Original Article

The role of predigested plant protein in enhancing nutritional value, feed utilization efficiency, and growth performance of Nile tilapia, *Oreochromis niloticus*

Subandiyono Subandiyono[1], Sri Hastuti[*1], Dicky Harwanto[1]

Department of Aquaculture, Faculty of Fisheries and Marine Science, Diponegoro University, Jl. Prof. Jacub Rais, UNDIP Tembalang Campus, Semarang, Central Java, Indonesia.

Abstract: This study evaluated the effect of incorporating predigested *Indigofera zollingeriana* leaf meal as a partial substitute for soybean meal in the diet of Nile tilapia (*Oreochromis niloticus*) on feed utilization efficiency, protein efficiency ratio, growth performance, and survival rate. Predigestion was conducted using cellulase enzyme (1.2 g kg^{-1}) to hydrolyze complex fiber and protein compounds into more digestible forms. A Completely Randomized Design (CRD) was employed, with four dietary treatments: 0 (control), 25, 50, and 75% substitution of soybean meal with predigested *Indigofera* leaf meal, each containing 30% crude protein and an energy-to-protein ratio of 8.5-9.0 kcal g^{-1}. The feeding trial lasted six weeks using juvenile tilapia (2.39±0.12 g) stocked at a density of 50 fish m^{-2} and fed three times daily to satiation. The results showed that predigestion enhanced the nutritional quality of Indigofera leaf meal, increasing protein content from 31.04 to 38.22% and reducing crude fiber from 15.53 to 13.10%. Increasing inclusion levels of predigested *Indigofera* leaf meal improved total feed intake (96.53-112.29 g), feed utilization efficiency (68.73-75.61%), protein efficiency ratio (2.29-2.52), and relative growth rate (4.22-5.84% day^{-1}). The highest final biomass (119.48 g) and survival rate (96.67%) were observed at the 75% substitution level, indicating that predigested *I. zollingeriana* meal supports efficient nutrient conversion and growth without adverse physiological effects. Water quality parameters, including temperature (24-28°C), pH (7.9-8.1), dissolved oxygen (3.5-4.7 mg L^{-1}), and ammonia (<0.15 mg L^{-1}), remained within optimal ranges throughout the rearing period. The findings demonstrate that predigested *I. zollingeriana* leaf meal can effectively replace up to 75% of soybean meal in Nile tilapia diets, improving feed efficiency, growth performance, and survival while supporting sustainable and cost-effective aquaculture practices.

Introduction

Fish feed plays a crucial role in growth, health, and economic sustainability in aquaculture (FAO, 2020). Understanding the specific nutritional requirements of each fish species and selecting appropriate feed ingredients can support more sustainable aquaculture practices (Gatlin et al., 2007). *Oreochromis niloticus* is a widely cultured freshwater fish species known for its high adaptability to diverse environmental conditions. It plays a vital role in the global aquaculture industry as a sustainable source of nutritious and safe food for humans (Subandiyono and Hastuti, 2020; El-Sayed and Fitzsimmons, 2023). The intensification of Nile tilapia farming has led to a growing dependence on cost-efficient, high-energy, and plant-based protein diets (Kiron et al., 2020; Xie et al., 2021; Xuan et al., 2024). However, the relatively undeveloped digestive system of early-stage fish makes them more susceptible to anti-nutritional factors (ANFs), and the prolonged use of plant-derived proteins has been associated with intestinal inflammation and reduced nutrient digestibility (Kiron et al., 2020; Xie et al., 2021; Zeng et al., 2024; Ibrahim et al., 2025). Moreover, fish health status plays a vital

---

*Correspondence: Sri Hastuti
E-mail: hastuti-hastuti@yahoo.com

role in determining their resistance and survival against pathogenic infections (Tao et al., 2022). To sustain and enhance the profitability and sustainability of aquaculture, improving feed quality and efficiency to deliver optimal nutrition and promote growth is essential (Akter et al., 2021). Therefore, investigating the potential use of predigested plant protein as a dietary strategy represents a promising approach to enhance nutrient utilization and growth performance in Nile tilapia.

In Nile tilapia production, feed accounts for the most significant operational expense, representing approximately 45-85% of total production costs (Komalasari et al., 2018; Fadum et al., 2024). The continuous rise in feed prices poses a major challenge to improving the sustainability and productivity of tilapia aquaculture. Therefore, developing cost-effective feed formulations using plant-based protein sources as alternatives to animal-derived proteins is essential. Plant-based protein ingredients used in tilapia feed formulation should ideally have good nutritional value, be readily available, easily processed, and affordable, enabling the development of low-cost yet nutritionally balanced alternative feeds (Zaenuri et al., 2014). The use of such ingredients is expected not only to reduce production costs but also to improve feed utilization efficiency and promote optimal growth performance in Nile tilapia.

Several feed ingredients with balanced nutritional profiles and relatively low cost include *Indigofera zollingeriana* leaf meal (Santi, 2017), *Ipomoea aquatica* leaf meal, and others. *Indigofera zollingeriana* is a leguminous plant known for its high protein content. Nutritionally, *I. zollingeriana* contains approximately 27.9% protein, 0.22% calcium, and 0.18% phosphorus (Mayasari and Ismiraj, 2019), while its leaf meal form contains about 28-34% protein (Suharlina and Sanusi, 2020). Meanwhile, *Ipomoea aquatica* leaf meal contains 24.12% protein and 12.70% crude fiber (Adrian, 2012). Therefore, both of these plant-based protein sources have strong potential as alternative ingredients in formulated feeds for Nile tilapia.

