RESEARCH ARTICLE
doi: 10.2306/scienceasia1513-1874.2022.096

ScienceAsia 48 (2022): **664–672**

Efficiency of homemade egg-based diet for male Siamese fighting fish (*Betta splendens*)

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Received 30 Dec 2021, Accepted 2 May 2022
Available online 6 Jul 2022

ABSTRACT: The proposed homemade egg-based diet (EBD) was studied to provide a suitable feed as an alternative for available instability of live diets and expensive commercial feeds for Siamese fighting fish (*Betta splendens*). The EBD was compared with the widely used commercial feeds, Sakura and Betta Bio-Gold. The 1.5-month-old solid-red male fish were individually reared and fed with the three alternative diets (EBD, Sakura, and Betta Bio-Gold) at 5% of body weight twice daily (09.00 and 17.00 h) for eight weeks. At the end of the trial, growth performances were measured in terms of final body weight and weight gain. The results showed that fish fed with the EBD and the Betta Bio-Gold had similar growth performances ($p > 0.05$), but both were significantly higher than those fed with the Sakura ($p < 0.05$). Feeding rates, feed conversion ratios, and protein efficiency ratios of the EBD and the Betta Bio-Gold fish groups were also superior to the Sakura. This corresponded with significantly higher specific activities of the intestinal protein-digesting enzymes (trypsin and chymotrypsin) ($p < 0.05$). However, their stomach protein-digesting enzyme (pepsin) and the activity ratio of amylase to trypsin were significantly lower ($p < 0.05$). Specific activities of amylase and lipase were similar among the three dietary groups. Fish coloration was not influenced by the diet types, while improved muscle protein synthesis capacity and carcass lipid were observed in the EBD group. These findings indicate the EBD as an advantageous diet for male Siamese fighting fish.

KEYWORDS: commercial diet, diet formulation, feed utilization, microwave-irradiated egg, steamed egg

INTRODUCTION

Siamese fighting fish (*Betta splendens*) is among the most important ornamental fish species in the global aquarium market. The habitats of wild population of the species are in Thailand and only a small region of Laos, while their domesticated ornamental forms (i.e., not for fighting) are traded worldwide [1]. The popularity of this fish species is owing to their multiple brilliant colors and fin shapes that are attractive to rearing, and only the male fish is a marketable target for ornamental fish culturists [2]. In Thailand, the *B. splendens* has been registered as the "National Aquatic Animal" due to its historical and cultural significance [3].

The quality of ornamental fish is directly influenced by nutrient quality in production [4]. For Siamese fighting fish, live diets include zooplankton, tubifex worms, *Daphnia*, mosquito larvae, and small aquatic insects, which are commonly used in rearing throughout its life span [5–8]. However, preparation of a live diet requires manpower, space, and is time consuming. Moreover, it is difficult to preserve the diet for a long time, or during transportation, without the use of a deep freezer [9]. Therefore, its artificial pellet diets have been formulated [4, 10–13]. However, the commercial pellet diets for Siamese fighting fish, for example Sakura, Tetra, Hikari, and Betta Bio-Gold, are relatively expensive compared with those for other ornamental fish such as guppy, molly, and goldfish [14].

Since Siamese fighting fish are naturally carnivorous, they prefer a live diet over an artificial pellet diet. In Thailand, all around 1000 Siamese fighting fish farms frequently use *Moina* sp. as the main diet during their growing phase, followed by traditional ground boiled eggs [3]. For newly hatched fish, feeding with ground boiled yolk and infusoria increased fish survival rate up to 83%, which is superior to the combination use of *Moina* sp. and ground boiled yolk (68%), other tested combinations, or a single feed type alone (66%) [15]. The findings indicated that cooked eggs are a reasonable alternative source of feed for Siamese fighting fish.

