



EFFECT OF AGRO-INDUSTRIAL WASTE FEEDS ON BIOMASS PRODUCTION AND NUTRIENT COMPOSITION OF *TUBIFEX* CULTURED IN A RECIRCULATING AQUACULTURE SYSTEM

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ARTICLE INFO	ABSTRACT
Article History: Received: 5 December 2024 Revised: 30 July 2026 Accepted: 6 August 2026 Published: 15 September 2026 Keywords: <i>Tubifex</i> culture, agro-industrial waste, biomass, proximate composition, RAS	<i>Tubifex</i> worms are widely used as live feed in aquaculture owing to their high nutritional value and suitability for fish larvae. However, conventional production systems rely heavily on organic manures, which raise biosecurity and environmental concerns. This study evaluated four agro-industrial by-products (rice bran, cassava pomace, wheat meal, and tofu dregs) as feed substrates for <i>Tubifex</i> cultured in a Recirculating Aquaculture System (RAS) a land-based, closed-loop technology that raises fish or other aquatic animals by continuously filtering and reusing 90% to 99% of its water. Water quality parameters remained stable within acceptable ranges throughout the experimental period, whereas feed type significantly affected biomass yield and proximate composition. Tofu dregs yielded the highest biomass, while cassava pomace produced worms with the highest crude protein content. Lipid concentrations did not differ significantly among treatments, but carbohydrate and ash contents varied according to the substrate provided. These results demonstrate that agro-industrial residues can be effectively repurposed as culture substrates, enhancing both the productivity and nutritional quality of <i>Tubifex</i>. In particular, tofu dregs exhibited superior performance and considerable potential for valorisation as an aquaculture resource. These findings highlight broader implications for sustainable aquaculture, indicating that waste-to-feed strategies can reduce environmental burdens, lower feed costs, and advance circular support circular bioeconomy practices. Future research should investigate the scalability of this approach, nutrient profiling at amino acid and fatty acid levels, and economic feasibility to facilitate wider adoption.

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Introduction

The aquaculture industry plays a pivotal role in supplying animal-derived protein for human consumption worldwide. By 2022, aquaculture production surpassed capture fisheries for the first time, reaching a total output of 130.9 million tonnes, with major contributions from China, Indonesia, India, Vietnam, Bangladesh, the Philippines, South Korea, Norway, Egypt, and Chile (Boyd *et al.*, 2022; FAO, 2024).

However, the long-term sustainability of this industry faces critical challenges, particularly regarding the steady supply of high-quality and sustainable feed resources. Live feed such as *Tubifex* worms constitutes an essential nutritional source for farmed aquatic species, especially during their sensitive larval and fry stages. Rich in proteins, essential fatty acids, and vitamins, *Tubifex* plays a fundamental role

in promoting early fish growth and survival (Das *et al.*, 2012; Simangunsong *et al.*, 2024).

Despite these advantages, conventional *Tubifex* production remains constrained by land availability, water quality fluctuations, and risk of disease outbreaks. Furthermore, using commercial feeds for worm culture increases operational costs and may contribute to environmental degradation. Consequently, developing more efficient, sustainable, and environmentally friendly culture techniques is urgently required (Strayer, 2006; Oplinger *et al.*, 2011).

One promising approach involves using agro-industrial waste as nutrient sources for *Tubifex*. Agro-industrial residues, including rice bran, wheat bran, tofu dregs, cassava pomace, and vegetable wastes, contain organic matter suitable as growth substrates (Haque & Hasan, 2022; Sharma *et al.*, 2023; Simangunsong *et al.*, 2023). Additionally, applying Recirculating Aquaculture Systems (RAS) enhances water use efficiency and mitigates environmental impacts (Ahmed & Turchini, 2021).

This study evaluates four specific agro-industrial by-products: Rice bran, cassava pomace, wheat meal, and tofu dregs. These materials were chosen because they are widely available locally as low-cost processing residues, offer complementary nutritional profiles across proteins, carbohydrates, and lipids, and present strong opportunities for waste valorisation. Rice bran provides a carbohydrate rich substrate supplying lipids and carbohydrates; cassava pomace contributes a carbohydrate rich matrix from tapioca extraction; wheat meal yields digestible carbohydrates and moderate protein; and tofu dregs serve as a protein-rich residue from tofu manufacturing. Previous research demonstrated that such residues can effectively support annelid culture while reducing organic waste disposal burdens (Ezejiofor *et al.*, 2014; Mandal *et al.*, 2016; Haque & Hasan, 2022). These materials were chosen to evaluate their effects on biomass production and nutrient modulation in *Tubifex* while advancing circular economy practices in aquaculture.

