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Research Article

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Optimation of tempe liquid waste usage for growth performances and feed uptake on *Oreochromis niloticus*

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ABSTRACT

Tempe liquid waste (TLW) contains nutrients such as fat, protein, carbohydrates, nitrogen, phosphate, and potassium that could be used in fish rearing. Moreover, it has lactic acid bacteria (LAB) belonging to gram-positive microorganisms, which could decompose organic and inorganic matter in water. Therefore, this study aimed to determine whether TLW could have the potential to enhance Tilapia (*Oreochromis niloticus*) growth and improve feed efficiency. The study used 180 fish (6.04 ± 0.13 g) reared in twelve aquariums and TLW was obtained from boiling soybeans during the tempe production process in the village of Sanan, Malang. The research design was Completely Randomized Design (CRD), which involved four treatments, T0 (no TLW), T1 (3%), T2 (5%), and T3 (7%) with three replications. The result showed that T2 resulted in the highest growth rate (11.22 ± 0.29 g), specific growth rate (2.07 ± 0.32% / day), feed conversion rate (1.54 ± 0.16), feed efficiency (56.02 ± 1.24%) and survival rate (91.11 ± 0.58%) compared to others. Nonetheless, there was no difference in the absolute length (Lm) parameter at the end of the research. The research results also found that water quality remained favourable for supporting tilapia cultivation under all observed conditions, except for the oxygen levels in the control group, which fell below the standard (<3 mg L^{-1}). Finally, the present study concluded that 5% of TLW could be the best; in contrast, the higher concentration of TLW decreased *O. niloticus* growth performance, feed efficiency, and survival rate.

Keywords: fish development, tilapia, wastewater, water qualities

ABSTRAK

Tempe liquid waste (TLW) mengandung nutrisi seperti lemak, protein, karbohidrat, nitrogen, fosfat, dan kalium yang dapat digunakan dalam budidaya ikan. Selain itu, TLW mengandung bakteri asam laktat (LAB) yang termasuk mikroorganisme gram-positif, yang dapat mendekomposisi bahan organik dan anorganik di dalam air. Oleh karena itu, penelitian ini bertujuan untuk menentukan apakah TLW memiliki potensi untuk meningkatkan pertumbuhan Tilapia (*Oreochromis niloticus*) dan meningkatkan efisiensi pakan. Penelitian ini menggunakan 180 *O. niloticus* (6.04 ± 0.13 g) yang dipelihara dalam dua belas akuarium, dan TLW diperoleh dari proses perebusan kedelai pada produksi tempe di desa Sanan, Malang. Desain penelitian merupakan Rancangan Acak Lengkap (RAL), yang terdiri dari empat perlakuan, T0 (tanpa TLW), T1 (3%), T2 (5%), dan T3 (7%) dengan tiga kali ulangan. Hasil penelitian menunjukkan bahwa T2 menghasilkan laju pertumbuhan tertinggi (11,22 ± 0,29 g), laju pertumbuhan spesifik (2,07 ± 0,32% / hari), rasio konversi pakan (1,54 ± 0,16), efisiensi pakan (56,02 ± 1,24%) dan kelulushidupan (91.11 ± 0.58%) dibandingkan dengan perlakuan lainnya. Namun, tidak ada perbedaan nyata pada parameter panjang mutlak (Lm). Hasil penelitian juga menemukan bahwa kualitas air masih dalam kondisi baik dalam mendukung budidaya ikan nila pada semua perlakuan selama pengamatan, kecuali pada kadar oksigen kelompok kontrol yang berada di bawah standar (<3 mg L^{-1}). Penelitian menyimpulkan bahwa 5% TLW merupakan perlakuan terbaik; sebaliknya, konsentrasi TLW yang lebih tinggi justru mengurangi kinerja pertumbuhan *O. niloticus*, efisiensi pakan, dan tingkat kelangsungan hidup.

