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Research article

Somatic responses of injured Siamese fighting fish (*Betta splendens*) raised using various recipes of Thai herbal fermented water

Saowalak Malawa[a], Nutt Nuntapong[a], Karun Thongprajukaew[b,c,*]

[a] *Aquatic Science and Innovative Management Division, Faculty of Natural Resources, Prince of Songkla University, Songkhla 90110, Thailand*

[b] *Division of Health and Applied Sciences, Faculty of Science, Prince of Songkla University, Songkhla 90110, Thailand*

[c] *Center of Excellence in Agricultural and Natural Resources Biotechnology Phase 3, Faculty of Natural Resources, Prince of Songkla University, Songkhla 90110, Thailand*

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Abstract

Importance of the work: Thai herbal remedies offer an alternative to chemicals to help in the recuperation of injured Siamese fighting fish (*Betta splendens*).

Objective: To evaluate fish survival and growth, feed utilization, skin coloration, digestive enzyme activity, muscle quality and whole-body composition at the end of herbal treatment for 5 wk.

Materials & Methods: Each male fighting fish had one-half of its caudal fin amputated and then was allocated to one of nine experimental groups. Each fish was reared separately for 5 wk in its own small tank. Treatments consisted of two controls, with one using non-fermented water (CT 1), while the other control consisted of fermented water (CT 2) and was mixed with various products—dried lemongrass leaves (1 g/L), dried clay (50 g/L) and salt (5 g/L). Subsequently, CT 2 was added to different combinations of dried Indian almond leaves (0.25 g/L), dried banana leaves (1.5 g/L) or dried papaya leaves (2.5 g/L) to make seven other treatments.

Results: No mortality occurred and fish growth performance was not influenced by the herbal treatments. The feed conversion ratio significantly ($p < 0.05$) increased in association with decreasing amylase-specific activity, whereas protein- and lipid-digesting enzymes remained consistent. Skin coloration parameters were either reduced or preserved due to the herbal treatments. Muscle quality was unchanged, whereas the crude protein, crude lipid and ash contents in whole-body were similar across the nine treatments.

Main findings: Whereas entire fish can compensate for growth normally, these findings indicated minor effects of long-term herbal treatments on the somatic changes in the injured fish. The results endorsed the therapeutic approach of fermented water of Thai herbs in Siamese fighting fish.

* Corresponding author.
E-mail address: karun.t@psu.ac.th (K. Thongprajukaew)

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Introduction

Betta splendens, also known as the Siamese fighting fish, is a small freshwater fish distinguished by its remarkable color variations and captivating fin morphological traits, whose origins can be traced back to Southeast Asia, notably within central Thailand (Goldstein, 2004; Monvises et al., 2009). Furthermore, Thailand's ornamental fish industry, exclusively targeting these male 'betta' fish, has witnessed substantial growth in captive breeding and international exports (Department of Fisheries, 2021). In the first half of 2021, betta was ranked first among 10 popular ornamental fish in Thailand, with the exported volume surpassing 10 million fish reported at Suvarnabhumi Airport (Department of Fisheries, 2021). The USA is the primary importer of Siamese fighting fish from Thailand, followed by China, France, Iran and Singapore (Sermwatanakul, 2019).

Inherently, these fish display agonistic behavior in confrontations associated with defending territory, be it between males and females or among individuals of the same gender, resulting in damaged bodies and torn fins (Malawa et al., 2023). Consequently, the breeders raise these fish in solitary captivity, commencing at approximately age 1.5 mth (Thongprajukaew et al., 2010a, b), to mitigate the adverse effects of their combative nature. In addition, these injuries can manifest during courtship and reproductive phases (Malawa et al., 2023).