While previous studies explored *Tubifex* culture using livestock manures and agricultural wastes (Singh *et al.*, 2010; Mandal *et al.*, 2016), limited information is available regarding the use of processed agro-industrial by-products under controlled RAS conditions. Furthermore, comparative assessments evaluating how different substrates influence both total biomass yield and the proximate body composition of *Tubifex* remain scarce. Evaluating both parameters is essential for optimising the productivity and nutritional value of live feed.

Therefore, this study aimed to evaluate the effects of four agro-industrial by-products (rice bran, cassava pomace, wheat meal, and tofu dregs) on the biomass production and proximate composition of *Tubifex* cultured in an RAS, in addition, the study assessed the potential of this culture system to support sustainable live-feed production. The findings provide valuable insights into the development of efficient culture techniques while promoting the valorisation of agro-industrial residues into value-added aquaculture resources.

Materials and Methods

Culture Media Preparation

Culture media were prepared by collecting field sludge from local rice fields. The sludge was sun-dried, ground, and passed through a 50-mesh sieve (nominal aperture $\approx 300 \mu\text{m}$) to remove coarse particles. The processed soil material was stored in sealed containers until use.

Feed Preparation

Four agro-industrial organic residues were evaluated as feed substrates: Rice bran, cassava pomace (tapioca waste), wheat meal, and tofu dregs. Rice bran was obtained from local rice milling operations. Cassava pomace was collected from a tapioca processing facility, wheat meal from a flour mill, and tofu dregs from a local tofu manufacturing plant. High moisture ingredients were oven dried to constant weight. The dried materials were ground into fine powders and passed through a 100-mesh

sieve (nominal aperture $\approx 150 \mu\text{m}$) to obtain uniform particle sizes suitable for ingestion by *Tubifex* (US standard sieve designations are used; nominal aperture sizes are provided for clarity). The prepared feed powders were stored in airtight plastic containers until use.

Culture Technique

Worm culture was conducted in a vertically arranged RAS. Water circulation was powered by a submersible pump (SAKKAI PRO ECO 105; flow rate: $2,600 \text{ L h}^{-1}$) with flow rates regulated by inline valves. Rearing basins were arranged vertically across four tiers. Water flowed continuously in series from the top tier down to a bottom reservoir equipped with a filter. Each rearing basin was filled with a 2 cm layer of crushed paddy field mud substrate and stocked with 25 g of an initial *Tubifex* colony. Daily feeding consisted of 1 g of organic feed powder per basin. Continuous supplemental aeration was provided to maintain adequate dissolved oxygen levels (Huang *et al.*, 2007). Partial water exchanges of 20% to 30% were performed regularly to maintain water quality and prevent metabolite accumulation (Datta, 2012).

Experimental Design

The experiment evaluated four feed treatments in triplicate, resulting in a total of 12 culture units in total. All units were maintained under identical RAS conditions with uniform aeration and partial water exchange. Each tank received one of the four agro-industrial feeds at a rate of 1 g day^{-1} . As no separate feed or commercial feed control was included, comparative evaluations were made strictly among the four feed treatments.

Biomass Production

Biomass harvest was performed on days 15 and 30. Water was drained from each tank, and the bottom medium was sectioned and washed through fine nylon mesh to separate *Tubifex* worms. Collected worms were rinsed in clean

water to remove remaining sediment. The harvested *Tubifex* biomass was gently blotted with absorbent paper to remove excess moisture and weighed on a precision balance.

Proximate Analysis

Moisture content was determined thermogravimetrically by oven drying samples at 105°C for three hours until constant weight. Total ash content was determined by initial heating at 200°C for one hour followed by muffle furnace incineration at 600°C for 30 minutes, after which samples cooled inside the furnace for five hours before weighing. Crude protein content was determined via the Kjeldahl method, and crude lipid was extracted using the Soxhlet solvent extraction method. Total carbohydrate content was calculated by difference: $100 - (\% \text{ moisture} + \% \text{ ash} + \% \text{ crude protein} + \% \text{ crude lipid})$. All proximate analyses were conducted in triplicate (Sharif *et al.*, 2015; Junianto *et al.*, 2020).

