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Research Article

Chaya leaf meal as a substitute for soybean meal in climbing perch (*Anabas testudineus* Bloch, 1792) diet

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Abstract

The sustainability and cost-effectiveness of aquafeeds are crucial research focus in the aquaculture industry. This study investigates Chaya leaf meal (CLM; *Cnidoscolus aconitifolius*) as an alternative to soybean meal (SBM) in climbing perch (*Anabas testudineus*) diet. Over a three-month feeding trial, three dietary modifications were tested, incorporating 0% (CLM 0, control group), 20% (CLM 20), and 40% (CLM 40) Chaya leaf meal. The growth performance parameters indicated no significant differences between the dietary groups ($p>0.05$). However, 20% CLM diet led to significantly improved survival rates ($p<0.05$). Detailed analysis of organosomatic indices, body composition, fillet proximate composition, hematological, and blood chemical indices demonstrated overall consistency across experimental diets ($p>0.05$), except for a notable variance in the crude lipid content. Likewise, digestive enzymes activity remained stable across the dietary groups. From an economic standpoint, 20% CLM diet exhibited a competitive profit index compared to the control, significantly outperforming 40% CLM diet ($p<0.05$). These findings support the viability of 20% CLM as a partial substitute for soybean meal in climbing perch diets, providing environmental and economic advantages. Nevertheless, additional research is essential to determine the optimal CLM inclusion level and understand its long-term impact on fish health and productivity.

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Introduction

Aquaculture faces a challenge the sourcing sustainable and cost-effective feed ingredients (Hua *et al.*, 2019; Boyd *et al.*, 2020; Kari *et al.*, 2023). The industry's reliance on fish meal (FM) has become increasingly problematic due to its limited supply and rising costs (Olsen and Hasan, 2012; Gasco *et al.*, 2018; Jannathulla *et al.*, 2019). Soybean meal (SBM) is a common alternative due to its high protein content and favorable amino acid profile (Daniel, 2018; Howlader *et al.*, 2023; Stockhausen *et al.*, 2023). However, its increasing price has led to exploring other plant protein sources such as rubber seed meal (Deng *et al.*, 2015), cashew nut meal (Iheanacho *et al.*, 2019), sacha inchi meal (Khieokhajonkhet *et al.*, 2021), canola meal (Zhou and Yue, 2010) and sesame seed cake (Hekmatpour *et al.*, 2023). Furthermore, plant-based protein sources, including leaf meals have been successfully included in fish diets without compromising growth performance (Yuangsoi and Masumoto, 2012; Dorothy *et al.*, 2018; Zeng *et al.*, 2021; Olude *et al.*, 2022)

Among the various alternatives explored, Panghal *et al.* (2021) suggested that Chaya leaf meal (CLM) has emerged as a potential candidate for fish feed formulation. Chaya or tree spinach (*Cnidoscolus aconitifolius*) belongs to the family Euphorbiaceae that is a tropical perennial shrub with a rapid growth in Mexico (Ross-Ibarra and Molina-Cruz, 2002). Chaya leaves, an edible plant with an intense umami flavor, rich in nutrients, including high protein content, essential amino acids, minerals (P, K, Ca, Mg, Zn, Fe, Cu, and Na), and vitamins (A, B, C, D, E, and K) (Jiyil *et al.*, 2021; Hutasingh *et al.*, 2023). The previous studies highlighted that Chaya possesses a notably higher concentration of vitamin C and β-carotene, ranging from 2-3 times more than that found in spinach and common green leafy vegetables (Kuti and Kuti, 1999; Kuti and Torres., 1996). Chaya leaves are rich in phytochemicals like phenolic acids, alkaloids, saponins, flavonoids, and terpenoids (Orji *et al.*, 2016). These compounds have antioxidant properties that can be beneficial for multiple purposes, including the prevention and treatment of diabetes, inhibition of cancer growth, anti-inflammatory, nourishment of the blood, improvement of blood circulation, and anti-bacterial activity (Lennox and John, 2018; Padilla-Camberos *et al.*, 2021; Mahammad *et al.*, 2023; Morales-guerrero *et al.*, 2023). Despite their potential to cause irritation in the digestive system and disrupt feeding patterns due to the presence of hydrocyanic glycosides and oxalates, these anti-nutritional components can be reduced through heating or boiling (Donkoh *et al.*, 1999; González-Laredo *et al.*, 2003; Babalola and Alabi, 2015). Overcoming the challenge, CLM has been successfully used in various animal feed formulations, such as chicken feed (Wongnhor *et al.*, 2023), cattle feed (Totakul *et al.*, 2021), Nile tilapia feed (Obasa *et al.*, 2007), and blue shrimp feed (Rocha Estrada *et al.*, 2012).