The use of *I. zollingeriana* or *I. aquatica* leaf meals

as alternative feed ingredients can support national food security and reduce dependence on imported feed materials such as fishmeal and soybean meal (Dawood et al., 2019; Kiron et al., 2020; Xie et al., 2021; Xuan et al., 2024). This approach aligns with government efforts to enhance food self-sufficiency, particularly within the aquaculture sector. Plant-based protein sources have been identified as the most promising alternatives to replace fishmeal and soybean meal in aquaculture feeds. However, plant ingredients generally contain high levels of carbohydrates, whereas fish have a limited capacity to use carbohydrates as an energy source for growth. One of the major carbohydrate components that poses a challenge is cellulose, which most fish species cannot digest. Therefore, this study aimed to assess the impact of predigested plant protein derived from *I. zollingeriana* leaf at different substitution levels on total feed intake (TFI), feed utilization efficiency (FUE), protein efficiency ratio (PER), and growth performance of omnivorous Nile tilapia.

Materials and Methods
Experimental procedure
Preparation phase: The experimental fish had an average total length of 5-7 cm and an average body weight of 2.39±0.12 g. The stocking density was 50 fish M^{-2} (Hastuti and Subandiyono, 2020a). The fish were acclimated to the experimental conditions by maintaining them in the experimental medium until they were fully adapted. Only healthy fish without visible signs of disease were selected as experimental animals. Prior to stocking, fish were fasted for 24 h to empty their digestive tracts, allowing better acceptance of the experimental diets.

Feed preparation began with the selection and weighing of ingredients, including fish meal, soybean meal, corn meal, rice bran meal, wheat flour, carboxymethyl cellulose (CMC), corn oil, fish oil, vitamin-mineral mix, *I. zollingeriana* leaf meal, and water (250 mL kg^{-1} feed). Proximate analyses of the feed ingredients were conducted prior to formulation to determine their nutrient composition (protein, lipid, fiber, and ash). Diets were formulated to contain 30%

crude protein, with the proximate composition serving as the basis for formulation. Animal-derived protein was obtained from fish meal, while plant-derived protein sources included soybean meal and *I. zollingeriana* leaf meal. The contribution of *I. zollingeriana* leaf meal to total plant protein ranged from 0% to 75% across treatments. The energy-to-protein (E/P) ratio for each diet was adjusted to 8.5-9.0 kcal g⁻¹. Lipid, crude fiber, and ash levels were determined in accordance with the Indonesian National Standard (SNI 7548:2009).

Prior to use, *I. zollingeriana* leaf meal was pre-digested using cellulase enzyme at 1.2 g kg⁻¹ and mixed with 30% water (Jefry et al., 2021). The mixture was thoroughly homogenized and incubated at room temperature for 24 h. The pre-digested meal was then oven-dried at 60°C for 1.5 h. The pre-digestion process aimed to reduce crude fiber and convert it into more digestible carbohydrates (Sofiana et al., 2023), while also improving nutrient digestibility and reducing anti-nutritional compounds (Susilowati et al., 2020).

All feed ingredients were subsequently mixed and extruded into pellets, which were dried to a moisture content of approximately 15%. Proximate analysis of the formulated diets was conducted to determine moisture, crude protein, lipid, ash, and carbohydrate contents. Analyses were conducted at the Chemistry Laboratory of the Agricultural Instrument Standardization Agency (BSIP) in Central Java, Indonesia.

Feeding and rearing: Selected tilapia were reared for 6 weeks at a stocking density of 50 fish m⁻² (Hastuti and Subandiyono, 2020a). Fish were fed to apparent satiation three times daily with the respective experimental diets. Water quality was maintained through regular water exchange, continuous aeration, and temperature control using a thermostat. Water exchange was performed to reduce ammonia accumulation, while aeration ensured optimal dissolved oxygen levels.

The experimental tanks measured 60×40×50 cm and contained 60 L of water. Fish were weighed at the beginning and end of the experiment. Water quality

parameters, i.e., temperature (°C), pH, and dissolved oxygen (mg L⁻¹), were monitored daily in the morning and afternoon.

Experimental design: The study was conducted using a Completely Randomized Design (CRD) with four dietary treatments and five replicates per treatment. The treatments varied in the level of substitution of predigested *I. zollingeriana* leaf meal as a plant-based protein source in the diet. The experimental diets were formulated to contain a constant crude protein level of 30% and an energy-to-protein ratio (E/P) of 8.5-9.0 kcal g⁻¹. The treatments differed in the level of substitution of pre-digested *I. zollingeriana* leaf meal as a plant-based protein source, replacing soybean meal protein in the diet.

The four dietary treatments were as follows: Treatment A (Control): Diet containing 0% substitution of pre-digested *I. zollingeriana* leaf meal (100% of plant protein derived from soybean meal); Treatment B: Diet containing 25% substitution of pre-digested *I. zollingeriana* leaf meal, replacing 25% of the total plant protein contribution from soybean meal; Treatment C: Diet containing 50% substitution of pre-digested *I. zollingeriana* leaf meal, replacing 50% of the total plant protein contribution from soybean meal; Treatment D: Diet containing 75% substitution of pre-digested *I. zollingeriana* leaf meal, replacing 75% of the total plant protein contribution from soybean meal. Each diet was formulated isonitrogenously and isoenergetically to meet the nutritional requirements of Nile tilapia as recommended by the National Research Council (NRC, 2011).

Measured parameters

Total Feed Intake (TFI): Total feed intake was calculated according to Pereira et al. (2007), using the formula of $TFI = F_1 - F_2$, where TFI = total feed intake (g), F_1 = total feed provided at the beginning (g), and F_2 = remaining feed at the end (g).

Feed Utilization Efficiency (FUE): Feed utilization efficiency was determined following NRC (2011): $FUE = ((W_t - W_0) / F)*100\%$, where FUE = feed utilization efficiency (%); W_t = final body weight (g); W_0 = initial body weight (g); F = total feed consumed (g).

Protein Efficiency Ratio (PER): Protein efficiency ratio was calculated using the NRC (2011) formula: PER = ((W_t - W_0) / P_i)*100%, where PER = protein efficiency ratio (%); W_t = final fish biomass (g); W_0 = initial fish biomass (g); P_i = total protein intake (g).