Kata kunci: air limbah, perkembangan ikan, kualitas air, tilapia

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1. Introduction

Soybeans are commonly used in traditional Indonesian meals, both in fermented and non-fermented forms Astuti et al. (2000). Tempeh, which originated in Java during the 1700s, represents an adaptation of tofu to Indonesia's tropical climate. It is produced through a controlled fermentation process that transforms soybeans into a cake-like form (Astuti et al., 2000; Wijaya, 2019). Most tempe is produced through small-scale home production, yielding between 10 kilograms and four metric tons of tempe daily (Ahnan-Winarno et al., 2021). However, this production process generates liquid waste, which poses environmental pollution challenges and is difficult to manage after contamination (Hartini et al., 2018; Putra et al., 2018). It is a byproduct of tempe production, resulting from soaking, boiling, washing, and stripping processes (Riva et al., 2014). Unfortunately, home-scale tempe industries struggle to handle wastewater, leading to the release of liquid waste into sewers and rivers (Anggraini, 2019; Setiawati et al., 2019). This liquid waste contains nitrogen (N_2), oxygen (O_2), hydrogen sulfide (H_2S), ammonia (NH_3) (Harahap, 2013), carbon dioxide (CO_2), and methane (CH_4) (Mahfoedz, 2014), contributing to river pollution and promoting algae blooms due to organic compound accumulation (Supinah et al., 2020; Utami et al., 2018).

Tempe liquid waste (TLW) is a by-product of tempe production that has also nutritional and microbial benefits. According to Prasetio and Widyastuti (2020), TLW contains 0.42 g of protein, 0.13 g of fat, 0.11 g of carbohydrate, 98.87 g of moisture, 13.6 ppm of calcium, 1.74 ppm of phosphorus, and 4.55 ppm of iron. Sari and Rahmawati (2020) further explained that the protein, carbohydrate, and fat content of TLW varies depending on the source of the liquid waste. They found that the cooking water of soybeans had higher levels of these nutrients (0.47 g, 4.06 g, and 0.04 g, respectively) than the soaking water (0.20 g, 1.47 g, and 0.02 g, respectively). Moreover, Amaliah et al. (2018) isolated eight bacterial strains from soybean-soaking liquid waste, seven of which were identified as lactic acid bacteria (LAB). Santosa and Retnaningrum (2020) also detected *Lactobacillus fermentum*, a type of LAB, in tempe liquid waste using Bergey's Manual of Systematic Bacteriology. Some arguments contemplated that LAB are gram-positive bacteria that can act as probiotics and aid in the digestive process, such as *Lactobacillus acidophilus* (Quinto et al., 2014; Soomro et al., 2002). They can also ferment organic matter into lactic acid compounds in the environment (Bujna et al., 2018) and enhance fish growth by optimizing nutrient absorption in the gut (Ringø et al., 2020). Therefore, TLW has the potential to improve fish growth through its prebiotic action, based on its nutritional and microbial properties.

In this study, we used tilapia (*Oreochromis niloticus*) as a sample to test the effect of TLW on fish growth. Tilapia is a valuable freshwater fish that can adapt to various water qualities and feed sources (El-Sayed, 2019; Gu et al., 2019; Montoya-Camacho et al., 2019). In addition, they have a high protein content and low-fat content, making them a nutritious fish product (Rieuwpassa, 2022; Zhang et al., 2020). Therefore, the demand for tilapia is increasing along with the global population growth and the awareness of the health benefits of consuming lean protein (Salsabila & Suprapto, 2018). Consequently, the rising tilapia population also implies an increased need for commercial feed, which accounts for about 60% or more of the production cost (Montoya-Camacho et al., 2019). Hence, finding alternative feed sources that are cheaper and richer in nutrients is crucial for sustainable tilapia culture. Based on the previous review, this study aimed to investigate the potential of TLW to enhance the growth and feed efficiency of *Oreochromis niloticus*. In addition, we also hypothesized that TLW might enhance those parameters through its prebiotic action, given its nutritional and microbial properties.

2. Material and Methods

2.1. Research design

This study was a follow-up of the research conducted by Heryanto (2020), which used a completely randomized design (CRD) with four treatments and three replications. Tempe liquid waste (TLW), obtained from the process of cooking soybeans to produce tempe was arranged into numerous concentrations. The study lasted for 30 days to observe the effect of TLW on *O. niloticus* growth which was measured at the beginning and the end of the study. On the other hand, the survival rate and water quality, such as pH, temperature, dissolved oxygen (DO), and ammonia, were monitored weekly.