Practically, cooked eggs for feeding Siamese fighting fish can be prepared in a microwave or by steaming. Cooked eggs contain essential amino acids, essential fatty acids, antioxidants, vitamins, minerals, antimicrobial agents, immunostimulants, and carotenoids [16–18]. However, little is known about the comparative efficiency of cooked eggs and commercial pellet diets for rearing Siamese fighting fish. Therefore, the efficiency of the proposed egg-based diet (EBD) was compared with globally available commercial pellet diets for Siamese fighting fish, Sakura and Betta Bio-Gold. Growth, feed utilization, digestive enzyme activities, skin coloration, muscle quality, and carcass

composition were used as assessment criteria. The homemade EBD formulation from the current study might be beneficially used in commercial production of male Siamese fighting fish.

MATERIALS AND METHODS

Animal ethics

Acclimatization, rearing, sampling, and euthanasia of Siamese fighting fish in the current study conformed to the "Ethical Principles and Guidelines for the Use of Animals for Scientific Purposes", National Research Council, Thailand (Application No. U1-06514-2560), and were approved by Institutional Animal Care and Use Committees, Prince of Songkla University (Project Code 2563-01-029).

Preparation of egg-based diet (EBD)

The EBD was prepared by mixing chicken egg (excluding eggshell) and distilled water at a ratio of 2:1 (w/w) in a 250 ml beaker, followed by adding 10% by weight of squid liver meal. The mixture was cooked in a microwave oven (MW 71B; Samsung, Selangor, Malaysia) at 800 W for 1 min. To maintain the quality of prepared EBD over the trial duration and to reduce the variation, the EBD was prepared from the same source of feed ingredients and then freeze-dried (Delta 2-24 LSC; Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany) to eliminate excess amount of moisture for 24 h. The dried EBD was ground and sieved through a 2 mm mesh, packed in bags, and kept at 4 °C.

Diet proximate chemical composition

Three experimental diets (EBD, Sakura, and Betta Bio-Gold) were homogenously minced and their proximate compositions, including moisture, crude protein, crude lipid, crude fiber, and ash, were determined as described by the AOAC [19]. Nitrogen-free extract (NFE, %) was calculated as [100 − (moisture + crude protein + crude lipid + crude fiber + ash)]. Gross energy (kcal/kg) was calculated from (crude protein ×4) + (crude lipid ×9) + (NFE ×4). All compositions were reported on a dry weight basis. The proximate chemical compositions of the three diets were summarized in Table 1.

Table 1 Ingredients and proximate chemical compositions of the proposed diet and the two commercial diets.

Experimental diet	EBD	Sakura†	Betta Bio-Gold†
Ingredient (% by fresh weight)			
Chicken egg	60		
Water	30		
Squid liver powder	10		
Chemical composition (% by dry weight)			
Moisture	4.24	7.48	7.44
Crude protein	51.0	34.0	39.4
Crude lipid	25.2	1.00	3.20
Crude fiber	1.95	5.40	1.97
Ash	5.43	9.55	7.72
NFE	12.2	42.7	40.3
Energy (kcal/kg)	4796	3150	3476

† The commercial diets include Sakura (U. Lek Trading Co., Ltd., Bangkok, Thailand) and Betta Bio-Gold (Kyorin Food Ind. Ltd., Himeji, Japan). EBD, egg-based diet; NFE, nitrogen-free extract.