In Thailand, immersion in herbal treatment is the preferred and practical treatment to heal external damage in injured fish rather than using antibiotics or chemicals that cause adverse health effects to the fish and (via the residues) in their surroundings (Malawa et al., 2022, 2023). Typically, some herbs, such as the dried leaves of the Indian almond (*Terminalia catappa*), banana (*Musa sapientum*), papaya (*Carica papaya*) and lemongrass (*Cymbopogon citratus*) or dried clay or salts, have been incorporated into the recipes used to treat the fish (Malawa et al., 2023). These ingredients have various phytoconstituents, such as tannin, flavonoid and phenolic compounds, differing in quantity and active ingredient type. For example, the dried leaves of the Indian almond aid in wound healing and act against harmful monogeneans and *Trichodina* spp. (Chitmanat et al., 2005; Ponpornpisit et al., 2007), while the dried leaves of banana, papaya and lemongrass provide anti-bacterial, anti-oxidant and anti-inflammatory activities that help to heal wounds (Wijaya et al., 2020; Maria and Veronica, 2021; Tanmoy et al., 2021), especially those caused during fish fighting. Dried clay and salt are added to help fish recover more rapidly, to heal wounds, to nourish the skin and to reduce pathogen growth (Putiyanan, 1999; Puello-Cruz et al., 2010; Chiarachavildham, 2016).

Other benefits have been reported regarding the use of certain herbs, specifically the optimal concentration of *T. catappa* leaf extract to enhance the survival rate, growth performance and feed utilization (Nugroho et al., 2016; Nurhidayat et al., 2016; Ramos et al., 2020). Additionally, this herb has been associated with improvements in digestive enzyme activities (Malawa et al., 2022) and skin coloration (Ardi et al., 2020; Malawa et al., 2022), but without affecting muscle quality and the whole-body composition in healthy fish (Malawa et al., 2022). However, an excessive concentration of its extract may adversely affect these parameters (Ikhwanuddin et al., 2014; Mininel et al., 2014).

Although herbal treatment involving Indian almond leaf has been documented for healthy Siamese fighting fish, there is a noticeable lack of information regarding using a combination of ingredients and their effects on somatic changes in injured fish. Therefore, the current study examined fish survival and growth, feed utilization, skin coloration, digestive enzyme activity, muscle quality and whole-body composition. Caudal fin amputation was used as a model to simulate a torn fin on the fish resulting from agonistic behavior. The findings from the current study might support using Thai herbal treatment in betta fish farms.

Materials and Methods

Ethics statements

The Institutional Animal Care and Use Committee at Prince of Songkla University approved the utilization of the animals in this scientific research (Project Code 2564-01-030). Transportation, acclimatization, surgery, husbandry and sampling were carried out under this agreement.

Preparation of herbal fermented water

Newly fallen leaves (Indian almond, banana and papaya), mature leaves (lemongrass) and clay were harvested from a few farms in Songkhla province, Thailand. The samples were cleaned, soaked in 5 mg/L of $KMnO_4$ for 15 min to eliminate attached microorganisms and then washed three times with distilled water. They were dried at 60°C for 12 hr, crushed using a grinder, sieved, homogenously mixed, packed in polyethylene bags, placed inside black plastic bags to exclude light and stored at 4°C until used.

The components of each herbal formula were detailed in Malawa et al. (2023). The chosen concentration of each ingredient was based on available documents (Banplakatrayong, 2022; Institute for Innovative Learning, 2022; Malawa et al., 2022; Rucksapram, 2007). Amounts of dried clay was estimated from Chiarachavildham (2016). Table salt was directly added to the rearing water to achieve a final concentration of 5 g/L (Puello-Cruz et al., 2010). Two control groups were established: one using non-fermented water (CT 1) and the other fermented water (CT 2), to which were added dried lemongrass leaves (1 g/L), dried clay (50 g/L), and salt (5 g/L). Three distinct treatments were created by incorporating dried Indian almond leaves (0.25 g/L), dried banana leaves (1.5 g/L), or dried papaya leaves (2.5 g/L) into CT 2. These treatments were denoted as IA, B and P, respectively. Additionally, three more treatments emerged by introducing combinations of dried Indian almond leaves and dried banana leaves, dried Indian almond leaves and dried papaya leaves, and dried banana leaves and dried papaya leaves to CT 2. These treatments were labeled as IAB, IP and BP, respectively. The seventh treatment was generated by adding dried Indian almond leaves, dried banana leaves and dried papaya leaves together to CT 2, and this treatment was designated as IBP. The fermentation was performed by soaking the relevant ingredients in dechlorinated water in 30 L plastic containers (31 cm width × 30 cm length × 49.7 cm height) at room temperature for 3 d, stirring twice daily. At the end of 3 d, the mixtures were passed through filter cloth and Whatman No. 4 paper. The obtained filtrate was used for fish rearing.