Relative Growth Rate (RGR): Relative growth rate was calculated according to Subandiyono and Hastuti (2016): RGR = ((W_t - W_0) / (W_0 - t) P_i)*100%, where: RGR = relative growth rate (% day^{-1}); W_t = final body weight (g); W_0 = initial body weight (g); t = rearing period (days).

Survival Rate (SR): Survival rate was calculated according to Panigrahi et al. (2017): SR = (N_t / N_0) *100%, where SR = survival rate (%); N_t = number of fish at the end of the experiment; N_0 = number of fish at the beginning of the experiment.

Water quality: The measured parameters were temperature (°C), pH, and dissolved oxygen (mg L^{-1}). Measurements were taken daily in the morning and evening throughout the experimental period.

Data analysis: Data on total feed intake, feed utilization efficiency, protein efficiency ratio, relative growth rate, and survival rate were analyzed using Analysis of Variance (ANOVA). Prior to ANOVA, data were tested for normality, additivity, and homogeneity of variances to ensure compliance with test assumptions. Differences among treatments were further analyzed using Duncan's Multiple Range Test (DMRT) at P = 0.05 significance level. Proximate composition data of feed ingredients and experimental diets were analyzed descriptively. Water quality data were also analyzed descriptively and compared with the standard optimal ranges for Nile tilapia culture.

Results

Role of predigested plant protein (*I. zollingeriana*) in experimental diets: The predigestion of *I. zollingeriana* leaf meal, performed with cellulase at 1.2 g kg^{-1}, effectively hydrolyzed complex protein and fiber compounds into simpler, more digestible forms. This bioconversion increased protein content from 31.04 to 38.22% (dry-weight basis) and reduced crude fiber from 15.53 to 13.10%, indicating enhanced nutritional quality and digestibility. Fat and nitrogen-free extract (NFE) decreased due to microbial utilization, while ash content slightly increased, reflecting higher mineral availability (Table 1).

The formulated experimental diets incorporated various inclusion levels of predigested *I. zollingeriana* leaf meal (0, 25, 50, and 75% substitution of plant-based protein). *Indigofera* progressively replaced soybean meal while maintaining a constant total dietary protein level of 30% (Table 2). The proximate composition of the experimental diets remained nutritionally balanced, with a gradual increase in digestible energy (from 249.42 to 251.85 kcal g^{-1}) and a decrease in crude fiber (from 8.24% to 7.30%) as *I. zollingeriana* inclusion increased (Table 3). These results indicate that fermented *Indigofera* meal can be used effectively as a sustainable protein source without compromising diet quality.

Biological response of Nile Tilapia fed diets containing predigested plant protein: Feeding trials revealed that the dietary inclusion of predigested *I. zollingeriana* improved growth

Table 1. Proximate composition of *Indigofera zollingeriana* leaf meal before and after predigestion with cellulase enzyme.

Parameter	% Wet Weight		% Dry Weight	
	Before predigestion	After predigestion with	Before predigestion	After predigestion
Protein	28.30	25.85	31.04	38.22
Fat	2.85	0.68	3.13	1.01
Crude Fiber	14.16	8.87	15.53	13.10
Ash Content	10.26	10.09	11.25	14.92
Moisture Content	8.83	32.36	–	–
NFE (Nitrogen-Free Extract)	35.60	22.15	39.05	32.75
Total	100.00	100.00	100.00	100.00

Table 2. Formulation of experimental diets.

Feed Ingredient	Diet Composition (per 100 g feed)			
	A (0%)	B (25%)	C (50%)	D (75%)
Fish meal	32.44	32.44	32.44	32.44
Soybean meal	25.35	17.84	10.30	2.78
Indigofera leaf meal	0.00	12.88	25.78	38.67
Corn meal	7.50	7.50	7.50	7.50
Rice bran	16.71	11.34	5.98	0.61
Wheat flour	9.00	9.00	9.00	9.00
CMC (binder)	1.00	1.00	1.00	1.00
Fish oil	2.50	2.50	2.50	2.50
Corn oil	2.50	2.50	2.50	2.50
Vitamin–mineral mix	3.00	3.00	3.00	3.00
Total (g)	100.00	100.00	100.00	100.00

Notes: A: Ratio of *Indigofera zollingeriana* protein to total plant-based protein = 0%; B: Ratio of *Indigofera* protein to total plant-based protein = 25%; C: Ratio of *Indigofera* protein to total plant-based protein = 50%; D: Ratio of *Indigofera* protein to total plant-based protein = 75%

Table 3. Proximate composition of experimental diets (%).

Nutrient	Diet A	Diet B	Diet C	Diet D
Protein*	30.00	30.00	30.00	30.00
Fat*	4.78	4.80	4.82	4.84
Crude Fiber*	8.24	7.92	7.62	7.30
Ash Content*	17.96	18.00	18.05	18.09
NFE (Nitrogen-Free Extract)	39.02	39.27	39.52	39.76
DE (kcal/g)*	249.42	250.24	251.03	251.85
E/P Ratio**	8.32	8.34	8.37	8.39
Total	100.00	100.00	100.00	100.00

Notes: * Proximate analysis conducted at the Chemistry Laboratory, Agricultural Instrument Standardization Agency (BSIP), Central Java. ** Based on the assumed DE (Digestible Energy) values: protein = 3.5 kcal/g; fat = 9.8 kcal/g; NFE = 2.5 kcal/g (Subandiyono et al., 2018).

Table 4. Biological Parameters of Nile Tilapia (*Oreochromis niloticus*) fed diets containing various levels of predigested plant protein from *Indigofera zollingeriana*.