Feeding trial

One-month-old solid-red Siamese fighting fish were purchased from a local fighting fish farm in Nakhon Si Thammarat Province in the southern Thailand. They were transported to Kidchakan Supamattaya Aquatic Animal Health Research Center, Faculty of Natural Resources, Prince of Songkla University. The fish were reared individually in a cylindrical polypropylene plastic cup (14.5 cm diameter × 11.5 cm height) containing 350 ml water under natural light (12-h light:12-h dark), and fed 5% of body weight (BW) twice daily (at 09.00 and 17.00 h). They were acclimatized for two weeks with a commercial diet (Sakura; U. Lek Trading Co., Ltd., Bangkok, Thailand). After that, 45 uniform sized fish (1.21 ± 0.03 g BW) were divided into three groups ($n = 15$ per group). They were fed with either EBD or a commercial diet, Sakura or Betta Bio-Gold (Kyorin Food Ind. Ltd., Himeji, Japan) over eight weeks of the feeding trial. The water was 80% changed every other day by stock dechlorinated water. The water quality was analyzed once a week by using a multi parameter water quality instrument (Pro Plus; YSI, Yellow Spring, Ohio, USA). During the 8-week trial, temperature (28.1 ± 0.4 °C), pH (7.85 ± 0.19), and dissolved oxygen (5.18 ± 0.23 mg/l) were maintained among the three dietary treated groups. The leftover feed was siphoned off after 30 min of feeding, and then dried at 60 °C until constant weight. The differences in the amounts of the diet provided and the uneaten diet were used to calculate feeding rate (FR), feed conversion ratio (FCR), and protein efficiency ratio (PER). The fish mortality was recorded daily. At the end of the 8-week feeding trial, the fish were starved for 24 h and then anesthetized using clove oil. Final fish body weight (FBW) and final standard length (FSL) were measured and used to calculate weight gain (WG) and condition factor (CF). Visceral organs were removed and then weighed for calculating viscerosomatic index (VSI). All the fish were used to assess growth, feed utilization, and coloration characteristics ($n = 15$). In each dietary treatment group, ten fish were used for measuring digestive enzyme activities and muscle quality ($n = 10$), while the five remaining fish were used for analyzing carcass chemical composition ($n = 5$).

Survival, growth performance, and feed utilization characteristics of the Siamese fighting fish at the end of

the 8-week feeding trial were calculated as follows:

$$\text{Survival}\,(\%) = 100 \times \frac{\text{final fish number}}{\text{initial fish number}}$$

$$\text{WG}\ (\text{g}) = W_t - W_0,$$

$$\text{CF} = 100 \times [W_t/\text{FSL}\ (\text{cm}^3)],$$

$$\text{VSI}\,(\%) = 100 \times \text{wet weight of visceral organs}\ (\text{g})/W_t,$$

$$\text{FR}\,(\%\ \text{body weight/day}) = 100 \times C/[(W_0 + W_t)/2]/t,$$

$$\text{FCR}\ (\text{g feed/g gain}) = \text{dry feed consumed}\ (\text{g})/\text{WG}\ (\text{g}),$$

$$\text{PER}\ (\text{g gain/g protein}) = \text{WG}\ (\text{g})/\text{protein intake}\ (\text{g}),$$

where C = daily feed consumption (g), W_0 = initial body weight (g), and W_t = FBW (g), and t = feeding duration (day).

Determination of digestive enzyme activities

Enzyme extraction and protein quantification in crude enzyme extracts

The whole visceral organ of Siamese fighting fish was mixed with cold 0.2 M Na_2HPO_4-NaH_2PO_4 buffer (pH 8) at a ratio of 1:10 (w/v) and then homogenized using a tissue micro-homogenizer (THP-220; Omni International, Kennesaw GA, USA) for 10 s. The homogenate was centrifuged at 15 000×g for 30 min at 4 °C. The supernatant was collected, aliquoted, and stored at −20 °C until use. The protein in the crude enzyme extracts was quantified using the method of Lowry et al [20] with bovine serum albumin as standard protein, and the protein concentration (mg/ml) was used for describing the specific activity of enzyme (U/mg protein). All enzyme and protein assays were performed within one month after extraction.

Determination of enzyme activities

The pepsin activity (EC 3.4.23.1) was assayed at pH 2 and 40 °C using hemoglobin as the substrate according to the method of Worthington [21]. One unit (U) of activity is defined as an increase of 1.0 in absorbance at 280 nm. The activities of trypsin (EC 3.4.21.4) and chymotrypsin (EC 3.4.21.1) were assayed at pH 8 and 50 °C according to the method of Rungruangsak-Torrissen et al [22] with *N*-benzoyl-*L*-Arg-*p*-nitroanilide and *N*-succinyl-Ala-Ala-Pro-Phe-*p*-nitroanilide as specific substrates, respectively, in comparison to the linear range of standard *p*-nitroanilide. The α-amylase activity (EC 3.2.1.1) was determined at pH 8 and 50 °C using soluble starch as substrate according to the method of Areekijseree et al [23], and the liberated product was compared against linear range of standard maltose. The lipase activity (EC 3.1.1.3) was determined at pH 8 and 40 °C using *p*-nitrophenyl palmitate as substrate according to the method of Winkler and Stuckmann [24], and the linear range of standard *p*-nitrophenol was used to quantify the amount of liberated product. One U of trypsin, chymotrypsin, amylase, and lipase are defined as the amount of relevant enzyme catalyzing the conversion of 1 μmol of substrate per minute. The activity of amylase was divided by the activity of trypsin from the same fish sample to obtain amylase/trypsin ratio (A/T ratio).