Herbal treatment trial

A completely randomized design was conducted using the nine herbal formulations, with 15 individual fish serving as experimental units. The fish preparation and fin amputation were performed as described by Malawa et al. (2023). First, each fish was anesthetized with a 15 mg/L solution of clove oil and then the caudal fin lengths were measured using a set of Vernier calipers. Subsequently, one-half of the caudal fin length was amputated in the vertical plane using a sterilized scalpel. The amputated fish (n = 135) with their fins excised were carefully carried and raised separately in cylindrical plastic containers measuring 9.3 cm in diameter, 12.5 cm in height and 8 cm in depth. These containers were filled with 350 mL of rearing water, comprised of either freshly fermented water obtained from the eight Thai herbal recipes (CT 2, IA, B, P, IAB, BP IP, and IBP) or dechlorinated tap water (CT 1). All fish were reared under a 12 hr light (0600–1800 hours)/ 12 hr dark cycle for 5 wk until there was complete regeneration of the caudal fin. During the herbal treatment period, all the fish were fed *ad libitum* using a commercial floating feed for fighting fish, which contained 39.4% crude protein (Betta Bio-Gold; Kyorin Food Ind. Ltd.; Himeji, Japan). Feeding occurred twice daily, at 0800 hours and 1700 hours. After each feeding session, any remaining feed was collected within 30 min and then dried at 60°C until it reached a constant weight. Obtained data were used to compute the feed conversion ratio (FCR) and the protein efficiency ratio (PER). Every 3 d, 80% of the rearing water was replaced with water containing freshly prepared fermented water. Twice a week, throughout the experimental period, various physio-chemical parameters of the water were measured: temperature (23.9±0.1°C), pH (6.51±0.03), conductivity (5.41±0.21 mS/cm), total dissolved solids (3.43±0.13 g/L), dissolved oxygen (5.71±0.06 mg/L), salinity (3.00±0.12 g/L), total alkalinity (129±5 mg/L), hardness (255±9 mg/L), total ammonia nitrogen (1.28±0.03 mg/L), nitrite (0.09±0.01 mg/L) and nitrate (0.86±0.15 mg/L).

Fish mortality was monitored daily throughout the experiment. At the end of the trial (5 wk), all the surviving fish (n = 15 per treatment) were starved for 24 hr and then sacrificed by chilling in ice before recording body weight (BW) and total length. Samples of viscera (n = 5 per treatment), digestive tracts (n = 5 per treatment) and white muscle (n = 5 per treatment) were gathered from the same group of fish to assess the viscerosomatic index (VSI), digestive enzyme activity and muscle quality, respectively. The whole-body composition analysis used 10 fish chosen from each treatment. Three or four fish were pooled into each lot to obtain one pooled sample (three pooled samples per treatment). The parameters describing survival, growth performance and feed conversion were computed using Equations 1–5:

$$\text{Survival (\%)} = 100 \times (\text{Final fish number} / \text{Initial fish number}) \quad (1)$$

$$\text{Weight gain} = \text{Final BW} - \text{Initial BW} \quad (2)$$

$$\text{VSI} = 100 \times [\text{Wet weight of viscera} / \text{BW}] \quad (3)$$

$$\text{FCR} = \text{Dry feed consumed} / \text{Wet weight gain} \quad (4)$$

$$\text{PER} = \text{Wet weight gain} / \text{Protein intake} \quad (5)$$

Where all parameters are measured in grams and the VSI is determined as a percentage.