However, a significant research gap exists regarding the utilization of CLM in fish feeds, particularly for climbing perch (*Anabas testudineus*) production. Climbing perch is a freshwater fish species with high economic value that has the potential for

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aquaculture development (Yulitine *et al.*, 2010; Syamsuddin *et al.*, 2019), and the industry is keen to investigate alternative feed ingredients for this species (Gokulakrishnan *et al.*, 2022; Al Mamun *et al.*, 2023). This study aims to fill this gap by investigating the potential of CLM as a viable substitute for soybean meal (SBM) in the diet of climbing perch.

The research question we address is whether CLM can partially replace SBM in climbing perch feed, thereby contributing to the sustainability and economic efficiency of aquaculture operations. Our study presents preliminary evidence that it can, provide valuable information for farmers to apply in their feed production using locally available raw materials. This approach could reduce production costs, promote sustainable aquaculture growth, and enhance global competitiveness. It represents an innovative change with the potential to significantly impact the aquaculture sector, promoting more sustainable and economically viable practices.

Materials and methods

Ethical statement

The experiment was conducted at the Fisheries Division laboratory of Mahasarakham University in Thailand. All experiments and protocols were strictly conducted following the guidelines outlined in the Code of Conduct for Animals in Scientific Research, established by the National Institute of Science and Technology. These procedures received approval under animal welfare license No. IACUC-MSU-28/2023, valid from October 3, 2022, to October 3, 2023, by the Institutional Animal Care and Use Committee of Mahasarakham University (IACUC-MSU).

Preparation and proximate composition of CLM

Fresh Chaya leaves were collected from the local gardens in Kantarawichai district located in Maha Sarakham province, Thailand. After sorting only green leaves, the leaves were thoroughly washed in clean water and chopped into small pieces approximately 1-2 mm. in size. The Chaya leaves were boiled in water for 5 minutes to reduce antinutrients (González-Laredo *et al.*, 2003). They were then dried (in a hot air oven 50°C for 48 h), ground, and sieved to obtain a meal. The provided CLM was stored in polyethylene zip lock bags at 4°C until it was used for the preparation of the experimental diets. Following the standard method of the Association of Official Analytical Chemistry (AOAC, 1990), CLM was subjected to proximate analysis to determine its composition. The analysis revealed values of 19.44±0.69%, 5.86 ± 0.38, 9.56±1.9%, and 8.14±5.27% for dry matter, crude protein, crude lipid, ash, and crude fiber, respectively.

Experimental design and diets

A completely randomized design with triplicates was employed in this study. Three experimental isonitrogenous and isoenergetic diets were formulated and produced for the experiment. These formulations were developed based on the nutritional requirement of climbing perch as described by Hossain *et al.* (2012). Soybean meal (SBM) was replaced in diets

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at 0, 20, and 40% by CLM in the balanced feed. The experimental groups were named accordingly as CLM 0 (0% SBM replacement as control treatment), CLM 20 (20% SBM replacement), and CLM 40 (40% SBM replacement). Before producing the diets, fish meal (with a crude protein content of 58% on a feed basis), SBM, rice bran (RB), cassava starch, and broken-milled rice meal (BMRM) were finely ground and sieved through a 0.5 mm mesh size. After accurate weighing, all ingredients were thoroughly mixed to ensure homogeneity. Soybean oil and premix were added, along with enough water to form a dough. The doughs were then pelletized by the extruder to obtain pellets with a diameter of approximately 2 mm. The pellets were dried in hot air oven for overnight at 60 °C and stored at 4°C in polyethylene zip lock bags until feeding. Each experimental diet was determined a feed cost per kg. The proximate composition of the experimental diets, including crude protein, crude lipid, ash, and crude fiber was determined and expressed in a dry matter basis according to the standard procedures described by AOAC (1990) (Table 1).

Table 1: Feed formulation and proximate composition (g kg^{-1}) of experimental diets.

Ingredient	Experimental diets		
	CLM0 (control)	CLM20	CLM40
Fishmeal (58% protein)	35	35	35
Soybean meal	30	24	18
Chaya leaf meal	0	14	28
Rice bran	9	10	6
Broken-milled rice meal	14	5	1
Cassava starch	5	5	5
Soybean oil	6	6	6
Mineral + Premix*	1	1	1
Proximate composition (g kg^{-1}dry matter basis) and feed cost			
DM	94.90	93.65	93.13
CP	35.79	35.35	35.09
CL	8.76	9.52	9.74
Ash	12.71	13.21	13.51
CF	4.90	4.74	4.57
NFE**	32.74	30.83	30.22
GE (kcal 100g^{-1}) ***	419.11	415.95	414.06
DE (kcal 100g^{-1}) ****	329.91	326.29	324.94
FC ($ kg^{-1}) *****	0.93	0.90	0.87

Abbreviations shown in the table indicate as follows: CLM 0 (0% SBM replacement), CLM 20 (20% SBM replacement), CLM 40 (40% SBM replacement), DM (Dry matter), CP (Crude protein), CL (Crude lipid), CF (crude fiber), NFE (Nitrogen-free extract), GE (gross energy), DE (Digestible energy) and FC (Feed cost).