Parameter	Experimental diets containing different levels of predigested plant protein from *Indigofera zollingeriana*			
	A (0%)	B (25%)	C (50%)	D (75%)
Initial body weight of tilapia (g)	2.50±0.18[a]	2.42±0.15[a]	2.33±0.07[a]	2.31±0.07[a]
Initial fish biomass (g)	37.49± 2.75[a]	36.36±2.25[a]	35.01±1.04[a]	34.65±1.06[a]
Final fish biomass (g)	103.88±12.21[a]	108.69±12.92[a]	114.11±14.29[a]	119.48±5.85[a]
Biomass weight gain (g)	66.39±11.23[b]	72.33±12.78[ab]	79.10±14.48[ab]	84.83±6.88[a]
Total feed consumption (g)	96.53±1.31[d]	102.88±4.92[c]	107.61±1.90[b]	112.29±2.48[a]
Feed utilization efficiency (%)	68.73±11.19[a]	70.13±11.04[a]	73.68±14.54[a]	75.61±6.89[a]
Protein efficiency ratio (%)	2.29±0.37[a]	2.34±0.37[a]	2.46±0.48[a]	2.52±0.23[a]
Relative growth rate (% day^{-1})	4.22±0.70[b]	4.75±0.93[ab]	5.39±1.03[ab]	5.84±0.66[a]
Survival rate (%)	86.67±14.14[a]	86.67±10.64[a]	88.22±11.90[a]	96.67±3.33[a]

Note: Values are presented as mean±standard deviation (SD). Different superscript letters (a, b, c, and d) in the same row indicate significant differences among treatments ($P<0.05$).

Table 5. Monitoring results of water temperature during 42 days of Nile tilapia (*Oreochromis niloticus*) rearing.

Treatment	Morning average (°C)	Morning range (°C)	Afternoon average (°C)	Afternoon range (°C)
A (0%)	24.74±0.25	24.0–25.1	27.50±0.37	26.6–27.9
B (25%)	24.76±0.31	24.1–25.4	27.53±0.35	26.4–28.0
C (50%)	24.77±0.27	24.2–25.2	27.55±0.32	26.8–28.0
D (75%)	24.75±0.32	24.0–25.4	27.56±0.35	26.9–28.0

Note: Values represent mean±standard deviation (SD). Morning and afternoon temperatures were recorded daily throughout the 42-day rearing period.

Table 6. Monitoring results of water pH during 42 days of Nile tilapia (*Oreochromis niloticus*) rearing.

Treatment	Morning average (pH)	Morning range (pH)	Afternoon average (pH)	Afternoon range (pH)
A (0%)	8.10±0.30	7.20–8.49	8.04±0.28	7.46–8.50
B (25%)	8.06±0.30	7.27–8.50	7.90±0.35	7.20 –8.42
C (50%)	8.00±0.37	7.06–8.52	7.91±0.34	7.12–8.50
D (75%)	7.92±0.40	7.04–8.46	7.94±0.36	6.97–8.41

Note: Values represent mean ± standard deviation (SD). Morning and afternoon pH were measured daily throughout the 42-day rearing period.

Table 7. Dissolved oxygen (DO) values measured in the morning and afternoon during 42 days of Nile tilapia (*Oreochromis niloticus*) rearing.

Treatment	Morning DO range (mg L⁻¹)	Morning DO average (mg L⁻¹)	Afternoon DO range (mg L⁻¹)	Afternoon DO average (mg L⁻¹)
A (0%)	3.50-4.61	4.04±0.30	3.44-4.59	3.71±0.21
B (25%)	3.50-4.70	3.97±0.32	3.60 –4.54	3.81±0.16
C (50%)	3.40-4.62	3.94±0.31	3.62–4.63	3.89±0.21
D (75%)	3.50-4.70	4.04±0.34	3.50–4.55	3.87±0.23

Note: Values represent mean±standard deviation (SD). Dissolved oxygen (DO) levels were recorded daily in the morning and afternoon throughout the 42-day rearing period.

performance and feed utilization in Nile tilapia (Table 4). Final biomass increased from 103.88 g (control) to 119.48 g (75% substitution), accompanied by a significant improvement in biomass gain (66.39-84.83 g) and total feed consumption (96.53-112.29 g). Feed utilization efficiency and protein efficiency ratio also improved (68.73-75.61% and 2.29-2.52, respectively). Relative growth rate (RGR) increased from 4.22 to 5.84% day⁻¹, while survival remained high across all treatments (86.67-96.67%). These findings suggest that up to 75% substitution of soybean meal with predigested I. *zollingeriana* meal enhances feed efficiency, growth rate, and survivability of Nile tilapia.

Water quality parameters: Water quality remained within the optimal range for tilapia culture throughout the 42-day trial. Morning temperatures averaged 24-25°C, while afternoon values reached 27–28°C with no significant differences among treatments (Table 5). pH levels were stable (7.9-8.1 in the morning and 7.9-8.0 in the afternoon) (Table 6). DO ranged between 3.5-4.7 mg L⁻¹ in the morning and 3.4–4.6 mg L⁻¹ in the afternoon, suitable for tilapia growth (Table 7). Ammonia concentrations fluctuated slightly over time but remained low (<0.15 mg L⁻¹) and decreased effectively after water exchange (Table 8). These results confirm that dietary treatments did not adversely affect the rearing water environment.

Table 8. Ammonia concentration (mg L⁻¹) measured weekly before and after water exchange during 42 days of Nile tilapia (*Oreochromis niloticus*) rearing.

Week	A (0%)		B (25%)		C (50%)		D (75%)	
	Before	After	Before	After	Before	After	Before	After
0	0.004	0.002	0.010	0.006	0.006	0.002	0.002	0.002
1	0.000	0.000	0.000	0.000	0.009	0.009	0.001	0.000
2	0.001	0.000	0.000	0.000	0.001	0.000	0.000	0.000
3	0.011	0.000	0.000	0.000	0.125	0.003	0.030	0.001
4	0.005	0.000	0.062	0.001	0.015	0.001	0.001	0.003
5	0.025	0.011	0.001	0.008	0.132	0.107	0.038	0.025

Discussions

Nutritional improvement of *I. zollingeriana* through predigestion: The use of *I. zollingeriana* as an alternative plant-based protein source in Nile tilapia diets shows promise, particularly when subjected to predigestion or fermentation. Fermentation acts to hydrolyze complex compounds—particularly proteins and crude fiber—into simpler, more digestible forms that the fish's digestive system can efficiently absorb. Initially, *I. zollingeriana* contained relatively high crude fiber (15.53% on a dry-weight basis), which could limit digestibility. However, following fermentation, crude fiber content decreased to 13.10%, while crude protein increased significantly from 31.04 to 38.22%. This indicates that fermentation enhances the nutritional quality of *Indigofera* by increasing protein and essential amino acid availability while reducing limiting factors such as fiber (Jefry et al., 2021; Mirzah et al., 2023).