Skin coloration

The fish skin was carefully cleaned using soft blotting paper. Subsequently, a portable spectrophotometer (MiniScan EZ 4500; Hunter Associates Laboratory; Reston, VA, USA) was calibrated using black and white standards before assessing the skin coloration in the mid region of the fish. The color parameters were digitally displayed on the LCD screen as L^*, a^*, h^* and C^*.

Determination of digestive enzyme activities

Enzyme extraction and protein quantification in crude extracts

The frozen gastrointestinal tract was extracted in cold 0.2 M Na_2HPO_4-NaH_2PO_4 buffer (1:15 weight per volume) using a tissue homogenizer (THP-220; Omni International; Kennesaw, GA, USA). The homogenate was centrifuged at 15,000×g and 4°C for 30 min to collect the supernatant from debris and then preserved at -20°C. The total protein concentration in the crude extract was assessed quantitatively based on the method outlined by Lowry et al. (1951) to determine the specific activity of the digestive enzymes (measured in units per milligram of protein).

Digestive enzyme assays

All enzyme assays of the betta fish were performed under optimized conditions of pH and temperature, as reported by Thongprajukaew et al. (2010a, b). Pepsin activity was examined according to the method of Worthington (1993). Trypsin and chymotrypsin activities were assessed based on Rungruangsak-Torrissen et al. (2006). Lipase activity was determined according to the method of Winkler and Stuckmann (1979). The alpha-amylase activity was assayed according to the method of Areekijseree et al. (2004). For these four enzyme tests, one unit (U) was determined as the catalyzed amount to convert 1 μmol of substrate per minute. The amylase-to-trypsin ratio was computed as the amylase activity divided by the trypsin activity from the same fish sample.

Muscle quality

RNA and the protein concentration were assayed according to the method of Rungruangsak-Torrissen (2007). Myosin, actin and the sarcoplasmic contents were monitored based on weighing defrosted white muscle (approximately 10 mg each), placing in an aluminum pan, sealing with an aluminum lid and then heating at a rate of 5°C/min relative to an empty pan. Muscle thermal characteristics were assessed through differential scanning calorimetry (DSC7; Perkin Elmer; Waltham, MA, USA) within the temperature range 20–100°C, to identify myosin, actin and sarcoplasmic protein based on the onset (T_o), peak (T_p) and conclusion (T_c) temperatures derived from thermograms as documented in Thongprajukaew et al. (2023) concerning betta fish muscle.

Whole-body composition

The whole body of each fish was uniformly minced before undergoing analysis for proximate composition, based on the moisture, ash, crude protein and crude lipid contents, following the standard procedures outlined in Association of Official Analytical Chemists (2005).

Statistical analysis

The trial used a completely randomized design comprising nine herbal formulations, with 15 individual fish as experimental units. The acquired data were presented as mean ± SD values. The data were subjected to one-way analysis of variance and mean comparisons were performed using Duncan's multiple range test at the $p < 0.05$ test level for significance. All statistical analyses were performed using the Statistical Package for Social Sciences version 22 (SPSS Inc.; Chicago, IL, USA).

Results

Survival, growth performance and feed utilization

No mortality was observed in the fish reared in any of the herbal treatments. The herbal treatments did not affect the growth and feed utilization parameters, except for FCR, which significantly increased in the fish raised in the CT 2, P, BP, IP and IBP treatments (Table 1).