*Vitamin and mineral mixer (IU or mg kg^{-1}): vitamin A (1,000,000 IU), vitamin D3 (2000,000 IU), vitamin E (5,000 mg), vitamin K3 (5,000 mg), vitamin B1 (3,000 mg), vitamin B2 (5,000 mg), vitamin B6 (3,000 mg), vitamin B12 (10 mg), vitamin C (10,000 mg), vitamin B3 (3,000 mg), vitamin B5 (1,000 mg), folic acid (1,000 mg), Mn (600 mg), Zn (8,000 mg), Cu (300 mg), Se (10 mg), Fe (300 mg), Co (330 mg), K (5,000 mg)

** % NFE = %DM – (% CP + % CL + % Ash + % CF)

*** GE (kcal 100g^{-1}) = %)CP × 5.64 %) + (NFE × 4.11) + (%CL×9.44)

**** DE(kcal 100g^{-1}) = (% protein × 3.5) + (% fat × 8.0) + (%NFE × 4.1)

***** Cost conversion rate: 1 US$ = 34.594 THB (2 June 2023)

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Proximate analysis

Chaya leaf meal (CLM), experimental diets, and fish fillets were analyzed for nutritional composition. Proximate composition was determined based on dry matter weight using AOAC official methods (AOAC, 1990). Crude protein content was measured by the Kjeldahl system (Gerhardt type vap. 40, Königswinter, Germany), crude lipid content by Soxhlet extraction (Büchi Extraction Unit E- 816 Hot Extraction) with hexane as the solvent, ash content by heating samples in a muffle furnace at a high temperature at 600°C for 2 h and the crude fiber (VELP® FIWE Raw Fiber Extractor) at a high temperature a 550°C content by acid and alkali digestion, with petroleum ether as the solvent.

Experimental fish and feeding

A total of 450 sex-reversed climbing perch fingerlings were acquired from a local farm in Mahasarakham province, Thailand. These fingerlings had initial weights measuring 3.32 ± 0.11 g and lengths measuring 5.22±0.74 cm. Before the experiment commenced, the fish were acclimatized in a cage for 2 weeks and fed a commercial fish feed (Charoen Pokphan Foods PCL., Thailand). Nine cages each measuring 1×1×1 m, were placed in a cement tank measuring 5×10×1.2 m which was equipped with an air-generated pump for oxygen circulation. The acclimatized fish were randomly divided and stocked at a density of 50 fish per cage (150 fish per treatment). The fish were fed either 5% of their body weight, twice daily (at 09:00 am and 4:00 pm) for 90 days. During the experimental trial, about one-third of the water was replaced biweekly and the water quality parameters including water temperature, dissolved oxygen, pH, and ammonia nitrogen were monitored weekly. These water quality parameters revealed an optimum level for fish culture according to the guidelines of water quality indices for aquaculture described by the Pollution control department of Pollution Control Department (PCD, 2022).

Sampling collection for analysis

Following the 90-day feeding trial, the fish were starved for a 24-h period in preparing the data collection. The survival fish in each cage were counted, and their length and weight were measured to evaluate growth performance, feed utilization, survival rate, and economic parameters. To further analysis, clove oil was employed as an anesthetic and euthanizing agent for the fish, with concentrations ranging from 50 to 150 mg L^{-1} according to Gokulakrishnan *et al.* (2022). Initially, a random selection of five fish per cage (totaling 15 fish per experimental diet) underwent anesthesia using clove oil at a concentration of 50 mg L^{-1} for 3 minutes. Subsequently, blood samples were collected from the anesthetized fish through caudal vein puncture using a 3 mL syringe. The obtained blood samples were divided into two portions to facilitate hematological and blood-chemical analyses. Approximately 0.5-1 mL of the first portion was stored in anticoagulant tubes containing EDTA for hematological analysis, while the remaining 1-2 mL was placed in clotted blood tubes without anticoagulant to evaluate blood chemistry. These blood

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sample tubes were then stored in a cooling box and promptly sent to the Vet Central Lab (Khonkaen, Thailand) within a 24-hour timeframe. Following the blood sample collection, the fish were euthanized using clove oil at a concentration of 150 mg L^{-1} for a duration of 10 minutes. Subsequently, the fish were dissected to evaluate their body composition, somatic parameters, enzyme digestibility, and the proximate composition of fish fillets.