Role of *I. zollingeriana* as a substitute for soybean meal: In feed formulations, *Indigofera* functions as a partial substitute for soybean meal, which remains the predominant plant protein source but faces issues of fluctuating availability and price (El-Sayed et al., 2015; Jefry et al., 2021; Mirzah et al., 2023; Suharlina and Sanusi, 2020). Formulation results indicate that increasing *Indigofera* inclusion from 0% to 75% of total plant protein maintained a constant dietary protein level of 30%. Meanwhile, *Indigofera*'s contribution to total protein content increased by up to 12%, indicating that it can effectively replace up to 12% of the soybean meal without disrupting the nutritional balance.

Proximate analysis results indicate that all feed formulations (A–D) contained equivalent crude protein levels (30%), whereas improvements were observed in other nutrient components. A decrease in crude fiber with higher *Indigofera* levels indicates improved digestibility, and digestible energy (DE) values increased from 249.42 to 251.85 kcal/g. This increase corresponds to higher nitrogen-free extract (NFE) content and lower fiber content, both of which enhance the feed's energy efficiency. Maintaining a balanced energy-to-protein (E/P) ratio is critical for optimizing tilapia growth, as sufficient digestible energy supports efficient metabolism without diverting protein to energy use (NRC, 2011; Subandiyono and Hastuti, 2022).

Growth performance and feed utilization efficiency: The inclusion of predigested *I. zollingeriana* protein improved the biological performance of *O. niloticus*. The final biomass increased with increasing *Indigofera* dosage, reaching the highest value (119.48 g) at the 75% inclusion level (Treatment D). This indicates that fermented *Indigofera* protein is effectively utilized for growth. Previous studies have demonstrated that fermentation enhances the digestibility of plant protein sources, thereby improving nutrient absorption and biomass accumulation (Hastuti and Subandiyono, 2020b; Jefry et al., 2021; Haetami et al., 2025).

Feed intake increased from 96.53 g in the control group to 112.29 g at the 75% level, indicating that the *Indigofera*-based diets were palatable and well accepted by fish. Feed utilization efficiency (FUE) improved from 68.73 to 75.61%, along with an increase in the protein efficiency ratio (PER) from 2.29 to 2.52. These results support that predigested protein enhances the conversion of feed nutrients into body tissue. According to El-Sayed et al. (2015), highly digestible and readily available protein plays a vital role in improving feed conversion efficiency in tilapia. The relative growth rate increased significantly from 4.22% per day (control) to 5.84% per day (75% *Indigofera* inclusion), confirming that

Indigofera serves as an effective alternative protein source, replacing a substantial proportion of soybean meal. As noted by Subandiyono and Hastuti (2020), maintaining a balanced energy–protein ratio is essential for maximizing tilapia growth, and the present findings confirm that this balance remains optimal even with high soybean substitution.

Survival and physiological response: Survival rates remained high across all treatments, ranging from 86.67 to 96.67%, with the highest in treatment D. This suggests that including up to 75% *Indigofera* protein did not cause stress or toxicity in fish. Similarly, Castillo and Gatlin (2015) and Zhou et al. (2013) reported that predigestion of plant materials not only improves digestibility but also reduces anti-nutritional factors that can interfere with fish metabolism. Overall, predigested *I. zollingeriana* can be incorporated into tilapia diets at up to 75% of total plant protein without adverse effects on growth, feed efficiency, or survival. On the contrary, it tends to enhance overall performance and supports more sustainable aquaculture practices.

Water quality dynamics during the feeding trial: Water quality monitoring throughout the 42-day experiment showed that temperature, pH, DO, and ammonia remained within optimal ranges for *O. niloticus* culture. Water temperature was stable, ranging from 24-25°C in the morning to 27-28°C in the afternoon—within the optimal range of 25-30°C for tilapia growth (El-Sayed, 2006). No significant differences in temperature were observed among treatments, indicating that dietary variation had a minimal effect on thermal conditions. The pH ranged from 6.97 to 8.52, averaging 7.92-8.10 in the morning and 7.90-8.04 in the afternoon. These values fall within the ideal range (6.5-9.0) for tilapia rearing (Boyd, 2015). DO ranged between 3.44 and 4.70 mg L^{-1}, with slightly higher readings in the morning (3.94-4.04 mg L^{-1}) than in the afternoon (3.71-3.89 mg L^{-1}). Although moderately low, these DO levels were still sufficient for tilapia survival, as the species can tolerate minimum levels of about 3 mg L^{-1} (Ngugi et al., 2007).

Ammonia concentrations fluctuated slightly over the culture period. During weeks 0–2, levels remained low or nearly undetectable. A slight increase was noted during weeks 3–5, particularly in treatments C (50%) and D (75%), with a maximum of 0.132 mg L^{-1}. Following water exchange, ammonia levels dropped sharply, demonstrating the effectiveness of water renewal in maintaining safe concentrations. According to Boyd (2015), un-ionized ammonia (NH$_3$) should remain below 0.05 mg L^{-1} and total ammonia below 1.0 mg L^{-1} for safe aquaculture conditions. Overall, water quality parameters remained within acceptable limits, ensuring optimal growth and health of Nile tilapia. However, regular monitoring of DO and ammonia is recommended to prevent potential stress under intensive culture conditions.