2.2. Fish preparation

Oreochromis niloticus, 6.04 ± 0.13 g in weight were obtained from a commercial freshwater fish farm at the Pendem Fish Seed Center in Junrejo District, Batu City, East Java, Indonesia. One hundred and eighty tilapia were acclimatised at 28°C to 30°C with continuous aeration and fed with commercial feed at 3% of their body weight per day for two weeks before the experiment in the Fishery Laboratory of the Aquaculture Department at the University of Muhammadiyah Malang. Then, 12 tanks (20 cm × 30 cm × 30 cm) containing 18 L of water with aeration and pH control were prepared for four treatments and 15 tilapia were randomly allocated to each tank.

2.3. Media preparation

The experimental medium consisted of fresh water and TLW with various concentrations, namely T0 (no TLW), T1 (3% TLW), T2 (5% TLW), and T3 (7% TLW). TLW was obtained from a tempe company in Sanan, Malang. The TLW was transported in sterile containers and stored at 4°C until use. The TLW was left for three days to reduce the dissolved organic matter in a tank. The TLW was diluted with water and aerated to decrease the concentration of nutrients and contaminants before being transferred to a separate container. This process aimed to break down organic matter and reduce the biological oxygen demand (BOD) and facilitate the decomposition of organic matter by aerobic bacteria. High BOD and organic matter levels, often associated with pollution, can lead to a reduction in dissolved oxygen concentration and impair the metabolism of aquatic organisms, which is vital for fish life cycles (Arvanitoyannis & Tserkezou, 2014; Nesje, 2018; Schindler Wildhaber et al., 2012). Afterwards, the TLW was adjusted to the desired concentrations and mixed with the water media in each tank. Moreover, TLW was applied one day before the research started and regularly added after changing the water media weekly during fish culture according to the treatments.

2.4. The effect of the TLW on *O. niloticus* growth performances

The growth performance of *O. niloticus* was evaluated, using various indicators such as growth rate (GR), specific growth rate (SGR), absolute length growth (Lm), and feed utilization parameters. Meanwhile, the feed utilization parameters included feed conversion rate (FCR) and feed efficiency (EF), which were calculated based on the formula given by Dangeubun et al. (2019).

$$\text{GR (g)} = \text{Wt} - \text{W0}$$

$$\text{SGR (\% d–1)} = \frac{\text{Ln Wt} - \text{Ln Wo}}{\text{t}}$$

$$\text{Lm (cm)} = \text{Lt} - \text{L0}$$

$$\text{FCR} = \frac{\text{F}}{\text{Wt} - \text{W0}}$$

$$\text{FE (\%)} = \frac{\text{Wt} - \text{W0}}{\text{F}}$$

Note:
GR = Growth rate (g)
SGR = Specific Growth Rate (% d^{-1})
FCR = Feed Conversion Ratio
Lm = Absolute length (cm)
F = Feed consumption during cultivation (kg)
W0 = Initial weight (g)
Wt = Final weight (g)
W0 = Initial length (cm)
Wt = Final length (cm)
t = Time (day)

The water quality parameters, such as acidity (pH), temperature (°C), dissolved oxygen (DO), and ammonia, were monitored weekly during the experiment utilizing a pH meter, a thermometer, and a DO meter type 5510, respectively. Additionally, ammonia was measured using the SERA Water Test Kit at the beginning and the end of the experiment (Hertika et al., 2021; Samsundari & Wirawan, 2013).

2.5. Statistical analysis

The present study used the SPPS tool to analyze variance (ANOVA) for the significance of all growth performance data caused by adding TLW to freshwater media. In addition, Duncan's Multiple Range Test was used to examine the data that revealed differences across treatments.