Water Quality

Water temperature and dissolved oxygen were measured twice daily at 08:00 and 16:00 using a YSI Pro2030 meter (YSI Incorporated, Yellow Springs, Ohio, USA). Water pH and total dissolved solids (TDS) were measured daily using a 5-in-1 TDS EC pH meter. Every three days, a 50% water exchange was performed along with siphoning of accumulated feed debris and faecal matter.

Statistical Analysis

Data for biomass production and proximate composition were analysed using one-way analysis of variance (ANOVA) and reported as mean $\pm$ standard deviation (SD). Differences among treatment means were evaluated using Duncan's Multiple Range Test (DMRT) at a significance level of $p < 0.05$. Water quality parameters are reported as minimum and maximum range values. All statistical analyses were conducted using IBM SPSS Statistics version 22.

Results

Tubifex Culture in Recirculating Aquaculture System (RAS)

The RAS configuration and the initial *Tubifex* culture setup are presented in Figure 1. Observations throughout the culture period indicated that the vertically configured RAS provided sufficient water circulation for *Tubifex* cultivation. The use of paddy-field mud as a rearing substrate alongside organic feed facilitated successful colony establishment and growth. Continuous aeration and regular partial water exchanges maintained stable environmental conditions, thereby creating a favourable culture environment for *Tubifex* development. Efficient water utilisation and stable environmental parameters within this system enhanced worm biomass productivity, demonstrating the effectiveness of the RAS for *Tubifex* culture.

Tubifex Biomass Production

Tubifex biomass production varied significantly across feed treatments and sampling intervals (Figure 2). On day 15, mean biomass ranged from 27.77 ± 1.52 g in the rice bran treatment to 46.17 ± 6.84 g in the tofu dregs treatment. No significant differences were observed among rice bran, cassava pomace, and wheat meal treatments at day 15, whereas the tofu dregs treatment yielded significantly higher biomass ($p < 0.05$). By day 30, biomass increased across all treatments. Tofu dregs produced the highest biomass at 95.60 ± 11.04 g, followed by cassava pomace (63.77 ± 6.56 g), rice bran (62.43 ± 4.10 g), and wheat meal (57.20 ± 3.32 g). Statistical analysis confirmed that biomass in the tofu dregs treatment was significantly greater than in all other treatments at day 30 ($p < 0.05$). Tofu dregs also achieved the highest relative biomass increase between day 15 and day 30, indicating superior growth support over time.

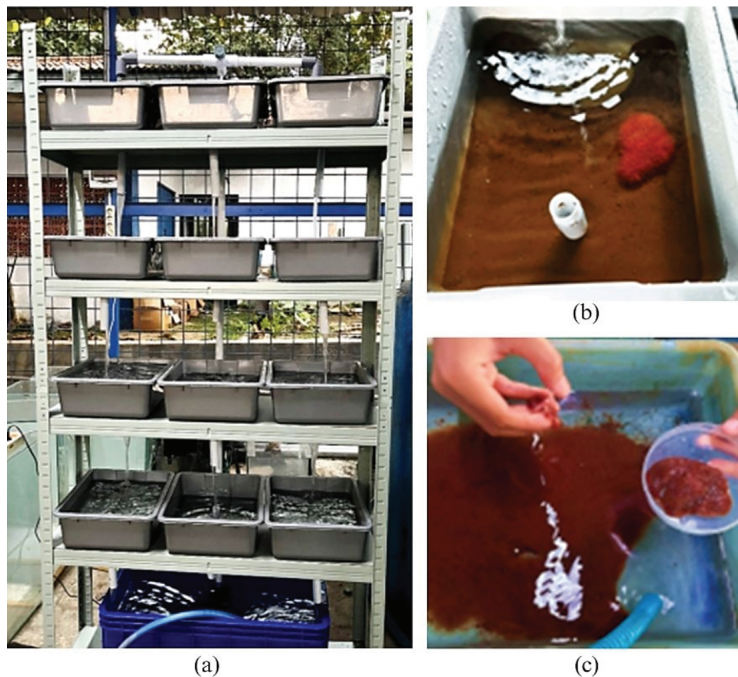


Figure 1: Recirculating aquaculture system (RAS) setup and *Tubifex* culture. (a) arrangement of culture units within the RAS; (b) initial culture of *Tubifex* in the system; (c) harvested *Tubifex* after 30 days of culture

