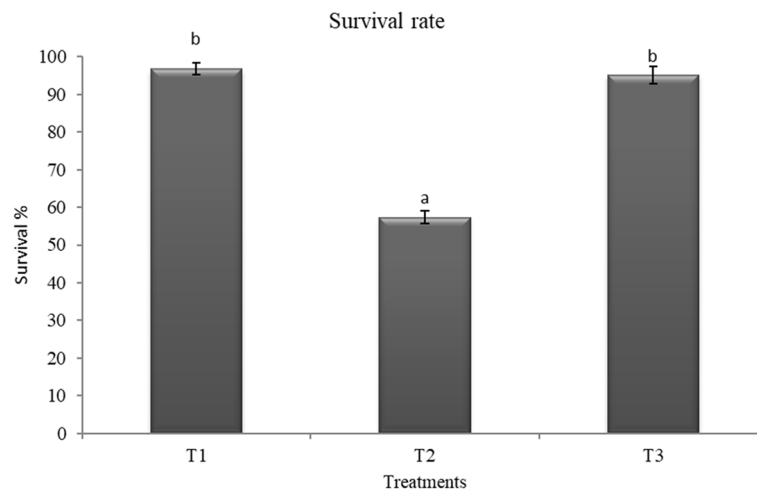


Fig. 1 Survival % of *Pangasianodon hypophthalmus* fed with different experimental diets

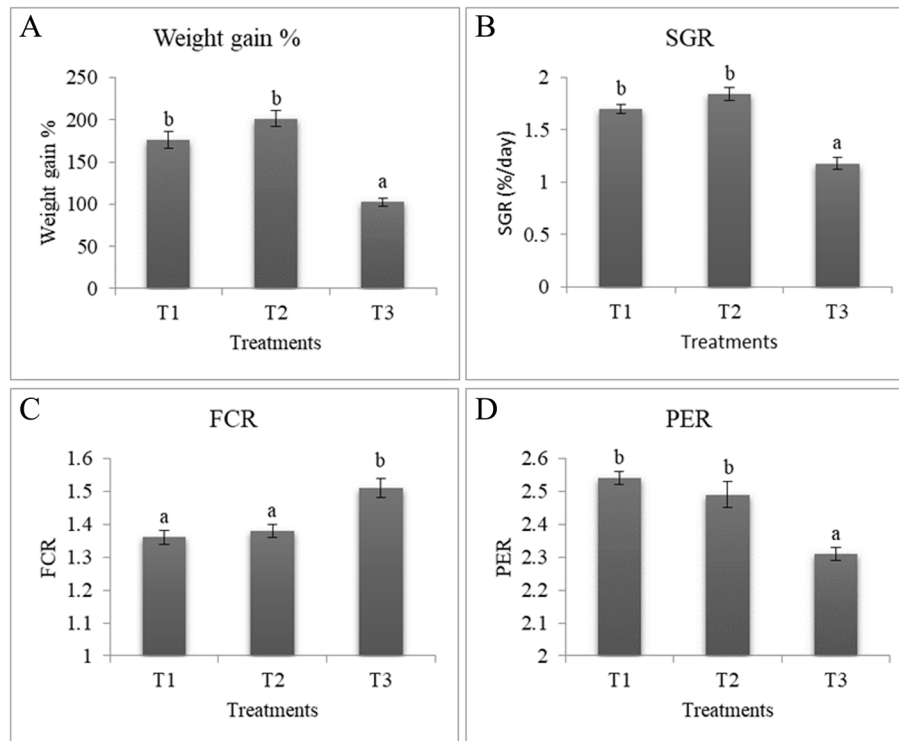


Fig. 2 A-D Weight gain %, SGR, FCR and PER of *Pangasianodon hypophthalmus* fed with different experimental diets

76.05 ± 0.96%, 13.23 ± 0.52%, 6.25 ± 0.31%, and 2.6 ± 0.22%, respectively. After 60 days of feeding, there was a significant variation ($p < 0.05$) in carcass moisture and lipid content between the treatments (Table 3). The carcass lipid content was lowest (10.15 ± 0.29%) and moisture content (72.5 ± 0.30%) was highest ($p < 0.05$) in fish fed the soybean-based floating feed (T1). However, there

was no significant variation ($p > 0.05$) in carcass lipid content or moisture content between fish fed the brewery waste-based floating and sinking feeds. Compared to the initial carcass lipid content, the body lipid content in all treatment groups was significantly higher ($p < 0.05$) after 60 days (Table 3). There was an inverse relationship between carcass crude lipid and moisture content. In