Skin coloration

The fish were dried by a soft blotting paper before the fish coloration was measured using a MiniScan EZ (Hunter Associates Laboratory, Reston VA, USA). The color instrument was calibrated to white and black standards before the measurements. Two sides on the middle part of each fish body were measured and summarized as mean for individual fish. The color coordinates recorded for skin were lightness (L^*), redness (a^*), chroma (C^*), and hue (H^*).

Muscle quality

The scales and skin of the fish were removed before the epaxial white muscle (below the dorsal fin) was collected. The total RNA and protein concentrations in the white muscle samples were determined as described by Rungruangsak-Torrissen [25]. A known amount of frozen muscle (approximately 30 mg) was mixed with TRIzol® reagent (Invitrogen, Carlsbad CA, USA) and sonicated (VCX; Sonic and Materials Inc., Newtown CT, USA) to obtain a pink transparent solution. The solution was mixed with chloroform, and then centrifuged to obtain upper and lower phases of RNA and protein, respectively. Isopropanol and ethanol were used in the precipitation steps. The RNA sediments were dissolved in sodium acetate and dried at 55 °C, while sodium dodecyl sulfate was applied to the protein sediments. The concentrations of RNA and protein were spectrophotometrically measured at 260 and 280 nm, and calculated based on the equations E_{260} = 40 μg RNA/ml and E_{280} = 2.1 mg protein/ml, respectively.

Carcass proximate chemical compositions

The fish carcasses were homogenously minced and their proximate compositions, including moisture, crude protein, crude lipid, and ash, were determined as described by the AOAC [19]. All compositions were reported on a wet weight basis.

Statistical analysis

Three dietary treatment groups comprising forty-five experimental units were conducted in completely randomized design. Statistical Package for Social Sciences, Version 14 (SPSS Inc., Chicago, USA) was used for all data analyses. The data were expressed as mean ± SEM. The arcsine square root transformation of percentage data was used. One-Way Analysis of

Variance was used to compare means, and the differences between means were statistically analyzed *post hoc* with Duncan's multiple range test ($p < 0.05$).

RESULTS

Proximate chemical compositions of the experimental diets

The EBD had higher contents in the crude protein, crude lipid, and energy but lower in crude fiber, ash, and NFE relative to the two commercial diets (Table 1). Betta Bio-Gold diet was higher in the crude protein, crude lipid, and energy contents but lower in crude fiber and ash relative to Sakura diet; however, they both had similar levels of NFE (Table 1).

Survival, growth performance, and feed utilization

No mortality was observed over the eight weeks of feeding trial. Growth performances (in terms of FBW and WG) of the EBD and the Betta Bio-Gold treated groups were similar ($p > 0.05$, but they were significantly higher than the group treated with the Sakura diet ($p < 0.05$, Table 2). FSL and VSI were higher in the Betta Bio-Gold group compared with those fed with EBD or Sakura ($p < 0.05$, Table 2). All three diets resulted in similar fish CF. Both the EBD and the Betta Bio-Gold groups exhibited desirable FR and FCR, while prominent PER was observed in the Betta Bio-Gold treatment compared with the EBD and the Sakura treatments (Table 2).

Specific activities of digestive enzymes

The diet types had significant effects on proteolytic enzymes in Siamese fighting fish. The fish fed with Sakura diet showed higher pepsin specific activity than those fed with EBD or Betta Bio-Gold (Fig. 1a). No differences in the specific activities of trypsin (Fig. 1b) and chymotrypsin (Fig. 1c) were found between the fish fed with EBD and Betta Bio-Gold, while the Sakura fed fish had the lowest activity. Specific activities of amylase (Fig. 1d) and lipase (Fig. 1e) did not differ among the three dietary treated groups. This resulted in the significantly higher A/T ratio in the Sakura treated group compared with the others ($p < 0.05$, Fig. 1f).