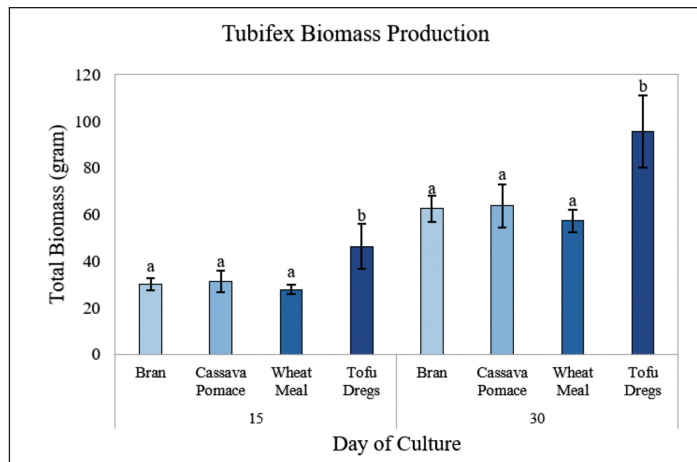


Figure 2: Total biomass production of *Tubifex* (g), all values are expressed as mean $\pm$ SD; different superscript letters on the chart indicate significant differences ($p < 0.05$)

Proximate Composition of *Tubifex*

The proximate composition of *Tubifex* cultured using different agro-industrial feed substrates is presented in Table 1. Moisture content ranged from $71.60\% \pm 0.70\%$ (tofu dregs) to $78.90\% \pm 0.97\%$ (rice bran). Ash content varied between $14.47\% \pm 0.53\%$ (rice bran) and $17.08\% \pm 0.48\%$ (wheat meal). Crude protein content ranged from $4.60\% \pm 0.11\%$ (rice bran) to $4.86\% \pm 0.06\%$ (cassava pomace). Lipid content did not differ significantly among treatments, ranging between 0.86% and 0.95% . Carbohydrate content was lowest in rice bran fed worms ($1.16\% \pm 0.46\%$) and highest in tofu dregs fed worms ($5.75\% \pm 0.45\%$). Significant differences ($p < 0.05$) were detected in moisture, ash, protein, and carbohydrate contents among treatments, whereas lipid content remained

statistically similar across all treatments. Overall, feed substrate influenced both biomass production and proximate composition, with observed variations reflecting differences in the nutritional characteristics of the respective substrates.

Water Quality

The Recirculating Aquaculture System (RAS) used in this study was able to maintain stable water quality throughout the culture period, providing suitable conditions for *Tubifex* rearing. The ranges of water quality parameters recorded across the different feed treatments are presented in Table 2. Temperature values were maintained between 24.2°C and 27.3°C ,

Table 1: Proximate composition of *Tubifex* worms under different treatments (mean $\pm$ SD, wet weight basis)

Parameter	Treatment			
	Bran	Cassava pomace	Wheat Meal	Tofu Dregs
Moisture (%)	78.90 ± 0.97^c	73.76 ± 0.49^b	74.30 ± 0.86^b	71.60 ± 0.70^a
Ash (%)	14.47 ± 0.53^a	16.64 ± 0.71^b	17.08 ± 0.48^b	17.03 ± 1.25^b
Protein (%)	4.60 ± 0.11^a	4.86 ± 0.06^b	4.69 ± 0.06^{ab}	4.76 ± 0.06^{ab}
Lipid (%)	0.87 ± 0.04^a	0.95 ± 0.01^a	0.86 ± 0.05^a	0.86 ± 0.04^a
Carbohydrate (%)	1.16 ± 0.46^a	3.79 ± 1.14^{bc}	3.07 ± 1.68^{ab}	5.75 ± 0.45^c

Note: Different superscript letters in the same row indicate significant differences ($p < 0.05$)

Table 2: Water quality parameters in *Tubifex* culture tanks with different treatments, all values are reported within as ranges

Parameter	Treatment			
	Bran	Cassava pomace	Wheat Meal	Tofu Dregs
Temperature (°C)	24.3–27.3	24.2–27.1	24.3–27.1	24.4–27.2
pH	7.3–7.8	7.3–7.7	7.3–7.7	7.3–7.7
DO (mg L ⁻¹)	4.40–6.20	4.42–6.11	4.44–6.05	4.40–6.23
TDS (ppm)	148–177	149–177	149–178	148–177

with minimal variation among treatments. The pH ranged from 7.3 to 7.8, indicating neutral to slightly alkaline conditions across all tanks. Dissolved Oxygen (DO) levels varied between 4.40 mg L⁻¹ and 6.23 mg L⁻¹, while total dissolved solids (TDS) were stable, ranging from 148 mg L⁻¹ to 178 mg L⁻¹. These values are within the general ranges previously reported to be adequate for *Tubifex* survival and culture. No mortality events or system failures were observed during the experiment, suggesting that the RAS configuration was effective in maintaining environmental stability.