Growth performance, feed utilization, body composition, and economic parameters

The collected data, including the total number, length, and weight of fish were used to calculate various parameters related to growth performance, feed utilization, survival rate, and economic parameters. The following formulas were employed for the calculations:

Weight gain (WG; g) = final body weight (g) - initial weight (g);
Specific growth rate (SGR; % d^{-1}) = 100 × [(ln final weight (g) - ln initial weight (g))/ days];
Average daily weight gain (ADWG; g day^{-1}) = (final weight (g) - initial weight (g))/ days;
Protein efficiency ratio (PER; %) = 100 × (weight gain (g)/protein intake (g));
Feed conversion ratio (FCR) = feed intake (g) /weight gain (g);
Survival rate (SR; %) = 100 × (final number of fish / initial number of fish);
Carcass yield (CY; %) = 100 × (carcass weight (g) / final weight (g));
Fillet yield (FY; %) = 100 × (flesh weight (g)/ final body weight (g));
Gonadosomatic index (GSI; %) = 100 × (gonad weight/body weight);
Hepatosomatic index (HSI; %) = 100 × (liver weight/body weight);
Viserosomatic index (VSI; %) = 100 × (visceral weight/body weight);
Economic conversion ratio (ECR; \$ kg^{-1}) = FCR × feed cost (\$ kg^{-1});
Economic profit index (EPI; \$ kg^{-1}) = (yield (kg) × value (\$ kg^{-1})) - ((yield (kg) × feed cost (\$ kg^{-1}))

Digestive enzymatic activity

After the 90-day experimental period, the digestive enzymatic activity of the climbing perch was analyzed. Fifteen fish were randomly selected from each group, and sampled from the stomach, proximal intestine, and distal intestine. These samples were pooled, homogenized using a 50 mM Tris-HCI pH 7.5 solution and centrifugated with speed 21,130 ×g at 4°C for 5 min. The resulting supernatant was stored at -20°C as a crude enzyme extract for further enzymatic analysis.

The protease activity was determined using the method established by Bezerra *et al.* (2005). The enzyme extract was incubated with Tris-HCl buffer (pH 7.2) and azocasein at 37°C. Subsequently, trichloroacetic acid was introduced, and the resulting absorbance was measured at 450 nm., then compared with the blank. The amylase activity was investigated following the modified method explained by Wangkahart *et al.* (2022), the enzyme extract was incubated with glycine NaOH buffer (pH 8.8) and a 1% starch solution at

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37 °C for an hour. Absorbance at 550 nm was measured and compared to a standard maltose sugar graph to determine maltose volume. The lipase activity was measured according to Iijima *et al.* (1998). The enzyme extract was incubated at 37°C for an hour after adding p-nitrophenyl palmitate and phosphate buffer (pH 7.0). Na_2CO_3 was added to stop the reaction, and p-nitrophenol volume was determined using a standard graph.

Hematological parameters

Total red blood cells (RBCs) and total white blood cells (WBCs) were counted using a Neubauer hemacytometer chamber after staining them with Wright-Giemsa (Natt and Herrick, 1952). Hematocrit (Hct) expressed as a percentage was evaluated through the microhematocrit method (Zhao *et al.*, 2018). Hemoglobin (Hb) was measured using an automated blood cell counter (Nihon Kohden model MEK-6550K). Different types of white blood cells (WBC) were counted by applying the Wright-Giemsa stain technique on a blood smear. Blood indices were assessed using the formulas (Saravanan *et al.*, 2011; Doan *et al.*, 2022) as follows:

Mean corpuscular volume (MCV; fL)= 10×(Hct (%)/RBCs (× 10^6 cells mm^3))

Mean corpuscular hemoglobin (MCH; pg)=10×(Hb (g dL^{-1}) /RBCs (× 10^6 cells mm^3))

Mean corpuscular hemoglobin concentration (MCHC; g dL^{-1})=100×(Hb (g dL^{-1}) /Hct (%))

Blood chemical profiles

The supernatant (150-200 µL) was carefully collected into sample cups after centrifugation the blood samples at 3,447 rpm for 10 minutes. Afterward, an automated A15 Biochemistry Analyzer (Biosystems S. A., Spain) was used to analyze the collected samples. The aspartate aminotransferase (AST), alanine aminotransferase (ALT), and blood urea nitrogen were evaluated using the kinetic method, following the guidelines established by the International Federation of Clinical Chemistry (IFCC) (Henley, 1980). The enzymatic method was used to assess glucose and cholesterol levels (Lott and Turner, 1975). Total protein and albumin levels were measured through a colorimetric method (Lowry *et al.*, 1951). Globulin level was derived by subtracting the albumin value from the total protein value.

Statistical analysis

The parameter values in this study are presented as Mean±SD. Before analysis, the data were subjected to normality and homogeneity tests to ensure compliance with the assumptions of the one-way ANOVA. Normality was assessed using the Kolmogorov-Smirnoff test, and homogeneity of variances was confirmed through Levene's test. Subsequently, ANOVA with a 95% confidence level was applied to determine any significant differences among the experimental diets. Post hoc analysis was conducted using Tukey's HSD test if significant differences were found in the ANOVA results.

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Results

The growth performance, feed utilization, and survival rate of sex-reversed climbing perch, which were fed three different experimental diets for a duration of 90 days, are presented in Table 2.

Table 2: Growth performances, feed utilizations, and survival rate of sex reversal climbing perch in the 90 days feeding trial.