Skin coloration

Siamese fighting fish coloration was not differed by the diet types. The L^*, a^*, C^*, and H^* were similar among the three dietary treatments (Table 3).

Muscle quality

The RNA concentrations in the white muscle were not different among the three treated groups (Fig. 2a). The protein concentrations observed in the Betta Bio-Gold ($p < 0.05$) and the Sakura ($p > 0.05$) treated groups were significantly higher than the EBD group (Fig. 2b) resulting in similar muscle RNA/protein ratios between the Sakura and the Betta Bio-Gold groups and significantly lower than the EBD group ($p < 0.05$, Fig. 2c).

Carcass compositions

Moisture and crude protein contents in the whole carcass of Siamese fighting fish did not differ among the three treated groups. However, the EBD group significantly had the highest crude lipid and the lowest ash content among the three ($p < 0.05$, Table 4).

DISCUSSION

The proposed EBD and the commercial Betta Bio-Gold diet provided significantly higher growth responses (WG values) in Siamese fighting fish due to being consumed (FR values) at significantly higher levels, compared with the commercial Sakura diet. Similar morphometric changes (CF values) among the three dietary groups were observed during growth, despite a significantly higher FSL in the Betta Bio-Gold fed fish. Theoretically, the optimal dietary protein levels for

Table 2 Survival, growth performance, and feed utilization of solid-red male Siamese fighting fish fed with egg-based diet compared with two commercial diets. The observed parameters were recorded at the end of the 8-week trial.

Parameter	EBD	Sakura[†]	Betta Bio-Gold[†]	*p*-value
Survival (%)	100	100	100	âĂŞ
FBW (g)	2.76 ± 0.04^{a}	2.40 ± 0.02^{b}	2.85 ± 0.06^{a}	< 0.001
WG (g)	1.63 ± 0.05^{a}	1.22 ± 0.02^{b}	1.60 ± 0.04^{a}	< 0.001
FSL (cm)	3.53 ± 0.04^{b}	3.43 ± 0.04^{b}	3.74 ± 0.03^{a}	< 0.001
CF	6.12 ± 0.16	6.03 ± 0.21	5.72 ± 0.12	0.228
VSI (%)	2.84 ± 0.27^{b}	2.31 ± 0.21^{b}	4.20 ± 0.37^{a}	0.001
FR (% BW/day)	2.92 ± 0.27^{b}	1.85 ± 0.25^{c}	4.18 ± 0.17^{a}	< 0.001
FCR (g feed/g gain)	2.38 ± 0.22^{b}	4.29 ± 0.50^{a}	1.97 ± 0.22^{b}	< 0.001
PER (g gain/g protein)	0.85 ± 0.11^{b}	0.77 ± 0.11^{b}	1.44 ± 0.18^{a}	0.012

[†] The commercial diets include Sakura (U. Lek Trading Co., Ltd., Bangkok, Thailand) and Betta Bio-Gold (Kyorin Food Ind. Ltd., Himeji, Japan). EBD, egg-based diet; FBW, final body weight; WG, weight gain; FSL, final standard length; CF, condition factor; VSI, viscerosomatic index; FR, feeding rate; FCR, feed conversion ratio; PER, protein efficiency ratio. Data are expressed as mean ± SEM ($n = 15$). Differences between means were tested with Duncan's multiple range test.