3. Result and Discussion

3.1. Growth performances

The growth parameters of tilapia, including growth rate (GR), specific growth rate (SGR), and

Table 1. Growth rate (GR), Specific daily growth (SGR), and absolute length

Parameter	***Treatment***			
	T0 (0 %)	***T1 (3 %)***	***T2 (5 %)***	***T3 (7 %)***
Growth rate (g)	7.12 ± 0.13^{a}	7.42 ± 1.16^{c}	11.22 ± 0.29^{d}	8.81 ± 0.58^{b}
Spesific growth rate (% / day)	0.55 ± 0.10^{a}	0.68 ± 0.32^{a}	2.07 ± 0.32^{c}	1.26 ± 0.02^{b}
Absolute length (cm)	4.26 ± 0.20^{a}	5.73 ± 0.28^{a}	6.43 ± 0.33^{a}	5.44 ± 0.31^{a}

The various letters in the same row indicate significant changes between treatments.

absolute length, were assessed for each treatment over a 30-day rearing period (Table 1). Statistical analysis revealed a significant difference ($p < 0.05$) among the treatments for these parameters. Over one month of rearing, T2 exhibited the highest growth rate for *O. niloticus* (11.22 ± 0.29 g), while the control group (T0) had the lowest growth rate (7.12 ± 0.13 g). In the specific growth rate (SGR) evaluation, T1 was the least recommended treatment, achieving only 0.68 ± 0.32% per day, which was statistically insignificant compared to T0 (the control group) at 0.55 ± 0.10% per day. Conversely, T2 demonstrated the highest SGR level at 2.07 ± 0.32% per day, while T3 stimulated tilapia SGR just below that of T2. Finally, there was no statistically significant effect on *O. niloticus* absolute length resulting from various TLW dosages ($p>0.05$).

Table 2 illustrates the impact of TLW on tilapia feed efficiency (FE) and Feed Conversion Ratio (FCR) after 30 days of rearing. Notably, all TLW dosages significantly outperformed the control group (T0), with T2 showing the most promising results. Regarding FE, all treatment data differed significantly from the control (T0) ($p<0.05$) and T2 achieved the highest FE at 56.02%, followed by T1 and T3. Similarly, in FCR evaluation, T2 stood out by reducing FCR by 1.54 over the 30-day study period, while statistically, T1 and T3 also performed similarly (b), differing from the control group. The study suggests that TLW enhances feed efficiency and FCR in fish by providing easily digestible nutrients, beneficial probiotics, and digestive enzymes (Amaliah et al., 2018; Prasetio & Widyastuti, 2020; Sari & Rahmawati, 2020). These components improve nutrient absorption, accelerate growth rates, and enhance overall fish health, resulting in more efficient feed utilization. Based on these findings, TLW is recommended for *O. niloticus* culture, particularly at a 5% TLW dosage.

The growth performance and feed utilization efficiency of juvenile fish, including *O. niloticus*, are influenced by factors such as dissolved oxygen, pH, temperature, nitrogen, genetics, food quantity and quality, and sex (Abdel-Tawwab et al., 2015; Eldridge et al., 2015; Lawrence et al., 2020; Madenjian et al., 2016; Parra & Baldisserotto, 2019; Tsai et al., 2015; Urbina & Glover, 2015; Workagegn et al., 2014). Our study specifically found that only T2, with 5% TLW, significantly affected the growth rate (GR), specific growth rate (SGR), feed efficiency (FE), and feed conversion ratio (FCR) of *O. niloticus*, except for length measurements (Lm). Interestingly, higher TLW concentrations were associated with reduced fish growth, as observed in the T3 data (Table 1). This phenomenon could be attributed to various harmful chemicals present in TLW, such as N_2, H_2S, NH_3, CO_2, and CH_4, despite undergoing treatment before use. For example, exposure to elevated CO_2 concentrations led to metabolic disorders, histopathological injuries, and growth retardation in *Scophthalmus maximus* (Guo et al., 2022). Similarly, NH_3 presence decreased growth and disrupted hormone systems involved in growth regulation, including the growth hormone/insulin-like growth factor (GH/IGF) and hypothalamic-pituitary-thyroid (HPT) axis, in Wuchang bream (Guo et al., 2021). Elevated NH_3 levels were also associated with higher fish mortality rates and hindered growth in young olive flounder (황인준 et al., 2009).