Parameter	Experimental diets			P-value
	CLM 0	CLM 20	CLM 40	
IBW (g)	3.23 ± 0.57	3.57 ± 0.35	3.08 ± 0.39	0.447
FBW(g)	21.47 ± 2.64	23.57 ± 1.86	18.47 ± 1.74	0.066
IL (cm)	5.13 ± 0.38	5.37 ± 0.25	5.10 ± 0.30	0.560
FL (cm)	9.83 ± 0.61	10.03 ± 0.85	9.23 ± 0.12	0.316
WG (g)	18.23 ± 2.40	19.97 ± 1.90	15.40 ± 1.32	0.070
SGR (%)	2.10 ± 0.20	2.10 ± 0.17	1.97 ± 0.06	0.521
ADWG (g day^{-1})	0.20 ± 0.03	0.22 ± 0.02	0.17 ± 0.02	0.067
PER (%)	1.60 ± 0.44	1.67 ± 0.38	1.23 ± 0.06	0.351
FCR	1.87 ± 0.42	1.83 ± 0.38	2.33 ± 0.21	0.310
SR (%)	74.67 ± 2.31^{b}	84.00 ± 5.29^{a}	67.33 ± 4.16^{b}	0.007

Abbreviations shown in the table indicate as follows: CLM 0 (0% SBM replacement), CLM 20 (20% SBM replacement), CLM 40 (40% SBM replacement), IBW (initial body weight), FBW (final body weight), IL (initial length), FL (final length), WG (weight gain), SGR (specific growth rate), ADWG (average daily weight gain), PER (protein efficiency ratio), FCR (feed conversion ratio) and SR (survival rate).
Values indicate the mean±SD (n=3). Values in the same row followed by the different superscripts are significantly difference at $p<0.05$.

Growth performance parameters including the final body weight (FBW), final length (FL), weight gain (WG), specific growth rate (SGR), average daily gain (ADWG), protein efficiency ratio (PER), and FCR no statistically significant differences among the fish fed with CLM 20, CLM 40, and the control group ($p>0.05$). However, the fish fed with CLM 20 exhibited a significantly higher survival rate (SR) compared to those fed with CLM 0 and CLM 40 ($p<0.05$).

The body composition, organosomatic indices, and fillet proximate composition (% dry weight basis) of sex-reversed climbing perch were analyzed after a 90-day feeding trial with three different experimental diets (Table 3). No significant differences were found in carcass yield, fillet yield, GSI, HIS, and VSI among the groups of fish fed with these diets ($p>0.05$). Although no significant differences were found in the dry matter, crude protein, and ash values in the fillets ($p>0.05$), there was a significant difference in crude lipid content ($p<0.05$).

The results of the hematological indices and blood chemical parameters are shown in Table 4. There were no significant differences in any of the hematological parameters, including red blood cell count, hemoglobin, hematocrit, white blood cell count, lymphocytes, mean cell volume (MCV), mean cell hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC), between the experimental diets ($p>0.05$). Similarly, there were no significant differences in total protein, albumin, globulin, aspartate aminotransferase (AST) and alanine aminotransferase (ALT), blood urea nitrogen, cholesterol, and glucose among

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the three experimental diet groups (p>0.05).

The enzyme digestive activity of fish that were fed different diets is displayed in Table 5. The parameters of digestive enzymatic activity, including protease, amylase, and lipase exhibited no significant differences among the groups of fish fed with the three experimental diets (p>0.05).

Table 3: Body composition and organosomatic indies and fillet proximate composition (g kg^{-1}) of sex reversal climbing perch in the 90-day feeding trial.

Parameter (%)	Experimental diets			P value
	CLM 0	CLM 20	CLM 40	
Carcass	46.78 ± 1.61	46.89 ± 1.18	46.99 ± 1.61	0.984
Fillet	32.67 ± 0.55	29.33 ± 2.57	30.43 ± 2.24	0.194
GSI	9.10 ± 1.44	11.17 ± 0.83	10.93 ± 1.90	0.243
HIS	1.57 ± 0.12	1.33 ± 0.15	1.27± 0.15	0.089
VSI	14.14 ± 1.80	16.26 ± 0.36	15.73 ± 1.83	0.272
Fillet proximate composition (g kg^{-1}dry matter basis)				
DM	93.97±0.18	93.82±0.44	93.94±0.03	0.637
CP	83.57±0.94	82.95±0.09	83.98±0.34	0.172
CL	4.10±0.13^{a}	3.83±0.03^{a}	2.64±0.40^{b}	0.001
Ash	15.63±0.15	15.19±0.68	15.56±0.49	0.534

Abbreviations shown in the table indicate as follows: CLM 0 (0% SBM replacement), CLM 20 (20% SBM replacement), CLM 40 (40% SBM replacement), GSI (Gonadosomatic index), HIS (Hepatosomatic index), VSI (Viscerosomatic index), DM (Dry matter), CP (Crude protein) and CL (Crude lipid).
Values indicate the mean±SD (n=3). Values in the same row followed by the different superscripts are significantly difference at p<0.05.