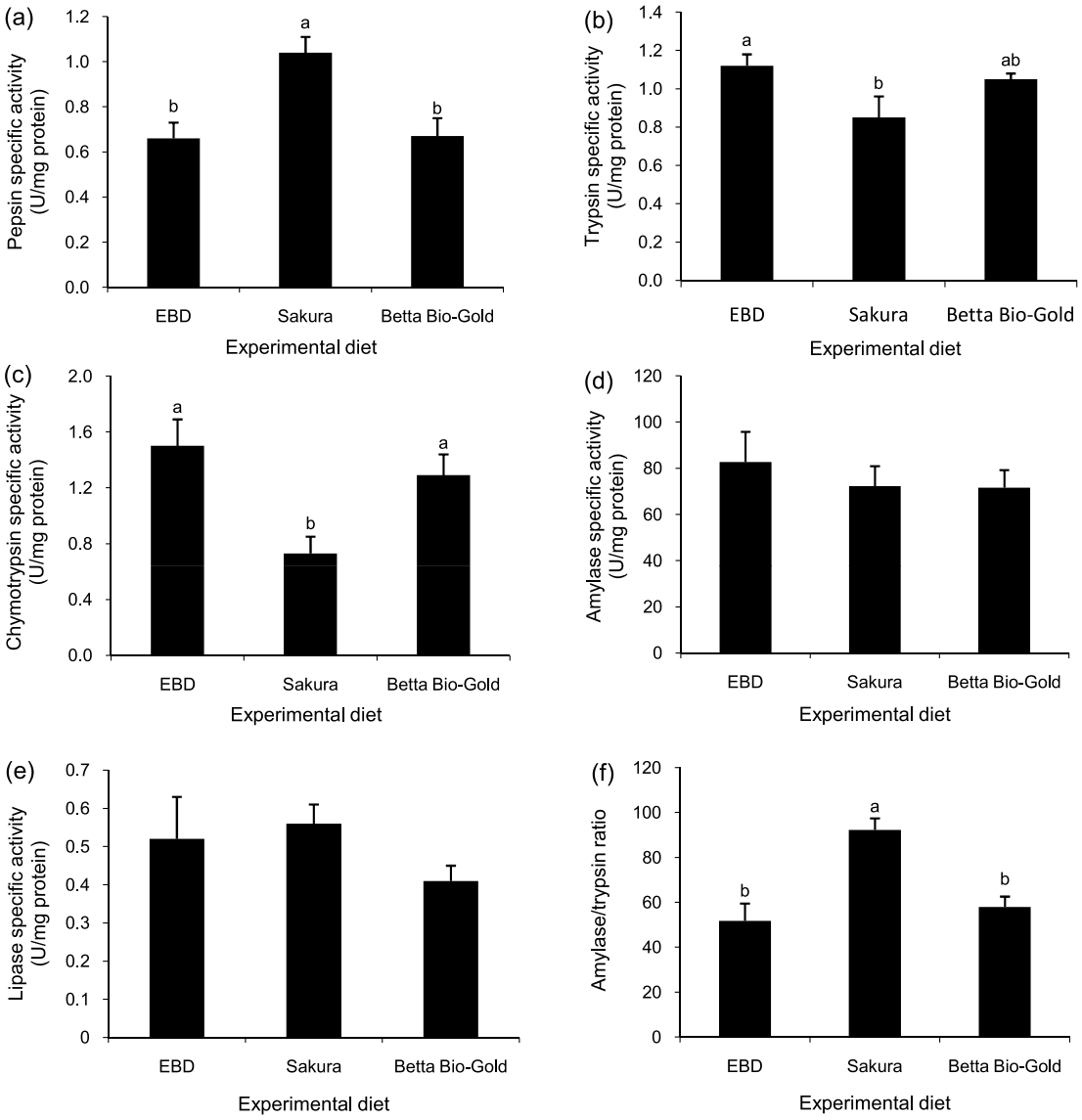

Fig. 1 Specific activities of pepsin (a), trypsin (b), chymotrypsin (c), amylase (d), lipase (e), and the activity ratio of amylase to trypsin as A/T ratio (f) in solid-red male Siamese fighting fish fed with the homemade egg-based diet (EBD) or commercial diets (Sakura or Betta Bio-Gold). Data are expressed as mean ± SEM ($n = 10$). Different superscripts indicate a significant difference ($p < 0.05$).

Table 3 Skin coloration parameters of solid-red male Siamese fighting fish fed with egg-based diet compared with two commercial diets. The observed parameters were recorded at the end of the 8-week trial.