Table 3 Biochemical composition of whole-body tissue of *Pangasianodon hypophthalmus* fingerlings fed with different experimental diets (% wet weight basis) for 60 days

	T1	T2	T3
Moisture	72.5 ± 0.30 ^b	70.14 ± 0.58 ^a	70.11 ± 0.51 ^a
Crude protein	12.12 ± 0.69 ^a	12.08 ± 0.52 ^a	11.06 ± 0.57 ^a
Crude lipid	10.15 ± 0.29 ^a	11.90 ± 0.41 ^b	11.80 ± 0.35 ^b
Ash	2.85 ± 0.11 ^a	3.09 ± 0.17 ^a	3.17 ± 0.18 ^a

Values are means ± SE of three replicates. Values within the same row not sharing a common superscript are significantly different ($p < 0.05$)

Table 4 Estimated feed cost per kg fish production

	T1	T2	T3
Feed required per kg fish production	1.36	1.38	1.51
Cost of 1 kg feed ^a (Rs)	28.29	19.11	17.98
Feed cost (Rs) per kg fish produced	38.47	26.37	27.15

^a Cost of 1 kg feed includes both ingredient cost and manufacturing cost

other words, the soybean-based floating feed produced fish with the lowest body lipid content and highest moisture content, while the brewery waste-based floating and sinking feeds produced fish with similar body lipid and moisture content. All of the feeds resulted in a significant increase in body lipid content over the course of the study. The inverse relationship between carcass crude lipid and moisture content suggests that as the fish accumulated more fat, they lost water.

Estimated feed cost per kg fish production

The cost of preparing 1 kg of soybean-based feed was Rs. 28.29, while the cost of preparing 1 kg of brewery waste-based floating and sinking feed was Rs. 19.11 and Rs. 17.98, respectively (Table 4). This means that by feeding fish brewery waste-based floating feed, the feed cost for producing 1 kg of fish can be reduced by 31.45% compared to feeding them soybean-based floating feed. Similarly, the feed cost for producing 1 kg of fish can be reduced by 29.5% by using brewery waste-based sinking feed compared to the reference diet (T1). In other words, brewery waste-based feed is significantly less expensive to produce than soybean-based feed, and this cost savings can be passed on to consumers in the form of lower fish prices.

Discussion

This study investigated the suitability of distiller's dried grains (DDGs) derived from the brewing industry as an alternative feed ingredient for *Pangasianodon hypophthalmus* in cage farming. Considering the increasing

demand for high-quality feed for this fish species, the objectives of this study focused on evaluating the impact of DDGs on growth, survival, flesh quality, and economic viability. Three primary criteria guided the selection of unconventional protein sources in fish feed: positive impact on growth and health, economic viability, and local availability. Distillery waste, a by-product of the brewing industry, meets these requirements due to its abundant availability, affordability, and proximity to fish farms.

After 60 days of feeding, no significant differences in survival rates were observed among the treatment groups, suggesting that the feed did not affect fish mortality. Growth and conversion efficiency were influenced by the feed form, with floating diets showing superior performance compared to sinking pellets. Specifically, the floating diet consisting of soybean meal and brewery waste yielded the best results in weight gain, specific growth rate, and protein efficiency ratio. These findings demonstrate that *Pangasianodon hypophthalmus* can effectively utilize plant-based ingredients, such as brewery waste, for growth and development, despite possessing a low metabolic rate for glucose. Moreover, the preference for floating feed indicates that collecting feed from the water surface is more convenient for this fish species. Brewery waste has previously proven to be a satisfactory replacement for soybean oilcake in the diet of *Labeo bata*, offering comparable growth performance and flesh quality [2, 3].

This study showcases the potential of using distillery by-products, particularly brewery waste, as a cost-effective and sustainable feed ingredient for *Pangasianodon hypophthalmus* fingerlings [16, 17]. The favorable growth and conversion efficiencies achieved with floating feed forms highlight the advantages of adopting this type of feed presentation for this particular fish species. The outcomes of this investigation contribute valuable information for fish farmers seeking to optimize production efficiency while minimizing production costs.