Table 4: Hematological and blood biochemical indices of sex reversal climbing perch in the 90-day feeding trial.

Parameter	Experimental diets			P value
	CLM 0	CLM 20	CLM 40	
Hematological index				
RBC (× 10^{6} cells µl^{-1})	4.20±0.38	4.07±0.46	4.09±0.18	0.891
WBC (× 10^{3} cells µl^{-1})	1.54±0.11	1.47±0.28	2.13±1.49	0.626
Hb (g dL^{-1})	13.60±1.11	12.83±1.07	13.67±0.67	0.541
Hct (%)	44.67±5.03	43.67±3.21	45.33±4.16	0.889
Lymphocytes (Lym; %)	82.00±8.72	81.00±7.55	80.67±4.04	0.972
MCV (fL)	106.23±5.45	107.37±4.04	110.40±5.61	0.609
MCH (pg)	32.33±1.19	31.57±1.03	33.90±1.51	0.148
MCHC (g dL^{-1})	30.50±0.95	29.37±0.55	30.02±1.14	0.511
Blood biochemical index				
Total protein (g dL^{-1})	3.67±0.21	3.60±0.17	3.60±0.20	0.891
Albumin (g dL^{-1})	1.47±0.12	1.37±0.06	1.40±0.10	0.464
Globulin (g dL^{-1})	2.20±0.10	2.23±0.15	2.20±0.10	0.927
AST (U L^{-1})	101.67±59.50	94.67±53.27	98.33±11.68	0.983
ALT (U L^{-1})	29.67±7.51	24.67±9.24	21.00±4.36	0.403
BUN (mg dL^{-1})	1.33±0.58	1.67±0.58	2.00±0.00	0.296
Cholesterol (mg dL^{-1})	267.00±7.21	260.00±21.63	276.33±15.04	0.488
Glucose (mg dL^{-1})	149.33±41.19	106.33±39.00	131.33±31.21	0.421

Abbreviations shown in the table indicate as follows: CLM 0 (0% SBM replacement), CLM 20 (20% SBM replacement), CLM 40 (40% SBM replacement), RCB (red blood cell), WBC (white blood cells), Hb (Hemoglobin), Hct (Hematocrit), MCV (mean cell volume), MCH (mean cell hemoglobin), MCHC (mean cell hemoglobin concentration), AST (Aspartate aminotransferase), ALT (Alanine aminotransferase), and BUN (Blood Urea Nitrogen).
Values indicate the mean±SD (n=3).

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Table 5: Enzyme digestive activity of sex reversal climbing perch in the 90-day feeding trial.

Parameter	Experimental diets			*P*-value
	CLM 0	CLM 20	CLM 40	
Protease (U/mg protein)	19.96 ± 0.01	19.96 ± 0.01	19.97 ± 0.01	0.422
Amylase (U/mg protein)	2.64 ± 0.13	2.74 ± 0.19	2.67 ± 0.05	0.678
Lipase (U/mg protein)	81.35 ± 28.54	101.50 ± 19.11	107.41± 7.62	0.326

Abbreviations shown in the table indicate as follows: CLM 0 (0% SBM replacement), CLM 20 (20% SBM replacement), CLM 40 (40% SBM replacement).

The results of the cost and profit analysis for the experimental trial are presented in Table 6. The highest ECR was observed in CLM 0, but it did not show a significant difference compared to the others. Conversely, CLM 20 demonstrated an EPI that was not significantly different from the control group (CLM 0). However, it was significantly higher than that of CLM 40 ($p<0.05$).

Table 6: Economic indicators of sex reversal climbing perch in the 90-day feeding trial.

Parameter	Experimental diets			*P*-value
	CLM 0	CLM 20	CLM 40	
ECR *	1.77±0.42	1.63±0.38	2.03±0.12	0.463
EPI	1.33±0.15^{ab}	1.67±0.21^{a}	1.07±0.15^{b}	0.022

Abbreviations shown in the table indicate as follows: Abbreviations shown in the table indicate as follows: CLM 0 (0% SBM replacement), CLM 20 (20% SBM replacement), CLM 40 (40% SBM replacement), ECR (Economic conversion ratio) and EPI (Economic profit index).
* Selling price on 2 June 2023 by 2.6 US dollar kg^{-1} (source: www.kasetprice.com)
Values indicate the mean±SD (n=3).

Discussion

Reliance on traditional protein sources like fishmeal and soybean meal in aquafeeds has raised concerns about sustainability and cost-effectiveness due to overfishing, environmental impacts, and fluctuating resource availability. Exploring alternative protein sources is therefore crucial for addressing these concerns and improving feed formulations in aquaculture. The current study sought to determine the feasibility of using CLM as an effective substitute for soybean meal in the diet of climbing perch (*A. testudineus*), with a focus on growth performance, feed utilization, digestive enzymes, body composition, hematological and blood biochemical values, and economic implications.