Table 1 Growth performance and feed utilization of male Siamese fighting fish reared in various medicinal recipes extract, where parameters were measured at end of experiment (at 5 wk)

Herbal recipe	Parameter					
	FBW (g)	WG (g)	FTL (cm)	VSI (%)	FCR	PER
CT 1	1.47±0.19	0.43±0.39	4.88±0.28	6.18±2.06	3.30±0.75[c]	1.08±0.26
CT 2	1.50±0.14	0.28±0.32	5.02±0.14	5.59±0.80	4.28±0.97[ab]	1.11±0.09
IA	1.56±0.07	0.27±0.06	5.01±0.18	6.73±0.82	3.24±0.44[c]	1.19±0.06
B	1.52±0.08	0.23±0.09	5.00±0.17	6.50±0.86	4.04±1.42[abc]	1.14±0.12
P	1.50±0.10	0.21±0.05	4.78±0.65	5.65±0.97	4.33±0.69[ab]	1.12±0.08
IAB	1.53±0.09	0.24±0.07	4.97±0.21	7.00±1.05	3.75±0.73[bc]	1.16±0.08
BP	1.42±0.14	0.30±0.34	4.88±0.19	5.05±0.55	4.69±1.11[a]	1.04±0.11
IP	1.53±0.23	0.31±0.33	5.02±0.24	7.01±1.97	4.32±0.70[ab]	1.10±0.06
IBP	1.47±0.12	0.35±0.35	4.98±0.14	6.54±1.10	4.35±1.28[ab]	1.12±0.08
p-Value	0.229	0.449	0.290	0.101	0.002	0.082

FBW = final body weight; WG = weight gain; FTL = final total length; VSI = viscerosomatic index; FCR = feed conversion ratio; PER = protein efficiency ratio; CT 1 = negative control (dechlorinated water); CT 2 = positive control (dried lemongrass leaves + dried clay + salt); IA = CT 2 + dried Indian almond (*Terminalia catappa*) leaf; B = CT 2 + dried banana (*Musa sapientum*) leaf; P = CT 2 + dried papaya (*Carica papaya*) leaf; IAB = CT 2 + dried Indian almond and banana leaves; BP = CT 2 + dried banana and papaya leaves; IP = CT 2 + dried Indian almond and papaya leaves; IBP = CT 2 + dried Indian almond, banana and papaya leaves.
Different lowercase superscripts in the same row indicate significant ($p < 0.05$) differences.

Skin coloration

There were significant differences in skin coloration among the fish in the various herbal treatments, except for L^* (Table 2). Relative to CT 1 and CT 2, there was a notable trend of either unchanged or reduced a^*, h^* and C^* values in all treatment groups ($p < 0.05$).

Table 2 Color parameters of male Siamese fighting fish reared in various treatments involving fermented water based on Thai herbal recipes, where parameters measured at end of experiment (at 5 wk)

Herbal recipe	Color parameter			
	L^*	a^*	h^*	C^*
CT 1	23.0±2.9	13.5±1.2[a]	0.51±0.06[a]	15.5±1.5[a]
CT 2	21.5±2.0	14.2±2.0[a]	0.50±0.05[ab]	16.2±2.1[a]
IA	23.5±2.8	12.2±2.0[b]	0.47±0.05[abc]	13.8±2.3[b]
B	24.3±2.6	11.4±1.3[bc]	0.45±0.04[bc]	12.7±1.6[bc]
P	23.3±2.6	11.0±1.4[bc]	0.43±0.09[c]	12.2±1.8[c]
IAB	23.7±2.6	11.6±1.4[bc]	0.46±0.07[abc]	13.0±1.7[bc]
BP	23.6±3.2	11.2±1.6[bc]	0.48±0.05[abc]	12.6±1.9[bc]
IP	23.0±1.1	11.4±1.3[bc]	0.49±0.04[ab]	13.0±1.6[bc]
IBP	22.8±2.1	10.6±1.5[c]	0.46±0.07[bc]	11.8±1.9[c]
p-value	0.208	< 0.001	0.012	< 0.001

L^* = lightness; a^* = redness; h^* = hue; C^* = chroma.
Details for each treatment are shown in Table 1 footnote.
Different lowercase superscripts in the same row indicate significant ($p < 0.05$) differences.