Discussion

This study evaluated the effects of four agro-industrial by-products (rice bran, cassava pomace, wheat meal, and tofu dregs) on the biomass production and proximate composition of *Tubifex* cultured in a recirculating aquaculture system (RAS). Overall, the findings demonstrate that feed type significantly influences both biomass yield and the nutritional quality of *Tubifex*. These results highlight the potential of agro-industrial residues as low-cost substrates for sustainable live feed production, while pointing to key practical and scientific considerations for system scaling and application.

Tubifex biomass increased steadily from day 15 to day 30 across all treatments, confirming that worms can achieve substantial growth in RAS when fed agro-industrial residues. However, the rate of biomass production differed among feed types. Tofu dregs produced the highest biomass yield, significantly outperforming all other treatments by day 30. This finding aligns

with previous studies identifying soybean-based residues as effective substrates owing to their high protein and carbohydrate contents, which provide essential energy and support tissue synthesis (Herawati *et al.*, 2016; Permatasari *et al.*, 2021). In contrast, rice bran, wheat meal, and cassava pomace resulted in lower biomass yields, though cassava pomace ranked second by day 30, suggesting its high carbohydrate composition supports growth during later culture stages. This growth pattern agrees with earlier reports showing that organic substrates such as livestock manures and agricultural residues produce variable biomass depending on nutrient availability and digestibility (Singh *et al.*, 2010; Mandal *et al.*, 2016; Khaerunnisa *et al.*, 2021). The high performance of tofu dregs holds notable sustainability value because this byproduct is generated in large quantities by tofu processing industries and often presents disposal challenges. Repurposing it for live feed production simultaneously alleviates waste burdens and enhances aquaculture feed sustainability (Ezejiofor *et al.*, 2014; Wadhwa & Bakshi, 2016).

Proximate analysis revealed that feed substrate affected both biomass yield and the nutritional profile of *Tubifex*. Moisture content was highest in worms fed rice bran, whereas worms fed tofu dregs exhibited the lowest moisture and highest carbohydrate content. These differences may reflect substrate water content as well as physiological adaptations of *Tubifex* to ingested feeds. Ash content was lowest in worms fed rice bran, potentially owing to lower mineral supply from bran or variations in mineral assimilation efficiency.

Protein content reached its highest level in worm fed cassava pomace, indicating that this substrate may facilitate nitrogen assimilation or offer higher amino acid digestibility. In contrast, lipid levels did not differ significantly among treatments, suggesting that lipid metabolism in *Tubifex* is less sensitive to dietary variation or that feed lipid levels were uniformly low. These findings agree with earlier reports demonstrating that dietary inputs can manipulate the nutrient composition of live feeds to meet the nutritional requirements of cultured fish larvae (Herawati *et al.*, 2016; Mandal *et al.*, 2018).

These results imply that feed substrates can be selected strategically to cultivate *Tubifex* with targeted nutrient profiles. Tofu dregs, for example, increase overall biomass while boosting carbohydrate reserves, which can enhance energy supply to fish larvae. In contrast, cassava pomace enriches protein content, improving the value of *Tubifex* as a protein source. Nutritional modulation offers clear advantages in aquaculture hatcheries, where larval fish require specific balances of protein, lipid, and carbohydrate for optimal growth and survival. Agro-industrial residues thus serve as functional tools to enhance both the productivity and quality of live feeds while advancing circular economy strategies.

Water quality parameters remained within acceptable ranges for *Tubifex* growth throughout the experiment. Temperature, pH, and Total Dissolved Solids (TDS) exhibited minor fluctuations, aligning with reports that RAS maintains stable environmental conditions (Badiola *et al.*, 2018; Goddek & Keesman, 2020). In contrast, Dissolved Oxygen (DO) levels varied notably among feed treatments. Lower DO concentrations in certain treatments were likely driven by higher organic loading, as elevated organic matter stimulates microbial respiration and increases oxygen consumption (Khursheed & Kazmi, 2011; Yohana *et al.*, 2024). Additionally, higher *Tubifex* density increases respiratory activity, compounding oxygen depletion (Oplinger *et al.*, 2011). Managing both feed substrate and stocking

density is therefore essential to maintain adequate oxygen availability, as insufficient DO can restrict growth performance (Pandian, 2019; Lal & Mogalekar, 2024).