Conclusion

The present study demonstrated that including predigested *I. zollingeriana* as a plant-based protein source in the diet of Nile tilapia can effectively replace a substantial proportion of conventional soybean meal without compromising growth performance, feed utilization, or survival. Fermentation significantly enhanced the nutritional quality of *Indigofera* by increasing crude protein content and reducing crude fiber, thereby improving digestibility and nutrient bioavailability. Biological performance data showed a consistent improvement with increasing levels of *Indigofera* inclusion. The highest inclusion level (75% of total plant protein) produced superior biomass gain, relative growth rate, and feed utilization efficiency compared to the control diet. These findings confirm that fermented *Indigofera* protein can be efficiently digested and metabolized by tilapia, thereby supporting optimal growth. The improved palatability and feed conversion efficiency also reflect the enhanced nutritional balance achieved through predigestion. The survival rate remained high (>86%) across all treatments, indicating that *Indigofera*-based diets were well tolerated and did not induce stress or toxicity. Furthermore, water quality parameters— including temperature, pH, dissolved oxygen, and ammonia—remained within acceptable limits

throughout the 42-day culture period, confirming that dietary treatments did not negatively affect the rearing environment.

In conclusion, predigested *I. zollingeriana* can be safely and effectively incorporated into Nile tilapia feed formulations up to 75% of total plant protein. This substitution not only enhances growth performance and feed efficiency but also provides a cost-effective, locally available, and sustainable alternative to imported soybean meal. Future research should focus on optimizing fermentation techniques, evaluating amino acid bioavailability, and assessing long-term physiological and economic impacts of *Indigofera*-based diets in commercial aquaculture settings.

References
Adrian. (2012). Microscopic description and mineral content of water spinach (Ipomoea aquatica Forsk.) [Undergraduate thesis]. Faculty of Fisheries and Marine Science, Bogor Agricultural University, Bogor. 32 p.

Akter M.N., Zahan K., Zafar M.A., Khatun N., Rana M.S., Mursalin M.I. (2021). Effects of dietary mannan oligosaccharide on growth performance, feed utilization, body composition, and haematological parameters in Asian catfish (*Clarias batrachus*) juveniles. Turkish Journal of Fisheries and Aquatic Sciences, 21(11): 559-567.

Boyd C.E. (2015). Water quality: An introduction. Springer. 374 p.

Castillo S., Gatlin D.M. (2015). Dietary supplementation of exogenous carbohydrase enzymes in fish nutrition: A review. Aquaculture, 435: 286-292.

Dawood M.A.O., Magouz F.I., Salem M.F.I., Abdel-Daim H.A. (2019). Modulation of digestive enzyme activity, blood health, oxidative responses, and growth-related gene expression in GIFT tilapia by heat-killed *Lactobacillus plantarum* (L-137). Aquaculture, 505: 127-136.

El-Sayed A.F.M. (2006). Tilapia culture. CABI Publishing, Wallingford. 277 p.

El-Sayed A.-F.M., Dickson M.W., El-Naggar G.O. (2015). Value chain analysis of the aquaculture feed sector in Egypt. Aquaculture, 437: 92-101.

El-Sayed A.F.M., Fitzsimmons K. (2023). From Africa to the world—The journey of Nile tilapia. Reviews in Aquaculture, 15(S1): 6-21.

Food and Agriculture Organization of the United Nations (FAO). (2020). The state of world fisheries and aquaculture (SOFIA). Rome, Italy.

Fadum J.M., Hall E.K., Litchman E., Zakem E.J. (2024). The aquaculture industry as a global network of perturbation experiments. Limnology and Oceanography Letters, 9(4): 317-323.

Gatlin D.M.III., Barrows F.T., Brown P., Dabrowski K., Gaylord T.G., Hardy R.W., Herman E., Hu G., Krogdahl Å., Nelson R., Overturf K., Rust M., Sealey W., Skonberg D., Souza E.J., Stone D., Wilson R., Wurtele E. (2007). Expanding the utilization of sustainable plant products in aquafeeds: A review. Aquaculture Research, 38: 551-579.

Haetami K., Aisyah, Abun. (2025). Physical processing of various forage plants and their application in aquafeed development. Jurnal Biologi Tropis, 25(3): 3141-3149.

Hastuti S., Subandiyono S. (2020a). Aminotransferase, hematological indices and growth of tilapia (*Oreochromis niloticus*) reared in various stocking densities in aquaponic systems. AACL Bioflux, 13(2): 813-824.

Hastuti S., Subandiyono S. (2020b). Blood serum biochemistry responses and digestive enzyme activities of tilapia (*Oreochromis niloticus*) according to different dietary protein level consumption. AACL Bioflux, 13(6): 3566-3573.

Ibrahim R.E., Elshopakey G.E., Younis E.M., Abdelwarith A.A., Yaseen A., Khamis T., Davies J.S., Rahman A.N.A. (2025). Peel of pumpkin (*Cucurbita pepo*) diets boost growth, digestive-absorptive functions, immune-antioxidant indices, and regulate immunomodulatory genes and mTOR/MAPK-P38/apoptosis signaling pathway in *Oreochromis niloticus*. Aquaculture Reports, 40: 102548.

Jefry J., Setiawati M., Jusadi D., Fauzi I.A. (2021). Cellulase-hydrolyzed *Indigofera zollingeriana* leaf utilization as a feed ingredient for gourami fingerlings.

Jurnal Akuakultur Indonesia, 20(2): 139-147.

Kiron V., Park Y., Siriyappagouder P., Dahle D., Vasanth G.K., Dias J., Fernandes J.M.O., Sørensen M., Trichet V.V. (2020). Intestinal transcriptome analysis reveals soy derivative-linked changes in Atlantic salmon. Frontiers in Immunology, 11: 596514.

Komalasari S.S., Subandiyono S., Hastuti S. (2018). The effect of vitamin C in commercial feed and fish density on survival and growth of tilapia (*Oreochromis niloticus*). Sains Akuakultur Tropis: Indonesian Journal of Tropical Aquaculture, 1(1): 31-41.

Mayasari N., Ismiraj M.R. (2019). Introduction of *Indigofera zollingeriana* utilization as a partial concentrate replacement for beef cattle at Putra Nusa farmers group, Kondangdjaja village, Cijulang district, Pangandaran regency. Dharmakarya: Jurnal Aplikasi Ipteks Untuk Masyarakat, 8(2): 105-110.