The key finding of this study is that incorporating CLM into the diet of climbing perch did not lead to any significant differences in crucial growth performance parameters, including FBW, FL, WG, SGR, ADWG, PER, and FCR. These findings suggest that CLM can serve as an effective substitute for soybean meal, supporting comparable growth performance in climbing perch. This aligns with similar results reported by Gokulakrishnan *et al.* (2022), where climbing perch fingerlings exhibited promising growth performances including weight gain and FCR improvement, with the inclusion of brewer's spent yeast in their diet. FCR To further contextualize these findings, we can draw comparisons with two notable studies that also explored the

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use of CLM in aquafeeds. The first study focusing on Nile tilapia (*Oreochromis niloticus*) demonstrated that a diet with 20% CLM achieved the best FCR and SGR (Obasa *et al.*, 2007). The second study by Rocha Estrada *et al.* (2012) investigated the nutritional value of CLM as an alternative diet for blue shrimp (*L. stylirostris*). The results showed that the 20% CLM and the commercial diet were optimum for feeding rate and WG. This indicates a parallel result to the current study, reinforcing the idea that CLM can be a viable replacement for soybean meal protein in the diet of various aquatic species. Similar positive outcomes were observed when alternative protein sources for SBM in aquafeeds. For instance, roquette (*Eruca sativa* Miller) seed meal can replace up to 20% of SBM in the diet of African catfish (*Clarius gariepinus*) without compromising growth and nutrient utilization (Fagbenro, 2004). Additionally, replacing 50% of SBM with cashew nut (*Anacardium occidentale*) meal improved the growth performance of African catfish (Iheanacho *et al*., 2019). The sunflower meal can replace up to 50% of SBM without a negative effect on the growth performance of grass carp (Shi *et al.*, 2023). These outcomes are highly encouraging, particularly considering the escalating demand for alternative protein sources in aquaculture feed formulations.

Furthermore, the current study revealed that the survival rate (SR) of fish fed with CLM 20 was significantly higher compared to those fed with CLM 0 and CLM 40, implying a potential positive effect of CLM on the overall health and survival of climbing perch. The diet of fish plays a significant role in their survival rate (Sultana *et al.*, 2018), and these findings are consistent with previous studies on Nile tilapia, rainbow trout (*Oncorhynchus mykiss*) and white shrimp (*Litopenaeus vannamei*). When Nile tilapia was fed with varying levels of CLM in their diet, they exhibited a high survival rate (Obasa *et al.*, 2007). Similarly, Yadollahi *et al.* (2018) documented that the survival rate of rainbow trout fed with a diet replacing 50% of SBM with guar meal was greater than the 70% substitution rate. Furthermore, Wang *et al.* (2020) observed a significant decrease in the survival rate of white shrimp when SBM was replaced by cottonseed meal.

Digestive enzymes aid in nutrient digestion, growth, and health, and are key for aquaculture management (Magouz *et al.*, 2020). In the present study, the digestive enzymatic activity parameters, including protease, amylase, and lipase, did not show any significant differences among the different diet groups. These results suggest that the inclusion of CLM in the diet did not significantly affect the digestive capacity of climbing perch. Similar findings have been reported in other studies investigating the use of alternative protein sources in fish diets. For example, studies on hybrid tilapia (*O. niloticus* × *O. aureus*) revealed that the inclusion of rubber seed meal did not significantly impact digestive enzyme activities (Deng *et al.*, 2015). This variation observed, however, could be attributed to differences in the species, environment, and diets. The inclusion of CLM in the blue shrimp (*Litopenaeus stylirostris*) diet revealed a significant effect on the activity of digestive enzymes (Rocha Estrada *et al.*, 2012). Pradhan *et al.* (2020) documented that a significant

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difference in the digestive enzymatic activity of Nile tilapia (*O. mossambicus*) fed with the inclusion of cashew nut meal diets were found. When the substitution level of SBM with sesame seed cake was increased up to 100% in the diet of juvenile common carp (*Cyprinus carpio*), a significant rise in the digestive enzyme activity was observed (Hekmatpour *et al.*, 2023).

Carcass composition parameters, including carcass yield, fillet yield, GSI, HSI, and VSI, showed no significant differences among fish fed a diet containing CLM compared to the control diet as indicated by the previous studies exploring alternative protein sources in diets. For instance, the somatic indices of red hybrid tilapia (*O. niloticus*×*O. mossambicus*) were unaffected by the replacement of SBM with sacha inchi meal (*Plukenetia v*olubilis L.) (Khieokhajonkhet *et al.,* 2021). Similarly, juvenile common carp (*Cyprinus carpio*) fed with different levels of sesame seed (*Sesamum indicum*) exhibited no significant differences in somatic indices among the treatments (Hekmatpour *et al.*, 2023). Furthermore, there were no significant differences among the dietary treatments of hybrid catfish (*Ictalurus punctatus* × *I. furcatus*) fed combinations of cottonseed meal and one or two other alternative protein sources, including distillers dried grains with solubles (DDGS), peanut meal, and porcine meat and bone meal (PMBM) in the diet (Li *et al.*, 2018). In another instance demonstrating a positive effect after incorporating an alternative protein source into the diet, Nile tilapia, when fed with 75% blanched duckweed meal, exhibited the highest carcass protein content of 60.80% (Abdullahi, 2023). These results affirm that a high level of plant protein in diets itself does not directly affect the overall body composition and organ development of fish.