Digestive enzyme activities

The herbal treatments had no significant effects on digestive enzyme-specific activities, except for amylase (Fig. 1), for which there was a significant reduction in the fish reared in treatments CT 2, BP, IP and IBP relative to the control group (dechlorinated water).

Protein synthesis capacity, and myosin and actin contents

There was no significant difference in the values for the average RNA (2,704±520 μg/g), protein (210±46 mg/g) or RNA-to-protein ratio (13.4±3.1 μg/mg) among the fish subjected to any of the herbal treatments. The principal muscle proteins, myosin, actin and sarcoplasmic protein levels exhibited temperature ranges of 41.5–53.4°C, 67.5–74.2°C and 81.0–85.5°C, respectively, from onset heating to the conclusion. Relative to the control group, there were no significant differences in the values for the average myosin (0.89±0.30 J/g), actin (0.35±0.12 J/g) and sarcoplasmic protein (0.10±0.04 J/g) contents, or their total amounts (1.34±0.36 J/g) or proportions (0.43±0.19 J/g) for all herbal treatments.

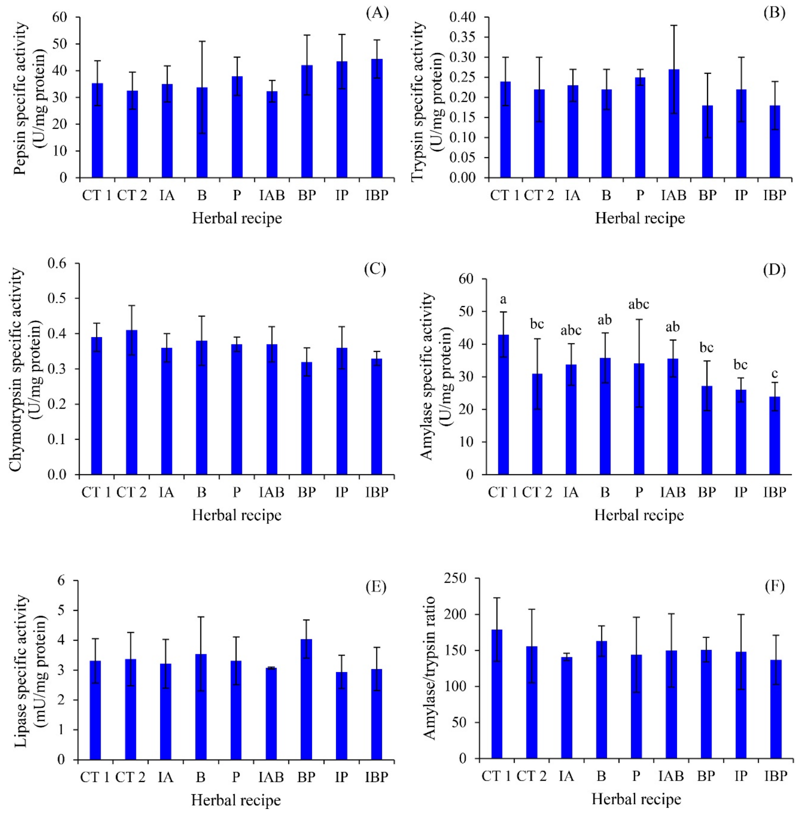

Fig. 1 Specific activities of digestive enzymes in male Siamese fighting fish reared in various treatments using water fermented with Thai herbal recipes: (A) pepsin; (B) trypsin; (C) chymotrypsin; (D) amylase; (E) lipase; (F) amylase-to-trypsin ratio, where parameters measured at end of experiment (at 5 wk), data =mean + SD values, different lowercase letters above columns indicate significant ($p < 0.05$) differences between treatments; details for each treatment are shown in Table 1 footnote.

Whole-body composition

Relative to CT 1, the moisture contents remained unchanged in all herbal treatments (Table 3, $p > 0.05$), except for the significant reduction in the fish in the BP treatment. No significant differences were observed in the crude protein, crude lipid and crude ash contents across the nine treatments.