Mirzah M., Hellyward J., Fajrona K., Herwanto T. (2023). Use of mixed meal of *Indigofera zollingeriana* leaves and fermented tofu waste with *Waretha* inoculum as a protein source in quail rations. Ternak Tropika: Journal of Tropical Animal Production, 24(2): 119-133.

National Research Council (NRC). (2011). Nutrient requirements of fish and shrimp. National Academies Press, Washington, D.C., USA.

Ngugi C.C., Bowman J.R., Omolo B.O. (2007). A new guide to fish farming in Kenya. Aquaculture Collaborative Research Support Program.

Panigrahi A., Sundaram M., Jebha J., Dayal J.S., Otta S.K., Bhuvaneswari T., Ravichandran P. (2017). Biofloc-based technology evaluation for nutrient-dense culture system for nursery and grow-out farming of Pacific white shrimp (*Litopenaeus vannamei*). Indian Journal of Fisheries, 64(1): 22-32.

Pereira L., Riquelme T., Hosokawa H. (2007). Effect of three photoperiod regimes on the growth and mortality of Japanese abalone (*Haliotis discus hannai* Ino). Kochi University, Aquaculture Department, Laboratory of Fish Nutrition, Japan, 26: 763-767.

Santi M.A. (2017). The use of *Indigofera zollingeriana* leaf meal as a substitute for soybean meal in broiler diets and its effect on broiler health. Jurnal Peternakan, 1(2): 17-21.

Sofiana S., Indariyanti N., Kurniawan A. (2023). The efficiency of *Indigofera* leaves meal hydrolysate utilization on growth performance of *Leptobarbus hoevenii*. Omni Akuatika, 19(1): 82-87.

Subandiyono, Hastuti S. (2016). Rabbitfish (*Siganus* sp.)

and the prospects of marine aquaculture in Indonesia. UNDIP Press, Semarang. ISBN 978-602-10653-89.

Subandiyono S., Sri H., Ristiawan A.N. (2018). Feed utilization efficiency and growth of Java barb (*Puntius javanicus*) fed on dietary pineapple extract. AACL Bioflux, 11(2): 309-318.

Subandiyono S., Hastuti S. (2020). Dietary protein levels affect growth and body composition of tilapia (*Oreochromis niloticus*). AACL Bioflux, 13(5): 2468-2476.

Subandiyono S., Hastuti S. (2022). Growth performance, feed utilization and hematological parameters of carp (*Cyprinus carpio*) according to dietary glutamate levels. AACL Bioflux, 15(2): 830-839.

Suharlina, Sanusi I. (2020). Nutritional quality of *Indigofera zollingeriana* forage supplemented with arbuscular mycorrhizal fungi biofertilizer. Jurnal Pertanian Terpadu, 8(1): 52-61.

Susilowati S., Wurlina, Mulyati S., Utama S., Meles D.K. (2020). Effects of silage, complete feed, and growth promoter on services per conception and milk production in repeat-breeder dairy cows. Ovozoa, 9: 28-34.

SNI 7548:2009. (2009). Artificial feed for catfish (*Pangasius* sp*.).* National Standardization Agency of Indonesia (BSN).

Tao J., Wang S., Qiu H., Xie R., Zhang H., Chen N., Li S. (2022). Modulation of growth performance, antioxidant capacity, non-specific immunity and disease resistance in largemouth bass (*Micropterus salmoides*) upon compound probiotic cultures inclusion. Fish and Shellfish Immunology, 127: 804-812.

Xie J., Li M., Ye W., Shan J., Zhao X., Duan Y., Liu Y., Unger B.H., Cheng Y., Zhang W., Wu N., Xia X.-Q. (2021). Sinomenine hydrochloride ameliorates fish foodborne enteritis via α7nAChR-mediated anti-inflammatory effect whilst altering microbiota composition. Frontiers in Immunology, 12: 766845.

Xuan C.L., Linh N.V., Wannavijit S., Outama P., Lubis A.R., Machimbirike V.I., Chromkaew Y., Phimolsiripol Y., Doan H.V. (2024). Enhancing growth, immunity, and gene expression in Nile tilapia (*Oreochromis niloticus*) through dietary supplementation with avocado (*Persea americana*) seed powder. Aquaculture Reports, 39: 102432.

Zeng X., Zheng X., Li C., Ming J., Dong H., Zhang J. (2024). Mechanisms of *Amomum villosum* essential oil in enhancing tilapia (*Oreochromis niloticus*) intestinal

health. Aquaculture Reports, 39: 102451.

Zaenuri R., Suharto B., Sutanhaji A.T. (2014). Quality of pellet-form fish feed made from agricultural waste. Jurnal Sumber Daya Alam dan Lingkungan, 1(1): 31-36.

Zhou Y., Yuan X., Liang X. F., Fang L., Li J., Guo X., Bai X., He S. (2013). Enhancement of growth and intestinal flora in grass carp: The effect of exogenous cellulase. Aquaculture, (416-417): 1-7.

Bock M.J., Jarvis G.C., Corey E.L., Stone E.E., Gribble K.E. (2019). Maternal age alters offspring lifespan, fitness, and lifespan extension under caloric restriction. Scientific Reports, 9: 31-38.

Chuah T.S., Loh J.Y., Hii Y.S. (2007). Acute and chronic effects of the insecticide-endosulfan on freshwater cladoceran, *Moina macrocopa straus*. Bulletin of Environmental Contamination and Toxicology, 79(5): 557-561.

Cardona E., Segret E., Cachelou Y., Vanderesse T., Larroquet L., Alexandre H. A., Surget A., Corraze G., Cachelou F., Bobe J., Skiba-Cassy S. (2022). Effect of micro-algae *Schizochytrium* sp. supplementation in plant diet on reproduction of female rainbow trout (*Oncorhynchus mykiss*): maternal programming impact of progeny. Journal of Animal Science and Biotechnology, 13: 1-33.