For the proximate composition of fish fillets, no significant differences were found in the dry matter, crude protein, and ash values among the different diet groups. However, notable variations were observed in the crude lipid content, with CLM 40 group showing a decrease in crude lipid content compared to the other groups. This reduction may be attributed to the different lipid content composition between Chaya leaves and soybean meal (Ayadi *et al.*, 2012; Jiyil *et al.*, 2021). This finding is consistent with the previous studies that have reported variations in the lipid content of fish fed with different plant protein sources. As an illustration, the substitution of soybean meal with guar meal in the diet of rainbow trout resulted in a noticeable increase in crude lipid content, as evidenced by Yadollahi *et al.* (2018). A similar finding was reported in African catfish fed with an incorporated cashew meal diet (Iheanacho *et al.*, 2019). Wang *et al.* (2020) documented that body composition of juvenile white shrimp, (*Litopenaeus vannamei*) was significantly increased with an increasing amount of cotton seed meal replacing SBM in their formulated diet. Several studies demonstrated that the inclusion of alternative protein sources has a non-significant difference in whole fillet composition across between treatments. Examples include protein enhanced copra meal (PECM®) for grouper (*Epinephelus*

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fuscoguttatus) (Mamauag *et al.*, 2019) fermented palm kernel meal for sex-reversed red tilapia (*Oreochromis niloticus*×*O. mossambicus*) (Wattanakul *et al.*, 2021) and sesame seed (*Sesamum indicum*) cake for juvenile common carp (*C. carpio*) (Hekmatpour *et al.*, 2023). The findings indicate that various fish species exhibit distinct responses to different dietary compositions, potentially influencing their body composition according to their specific nutritional requirements (Lall and Dumas, 2015).

The hematological and blood biochemical indices play a crucial role as potential diagnostic tools for monitoring fish health status and responding to nutritional alterations (Fazio, 2019).

In the present study, the blood parameters did not show any significant variations among the different diet groups. Similar findings were reported for *Clarias gariepinus* fingering fed diet containing sicklepod leaf (*Cassis tora*) meal (Jibrin *et al.*, 2021) and roselle seeds (*Hibiscus sabdariffa*) (Usman *et al.*, 2023). Moreover, hematological and blood biochemical profiles of red hybrid tilapia (*O. niloticus* × *O. mossambicus*) exhibited no significant differences among groups and basal diet when fed with varying levels of sacha inchi meal (*P. volubilis* L.) (Khieokhajonkhet *et al.*, 2021). These results suggest that the inclusion of CLM in the diet did not significantly affect the health of fish.

Regarding economic profitability, the highest ECR was observed in the control group (CLM 0), indicating a higher cost of feed required for producing a unit of fish biomass. However, this difference was not statistically significant compared to CLM 20 and CLM 40 groups. Notably, CLM 20 exhibited an EPI that was significantly higher than that of CLM 40. Similar economic benefits have been reported by Poot-López and Gasca-Leyva (2009) that the substitution of chaya leaves in the balanced feed of Nile tilapia at up to 50% provided the lowest production costs. These economic findings suggest that the inclusion of CLM in the diet can potentially improve the cost-effectiveness of climbing perch production.

In conclusion, the present study provides evidence supporting the potential of CLM as an effective substitute for soybean meal in the climbing perch diet. The results demonstrate that CLM inclusion did not significantly affect the growth performance, digestive enzymatic activity, carcass composition, hematological parameters, and blood biochemical indices of climbing perch. Moreover, the inclusion of CLM in the diet resulted in a significantly higher survival rate and improved economic profitability compared to the control group and a higher economic profit index compared to CLM 40 group. These findings highlight the feasibility of utilizing CLM as a sustainable alternative protein source in fish diets, contributing to the development of cost-effective and environmentally friendly aquaculture practices.

Further studies are recommended to investigate the optimal inclusion levels of CLM in the diet of climbing perch to maximize growth performance while maintaining high survival rates and economic profitability. Additionally, further research should explore the effects

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of CLM on fish survival rate, economic efficiency, health, immunity, and product quality. This study could provide valuable insights into using CLM as a soybean meal alternative in aquaculture, contributing to sustainable fish feed development and emphasizing CLM's potential as a viable protein source for sustainable fish farming.

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Conflicts of interest

The authors declare no conflicts of interest.

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