Furthermore, feed conversion ratio (FCR) and feed efficiency (FE) were evaluated consistently with growth performance assessments. Among the treatments, T2 demonstrated the highest efficacy in optimizing feed utilization (as shown in Table 2). Alavi et al. (2019) define FCR as the ratio of feed consumed to total fish weight. Meanwhile, FE represents the percentage of feed intake relative to body weight gain (de Verdal et al., 2018). Omasaki et al. (2017) emphasize that the FCR value provides insight into the proportion of feed converted to fish weight.

Table 2. The feed efficiency and feed conversion ratio of tilapia

Treatment	*FE (%)*	*FCR*
T0	35.1 ± 1.23^{a}	2.23 ± 0.37^{c}
T1	50.53 ± 1.61^{b}	2.12 ± 0.23^{b}
T2	56.02 ± 1.24^{c}	1.54 ± 0.16^{a}
T3	53.07 ± 2.00^{b}	2.00 ± 0.18^{b}

The various letters in the same column indicate significant changes between treatments

3.2. Survival Rate

The survival rate (SR) data displayed a statistically significant difference among treatments during the study. Overall, all treatments led to a slight decrease in SR by the end of the period, as indicated in Table 3. Notably, T2 remains the recommended treatment for optimizing *O. niloticus* survival rates (91.11 percent). Conversely, yield statistics revealed that T3 had the poorest SR performance. Our findings suggest that *O. niloticus* can tolerate low TLW concentrations but not high ones.

Table 3. The survival rate of *O. niloticus* after 30 days of TLW application

Treatment	***Week (%)***			
	1	***2***	***3***	***4***
T0	95.56 ± 0.58[b]	91.11 ± 0.58[b]	88.89 ± 0.58[b]	84.44 ± 0.58[b]
T1	95.56 ± 1.54[b]	91.11 ± 0.58[b]	88.89 ± 0.58[b]	88.89 ± 0.58[b]
T2	95.56 ± 0.58[b]	93.33 ± 1.00[c]	91.11 ± 0.58[c]	91.11 ± 0.58[c]
T3	93.33 ± 1.00[a]	86.67 ± 1.00[a]	84.44 ± 0.58[a]	80.00 ± 1.00[a]

The various letters in the same column indicate significant changes between treatments

Tempe liquid waste (TLW) contains various chemical compounds, including N_2, O_2, H_2S, NH_3 (Harahap, 2013), CO_2, and CH_4 (Mahfoedz, 2014). At high concentrations, TLW adversely affects fish health and can even lead to mortality (Rajabiesterabadi et al., 2020; Sheikh et al., 2017; Zhang et al., 2018). Consequently, these references provide insights into the data observed for T3 (8%), which exhibited the lowest survival rate (SR) (Table 3) and growth performance (Table 1) at the end of the study period. Notably, chemical substances like NH_3 inhibit the growth of *O. niloticus*. Elevated ammonia levels are toxic to fish, resulting in growth retardation, tissue necrosis, reduced expression of the growth hormone/insulin-like growth factor (GH/IGF) axis genes, and immunosuppression (Yu et al., 2021). In comparison to other studies, *Megalobrama amblycephala* experienced a slight growth decline of 3 g compared to the control group (45.34 g) when exposed to 5 mg L^{-1} of NH_3. Furthermore, using 10 mg L^{-1} of NH3 led to a more pronounced drop in *M. amblycephala* growth (Zhang et al., 2019). Nursyam (2017) highlights that NH_3 compounds significantly impact aquatic biota growth at concentrations exceeding 0.45 mg L–1, exerting a 50% effect. Thus, this explains the observed decline in *O. niloticus* growth over the 30 days for T3.