Table 3 Whole-body composition of male Siamese fighting fish reared in treatments using water fermented with Thai herbal recipes, where parameters measured at end of experiment (at 5 wk)

Herbal recipe	Composition (% of wet weight)			
	Moisture	Crude protein	Crude lipid	Ash
CT 1	70.1 ± 1.4^{ab}	17.2±1.2	6.22±0.27	3.82±0.23
CT 2	71.5 ± 2.4^{a}	16.2±1.2	5.52±0.73	4.41±0.51
IA	68.8 ± 0.9^{bc}	16.6±0.6	6.59±0.50	5.12±0.98
B	71.2 ± 1.0^{ab}	15.6±0.6	5.62±1.01	4.25±0.13
P	70.2 ± 0.5^{ab}	16.0±1.0	5.46±0.69	5.12±0.53
IAB	70.6 ± 1.0^{ab}	15.5±0.6	6.24±0.74	4.20±0.28
BP	67.4 ± 1.6^{c}	17.1±1.0	6.73±1.00	5.13±0.63
IP	71.9 ± 0.3^{a}	15.6±0.1	4.82±0.29	5.45±1.68
IBP	69.7 ± 1.9^{abc}	16.0±1.1	6.12±1.41	4.79±0.53
p-value	0.020	0.210	0.219	0.199

Details for each treatment are shown in Table 1 footnote.
Different lowercase superscripts in the same row indicate significant ($p < 0.05$) differences.

Discussion

Another study reported enhancements in growth parameters across several species following rearing in Indian almond leaf extract at appropriate concentrations (Ikhwanuddin et al., 2014; Nurhidayat et al., 2016; Ramos et al., 2020). Nevertheless, excessive concentrations of these extracts have been proven detrimental to aquatic fauna, such as the neon tetra fish, *Paracheirodon innesi* (Ardi et al., 2020), the black tiger shrimp, *Penaeus monodon* (Ikhwanuddin et al., 2014), the Siamese fighting fish, *Betta* sp. (Nugroho et al., 2016, 2017; Malawa et al., 2022), the cardinal tetra, *Paracheirodon axelrodi* (Nurhidayat et al., 2016) and the Amazon leaf fish, *Monocirrhus polyacanthus* (Ramos et al., 2020).

In the current study, there were no fatalities among the injured fish in all herbal treatments throughout the experimental period (5 wk). Furthermore, the growth-related parameters exhibited no significant variations relative to the control groups (CT 1 and CT 2). Regarding feed utilization, PER remained consistent, aligning with the unchanged specific activities of protein-digesting enzymes (pepsin, trypsin and chymotrypsin). In addition, the lipid-digesting enzyme (lipase) maintained its activity. These results were consistent with the findings reported by Malawa et al. (2022) where 0.25 g/L of *T. catappa* leaf extract had no impact on the proteolytic-specific activity but led to lipase-specific activity improvement, indicating normal functioning of gastrointestinal digestion for both proteins and lipids.

Notably, there was a significant increase in the FCR among the injured fish reared in the CT 2, P, BP, IP and IBP treatments. This was consistent with a decrease in the amylase-specific activity, except for the P treatment relative to CT 1. However, the amylase-to-trypsin ratio remained constant, suggesting the utilization of available carbohydrates per unit of protein in all herbal remedies, operating in a usual way (Thongprajukaew et al., 2011). These findings aligned with those reported by Malawa et al. (2022) on fish immersed in 0.25 g/L of *T. catappa* leaf extract or even for *in vitro* studies with incorporated tannin. Additionally, findings by Maitra and Ray (2003) and Mandal and Ghosh (2010) noted a reduction in the performance of intestinal enzyme activities, including protease, amylase and lipase. This could be attributed to the herbs selected in the experiment, which contained phytochemical constituents, namely polyphenols categorized into phenolic acids, flavonoids or non-flavonoids (Vuong et al., 2013; Raphaël et al., 2019; Kumar et al., 2021; Muala et al., 2021). These polyphenols, particularly the tannin contents, disrupt protein and dry matter digestibility by creating indigestible complexes (Krogdahl, 1989). However, betta fish are naturally carnivorous and can effectively utilize the protein in their diet. Therefore, herbal treatments are less effective with carnivorous species than with herbivorous fish, specifically in terms of amylase activity, as observed in the study by Mandal and Ghosh (2010). In the current study, an immersion method was used to deliver the herbal extract, resulting in a diminished impact on fish compared to direct dietary intake (Prusty et al., 2007).