Carli A., Mariottini G., Pane L. (1995). Influence of nutrition on fecundity and survival in *Tigriopus fulvus* Fischer (Copepoda: Harpacticoida). Aquaculture, 134: 113-119.

Choedchim W., Maiphae S. (2023). Diversity and distribution of the *Cladocerans* (Crustacea, Branchiopoda) in Thailand. Biodiversity Data Journal, 11: e103553.

El-khodary G.M., Mona M.M., El-Sayed H.S., Ghoneim A.Z. (2020). Phylogenetic identification and assessment of the nutritional value of different diets for a copepod species isolated from Eastern Harbor coastal region. Egyptian Journal of Aquatic Research, 46: 173-180.

Farrer L., Cupples A., Kiely D.K., Conneally P.M., Myers R.H. (1992). Inverse relationship between age at onset of huntington disease and paternal age suggests involvement of genetic imprinting. American Journal of Human Genetics, 50: 528-535

He Z.H., Qin I.G., Wang Y., Jiang H., Wen Z. (2001). Biology of *Moina mongolica* (Moinidae, Cladocera)and perspective as live food for marine fish larvae: A review. Hydrobiologia, 457: 25-37.

Kohyama T.S., Kohyama T.I., Sheil D. (2017). Definition and estimation of vital rates from repeated censuses: Choices, comparisons and bias corrections focusing on trees. Methods in Ecology and Evolution, 9: 809-821.

Li M.H., Robinson E.H., Tucker C.S., Manning B.B., Khoo L. (2009). Effects of dried algae *Schizochytrium* sp., a rich source of docosahexaenoic acid, on growth, fatty acid composition, and sensory quality of channel catfish *Ictalurus punctatus*. Aquaculture, 292(3): 232-236.

Liguori A., Korm S., Gribble E.K., Profetto A., Richters E. (2023). Maternal age effects on offspring lifespan and reproduction vary within a species. Ecology and Evolution, 14(5): e11287.

Lin Q., Gao Y., Sheng J., Chen Q., Zhang B., Lu J. (2007). The effects of food and the sum of effective temperature on the embryonic development of the seahorse, *Hippocampus kuda* Bleeker. Aquaculture, 262: 481-492.

Magouz F.I., Essa M.A., Matter M., Mansour A.T., Alkafafy M., Ashour M. (2021). Population Dynamics, Fecundity and Fatty Acid Composition of *Oithona nana* (Cyclopoida, Copepoda), fed on different diets. Animals, 11(5): 1188.

Makrushin A.V. (2011). Senescence of *Moina macrocopa* (Cladocera, Crustacea). Advances in Gerontology, 24(1): 24-25.

Murugan A., Dhanya S., Sreepada R. A., Rajagopal S., Balasubramanian T. (2009). Breeding and mass-scale rearing of three spotted seahorse, *Hippocampus trimaculatus* Leach under captive conditions. Aquaculture, 290(1-2): 87-96.

Neri T.A., Rohmah Z.F., Ticar B.F., Choi B. (2020). Effect of different culture conditions on nutritional value of *Moina macrocopa* as a live feed for fish fry production. Journal of Agriculture and Life Science, 54(6): 91-98.

New M.B., Valenti W.C., James H.T., D'Abramo L.R., Kutty M. (2010). Freshwater Prawns Biology and Farming. Wiley-Blackwell. United Kingdom. 570 p.

Newcombe C.P., Macdonald D.D. (1991). Effects of suspended sediments on aquatic ecosystems. North American Journal of Fish Management, 11(1): 72-82.

Pan Y.J., Souissi A., Souissi S., Hwang J.S. (2016). Effects of salinity on the reproductive performance of *Apocyclops royi* (Copepoda, Cyclopoida). Journal of Experimental Marine Biology and Ecology, 475: 108-113.

Payne M., Rippingale R. (2000). Evaluation of diets for culture of the calanoid copepod *Gladioferens*

imparipes. Aquaculture, 187(1): 85-96.

Razlutskii V.I. (1992). Effect of trophic conditions on the rate of biological processes in Moina macrocopa Straus and *Moina rectirostris* Leydig. Gidrobiologicheskii Zhurnal, 28(1): 53-59.

Rocca W.A., van Duijn C.M., Clayton D., Chandra V., Fratiglioni L., Graves A.B., Heyman A., Jorm A.F., Kokmen E., Kondo K. (1992). Maternal age and alzheimer's disease: A collaborative re-analysis of case-control studies. International Journal of Epidemiology, 20(2): S21-S27.

Rodmongkoldee M., Taperhudee W., Saengphan N. (2020). Laboratory study on life history of three water flea species (Cladocera: Moinidae) in Thailand. Burapha Science Journal, 25(1): 129-140

Roff D.A. (2001). Age and size at maturity. In: C.W. Fox,

D.A. Roff, D.J. Fairbairn (Eds.), Evolutionary Ecology. Concepts and Case Studies. No. 448. New York: Oxford University Press. pp: 99-127.

Smirnov N.N. (2013). Physiology of the Cladocera. Academic Press, San Diego, USA. 352 p.

Støttrup J.G., Jensen J. (1990). Influence of algal diet on feeding and egg-production of the calanoid copepod *Acartia tonsa* Dana. Journal of Experimental Marine Biology and Ecology, 141(2-3): 87-105.

Sushchenya L.I., Semrnchenko V.P., Semenyuk G.A., Trubeckova I.L. (1990). Production of planktonic crustacean and factors of environment. Navuka I technika, Minks. pp: 133-141

Voronin V.N., Makrushin A.V. (2006). The artificial infection of the cladoceran *Moina macrocopa* (Crustacea: Phyllopoda) with the microsporidia *Gurleya* sp. (Microsporidia: Gurleyidae). Parazitologiia, 40(5): 462-471.

Venkataraman K. (1990). Biology of *Moina weismanni* Ishikava Cladocera Crustacea under laboratory conditions. Journal of the Andaman Science Association, 6(1): 60-62.