3.3. Water quality

Based on Table 4, the study examined water quality parameters, including temperature (ºC), pH, dissolved oxygen (O_2), and ammonia (NH_3). *Oreochromis niloticus* thrived when cultured in the T2 medium supplemented with 5% TLW for 30 days. On the first day of the study, the NH_3 content in T2 and T3 reached 0.5 mg L^{-1} and 1 mg L^{-1}, respectively. However, by the end of the experimental period, these levels decreased to 0.3 mg L^{-1} (as shown in Table 4). Based on these findings, our study posits that microorganisms present in TLW can effectively remediate wastewater and support the development of *O. niloticus*. This conclusion aligns with the work of Amaliah et al. (2018), who investigated various types of lactic acid bacteria (LAB) derived from TLW. Novik et al. (2017) also emphasized the critical role of LAB in organic material degradation and water quality improvement. Notably, LAB acts as a decomposer in aqueous environments (Hadioetomo, 1990; Markowiak & Śliżewska, 2017; Paladhi et al., 2022).

An illustrative example comes from Kamaruddin et al. (2013), who demonstrated that LAB effectively reduced chemical oxygen demand (COD) and NH_3 levels in industrial wastewater over 14 days. Specifically, 100 mL of LAB led to COD and NH_3 removal percentages of 65.5% and 60.2%, respectively. Additionally, LAB strains such as *L. acidophilus*, *L. bulgaricus*, *L. casei*, *L. fermentum*, and *L. plantarum* have been successful in bio coagulation of dairy wastewater using a micro-aerobic system (Seesuriyachan et al., 2009; Shah & Patel, 2013).

In summary, our investigation highlights that a 5% concentration of tempe liquid waste positively influenced *O. niloticus* growth and contributed to improved water quality. However, caution should be exercised with higher TLW doses, as they may adversely affect *O. niloticus* development. Based on our findings, we recommend that freshwater fish farmers consider utilizing 5% TLW to significantly enhance fish growth.

Throughout the trial, the pH ranged between 6.5 and 8.7, which tilapia tolerated well. According to Efendi (2003), the optimal pH range for tilapia aquaculture is 6 to 8.5, while another study suggests that a pH range of 6 to 9 remains acceptable (Fradina & Latuconsina, 2022). Prolonged exposure to water more acidic than pH 6.5 or more alkaline than pH 9.0 can inhibit reproduction and growth (Ndubuisi et al., 2015). Temperature is a critical factor in fish farming, directly impacting fish metabolic rates. In our study, the temperature range across all treatments was approximately 25°C to 29°C. Mulqan et al. (2017) identified the optimum temperature conditions for fish growth as 25°C to

Table 4. Water quality during the study

Parameter		Water quality parameters				Standard	Reference
		T0	T1	T2	T3		
Temperature (ºC)		25 – 29	25 – 29	25 – 29	25 – 29	25 – 32	(Fradina & Latuconsina, 2022)
pH		6.6 – 8.7	6.8 – 8.7	7.0 – 8.6	7.2 – 8.6	6.5 – 9.0	(Fradina & Latuconsina, 2022)
Dissolved oxygen ($mg\ L^{-1}$)		2.0 – 2.5	2.1 – 3.0	2.2 – 3.0	2.0 – 3.2	≥3	(Fradina & Latuconsina, 2022)
Ammonia ($mg\ L^{-1}$)	**Day 0**	0.0	0.3	0.5	1.0	>1	(Cokrowati, 2021)
	Day 30	0.40	0.30	0.30	0.30		

The various letters in the same column indicate significant changes between treatments

32°C. An increase in temperature leads to higher metabolic rates (RMR) in fish (Xie et al., 2017). Dissolved oxygen (DO) is vital for fish respiration and overall metabolism. Our study maintained good DO concentrations across all treatments, ranging from 2.0 $mg\ L^{-1}$ to 3.0 $mg\ L^{-1}$. Popma and Masser (1999) reported that tilapia can survive in DO levels exceeding 0.3 $mg\ L^{-1}$. However, it's essential to note that maintaining oxygen levels near saturation enhances growth rates, food conversion ratios, and overall fish output, while excessive oxygen can be detrimental (Mallya, 2007).

4. Conclusions

According to the study, *O. niloticus'* growth and feed consumption improved when using 5% of TLW. However, increased waste concentration negatively impacted the growth and survival rate of *O. niloticus*. Therefore, the study suggests that applying 5% TLW could support Tilapia culture and should be explored for other freshwater fish species.

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