Brewery waste proves a sustainable and cost-effective fish feed additive, as demonstrated by several studies. Nile tilapia (*Oreochromis niloticus*) fed diets with cotton and brewery waste exhibit enhanced feed conversion ratios (FCR) [18]. Channel catfish weight gain and feed conversion efficiency remained unaffected when fish meal was wholly replaced with distiller's grain soluble (DGS) and soybean meal [19, 20]. Optimal tilapia weight gain occurred with 35% corn DGS in their diet [21]. Nile tilapia fed 82% DGS with lysine and tryptophan supplements boasted diminished FCR and heightened PER [22]. Kaur and Saxena [2] verified that brewery waste supplanting rice bran in fish diets boosted growth in *Catla catla*, *Labeo rohita*, and *Cirrhinus mrigala*. In the presented

study, *Pangasianodon hypophthalmus* fingerlings' whole-body tissue analysis exposed significant ($p < 0.05$) variations in moisture and lipid content between treatments. Lipid content surged considerably post-feeding trial in all treatment groups versus initial amounts. This outcome mirrors *Labeo bata* cultivated in cages [3], potentially ascribable to the feed's elevated Nitrogen Free Extract (NFE) content or restricted fish movement within cages. Physico-chemical parameters, including water temperature, pH, conductivity, and dissolved oxygen, fell within acceptable limits for *P. hypophthalmus* cultivation. Minimal fluctuation in transparency and primary productivity ensued throughout the experiment, signaling negligible impact on fish growth. Regarding finances, implementing treatments T2 and T3 enabled feed cost reductions of 31.45% and 29.5%, respectively, vis-à-vis treatment T1 for every kilogram of produced fish. Consistent with Hassan et al.'s earlier study on *Labeo bata* [3], the feed expense dropped by 33.8% for 1 kg of brewery-based floating feed compared to soybean oilcake counterparts. The lower-priced brewery-derived feed substantiates the potential of employing brewery waste as a protein component in fish diets. Consequently, fish farmers benefit financially, doubling their earnings and fortifying nutritional security nationwide. Moreover, brewery waste holds promise as a renewable feed element, sourced from the brewing industry without extra land or resources. Apart from fish feed, BW yields added-value commodities like bioethanol and biogas, assisting waste reduction and augmenting revenue generation for fish producers [22–24]. All things considered, harnessing BW as a sustainable feed substance seems auspicious. Its capability to enhance growth, feed effectiveness, and farmer profits renders it an appealing option for fish breeders. Additional exploration remains warranted to pinpoint ideal BW proportions in diets catering to diverse species and development phases.

Conclusion

Brewery waste-based floating feed is the most cost-effective and sustainable option for *Pangasianodon hypophthalmus* cage farming. It can also be used as a sinking feed alternative for small-scale or medium-scale farmers. Both brewery waste-based floating and sinking feeds can effectively replace expensive soybean meal-based feed without adversely affecting growth or flesh quality. This study demonstrates that brewery waste is a promising alternative protein source for fish feed, reducing production costs and environmental waste.

Acknowledgements

The authors are grateful to Director, ICAR-CIFRI, Barrackpore for providing the facilities for carrying out the research work.

Authors' contributions

M.A. Hassan-Project monitoring, conceptualisation, MS editing; Md. Aftabuddin- article Review, Editing, D.K. Meena-data analysis, original draft preparation, Mishal P- review and writing, B.K. Das- review and editing, conceptualisation, A.P. Sharma- Fund acquisition, review and editing.

Funding

The study was funded by Indian Council of Agricultural Research, New Delhi under the ICAR outreach activity on Fish Feed.

Availability of data and materials

All the data pertaining to the study are available with the first author and corresponding author and will be available on request.

Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

The Institute Research Committee (IRC) of ICAR-Central Inland Fisheries Research Institute, Kolkata considering the animal care and ethical issues approved the research program and sampling methodology. All the authors consented to participate in the process of publication of the paper.

Consent for publication

All the authors consented to publish the findings of the study.

Competing interests

The authors declare no competing interests.

Received: 27 January 2024 Accepted: 2 September 2024

Published online: 04 December 2024

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