Although nitrogenous compounds such as ammonia, nitrite, and nitrate were not directly measured, monitoring these parameters in future studies is crucial because they directly reflect organic matter decomposition and nitrification dynamics (de Valk, 2021; Garai *et al.*, 2022; Lindholm-Lehto, 2023). However, nitrite accumulation also needs to be avoided as it may inhibit *Tubifex* growth. Therefore, regular monitoring of ammonia, nitrite, and nitrate parameters is essential to maintain water quality and support optimal growth of *Tubifex* (Tian & Lu, 2010; Owalo *et al.*, 2022).

Despite these promising results, several limitations should be acknowledged. First, the absence of a dedicated control group, such as a no feed baseline or commercial diet benchmark, limits the ability to quantify the absolute performance of agro-industrial substrates. Second, the 30 day culture period was relatively short, necessitating longer term evaluations to verify whether these growth trends persist across extended culture cycles. Third, the proximate analysis did not evaluate amino acid or fatty acid profiles, which are necessary to fully assess the nutritional composition of *Tubifex*. Fourth, microbial community dynamics were not examined, even though microbial activity drives organic matter decomposition and strongly influences water quality and worm nutrition. Finally, the practical scalability of tofu dregs warrants further consideration, as collection, storage, and transport logistics may affect substrate feasibility in commercial scale operations, particularly in regions where tofu processing is seasonal or decentralised.

From a system perspective, this study demonstrated the utility of RAS for *Tubifex* cultivation. RAS provides superior water quality control relative to traditional flow through or static systems, although it demands higher capital and operational expenditure (Badiola *et al.*, 2018; Ahmed & Turchini, 2021). Further

comparative evaluations between RAS and conventional culture methods are necessary to assess the cost effectiveness and ecological efficiency of each approach for *Tubifex* production. Although RAS offers distinct advantages in water conservation and effluent management, traditional culture methods may remain more practical for small scale or resource limited farmers. Coupling economic feasibility assessments with biological performance will provide a more robust basis for decision making in sustainable aquaculture.

In summary, this study confirms that agro-industrial by-products can effectively support *Tubifex* growth and nutritional enhancement in RAS. Tofu dregs proved to be the most effective substrate for biomass production, whereas cassava pomace enhanced protein content. These findings reinforce the potential of waste-to-feed strategies in advancing sustainable aquaculture, while highlighting the need to address practical constraints such as feed supply logistics, system scalability, and long-term nutrient evaluation. Future research should incorporate appropriate control groups, extended culture periods, amino acid and fatty acid profiling, and economic assessments of substrate utilization. Addressing these factors will optimise *Tubifex* production, improve live feed quality, and contributing to sustainable food systems.

Conclusions

This study demonstrated that agro-industrial by-products serve as effective substrates for *Tubifex* production in RAS, with tofu dregs yielding the highest biomass and cassava pomace resulting in the highest crude protein content. These results highlight the dual benefits of repurposing food-processing residues: Improving live feed production while supporting waste reduction and circular bioeconomy objectives. Future research should evaluate the commercial scalability of this approach, incorporating cost-benefit analyses, long-term culture performance, and detailed nutrient profiling at the amino acid and

fatty acid levels. Attention should also be given to handling logistical considerations such as the collection and storage of tofu dregs. Integrating waste-to-feed strategies enables aquaculture operations to enhance sustainability, reduce dependence on conventional feed resources, and minimise environmental impacts.

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Author Contributions Statement

TS conceptualised the study, designed the experimental framework, carried out the research, and wrote and revised the manuscript. RW, ZNR, and DMRW conducted the culture experiments, collected the data, and contributed to data analysis. JA assisted with experimental work, analysed the results, and contributed to manuscript preparation. AE performed the statistical analysis and refined the methodology. EL conducted the proximate composition analysis and assisted in interpreting the results. RF supported data collection, literature review, and the preparation of figures and tables. PS contributed to manuscript editing, critical revision, and approved the final version of the manuscript.

Conflict of Interest Statement

The authors declare that there are no conflicts of interest regarding the publication of this article, including financial, institutional, or funder-related conflicts.

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