Furthermore, the coloration of ornamental fish can serve as a crucial indicator not only of their aesthetic quality but also of their health status (Yi et al., 2021). Nonetheless, the injury stress that occurred during the caudal fin regeneration and color development processes, as documented in the report by Malawa et al. (2023), is a possible factor influencing various physiological processes, including altered shades of body color (Ardi et al., 2020). The current findings identified notable changes, such as significant decreases in some body color parameters (a^*, h^*, C^*) in the injured fish in all herbal treatments, except for CT 2 relative to CT 1. However, the L^* values remained steady. These findings conflicted with the reports by Ardi et al. (2020) and Malawa et al. (2022) that *T. catappa* leaf extracts improved body color quality in healthy fish. The decrease in these values may have been impacted by the color of the fermented herbal water that ranged from deep brown, dark brown and dark gray to dark magenta (Fig. S1). Consequently, the body color of the fish shifted to a shade of brown. In contrast, CT 2 produced a lighter yellow-colored skin than CT 1. This finding was consistent with other research results concerning the utilization of tannin extracted from various leaves as a natural dye, resulting in brown hues, such as those obtained from Indian almond, papaya or banana leaves (Chitnis, 2013; Saleh et al., 2013; Rani et al., 2020).

There were no significant differences in the RNA, protein and RNA-to-protein ratio among the fish subjected to any of the herbal treatments compared to CT 1 and CT 2. Additionally, these results corresponded with the stability of the chief muscle components, such as myosin, actin and sarcoplasmic protein and of their total amounts and proportions, suggesting that the herbal treatments had no substantial impact on general physiological movement in the fish. These findings matched well with the unchanged whole-body protein contents. In addition, compensation in the whole-body lipid and ash contents was observed in all the herbal treatments, whereas the moisture content changed significantly only in fish in the BP treatment. Since the biochemical composition would be influenced by the surrounding water in which the fish were reared, the fish immersed in an external solution with a combined concentration (1.5 g/L BL versus 2.5 g/L PL) that may have been higher than their body fluids, might have caused water to leave the fish cells through osmosis. In contrast, IAL (0.25 g/L) alone (Malawa et al., 2022), or perhaps in combination, in a remedy had no impact on the overall whole-body composition.

Although some herbal recipes have reportedly improved fin regeneration and bubble-nest creation in injured male betta fish (Malawa et al., 2023), some treatments in the current study had negative effects due to lowering feed utilization, as indicated by the FCR, amylase specific activity and skin coloration. Maintained feed utilization without changed growth performance, muscle quality and whole-body composition (albeit with a significant change in whole-body moisture content) were also observed in fish in the water fermentation treatment of 1 g/L of lemongrass, 50 g/L of dried clay and 5 g/L of salt with or without singly 0.25 g/L of dried Indian almond leaf, 1.5 g/L of dried banana leaf, 2.5 g/L of dried papaya leaf or their combinations, except for the mixture between dried banana and papaya leaves. However, as somatic growth is not a main assessment criterion during fish injury, the current results still endorse using fermented water of Thai herbs for short-term wound healing in betta fish rather than relying on antibiotics or chemical products that can adversely affect aquatic fauna or their surroundings, or both. Additionally, the active ingredients found in each herb may be affected by the leaf age, extraction solvent, extraction protocol, seasonal variation, geographical location and soil fertility. Differences in these variables could result in differences in the observed outcomes in fish.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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