Parameter	EBD	Sakura[†]	Betta Bio-Gold[†]	*p*-value
L^*	12.9 ± 1.0	11.4 ± 2.2	13.1 ± 0.9	0.658
a^*	22.0 ± 1.4	18.2 ± 0.8	18.8 ± 1.2	0.088
C^*	24.2 ± 1.5	20.4 ± 1.0	20.9 ± 1.4	0.120
H^*	28.6 ± 2.5	21.5 ± 2.0	25.1 ± 2.6	0.141

[†] The commercial diets include Sakura (U. Lek Trading Co., Ltd., Bangkok, Thailand) and Betta Bio-Gold (Kyorin Food Ind. Ltd., Himeji, Japan). EBD, egg-based diet; L^*, lightness; a^*, redness/greenness; C^*, chroma; H^*, hue. Data are expressed as mean ± SEM ($n = 15$). Differences between means were tested with Duncan's multiple range test.

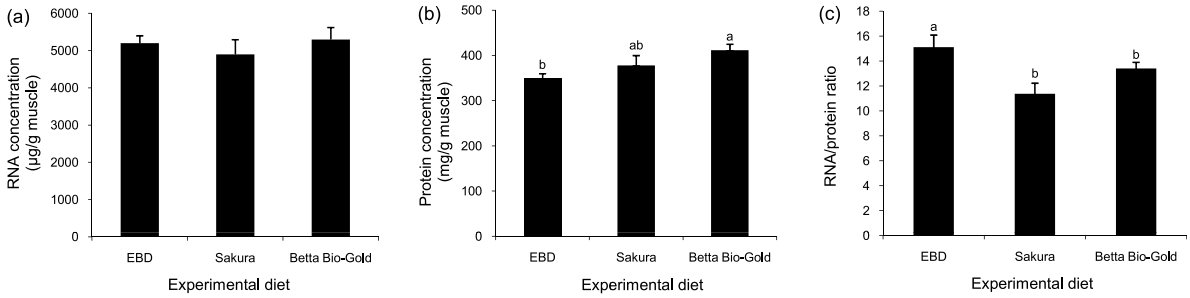

Fig. 2 RNA concentration (a), protein concentration (b), and RNA/protein ratio (c) in solid-red male Siamese fighting fish fed with the homemade egg-based diet (EBD) or commercial diets (Sakura or Betta Bio-Gold). Data are expressed as mean ± SEM ($n = 10$). Different superscripts indicate a significant difference ($p < 0.05$).

Table 4 Proximate chemical compositions (% FW) of whole carcass of solid-red male Siamese fighting fish fed with egg-based diet compared with two commercial diets. The observed parameters were recorded at the end of the 8-week trial.

Parameter	EBD	Sakura†	Betta Bio-Gold†	*p*-value
Moisture	72.5 ± 0.7	72.4 ± 1.3	71.4 ± 1.2	0.699
Crude protein	15.6 ± 0.7	15.4 ± 0.5	16.2 ± 2.8	0.928
Crude lipid	1.89 ± 0.14^{a}	0.80 ± 0.30^{b}	0.70 ± 0.14^{b}	0.001
Ash	4.71 ± 0.24^{b}	7.05 ± 0.51^{a}	6.21 ± 0.45^{a}	0.002

† The commercial diets include Sakura (U. Lek Trading Co., Ltd., Bangkok, Thailand) and Betta Bio-Gold (Kyorin Food Ind. Ltd., Himeji, Japan). FW, fresh weight; EBD, egg-based diet. Data are expressed as mean ± SEM ($n = 5$). Differences between means were tested with Duncan's multiple range test.

rearing Siamese fighting fish are in the range from 28.0 to 33.4% [10, 11, 13], which is similar to the observed protein content in the Sakura group (34.0%), but not in the Betta Bio-Gold (39.4%) and the EBD (51.0%). However, the EBD might be suitable for carnivorous fish because of its excess amounts of protein and lipid with a low level of NFE corresponding well with the chemical compositions of live diets for Siamese fighting fish [26]. Higher dietary protein levels resulted in higher body length and protein deposition in fish [27, 28]. Since variations in the dietary protein levels between the EBD and the Betta Bio-Gold diet did not correspond with the FSL and the optimal protein levels in the white muscle of the fish, the variations in the dietary protein quality affecting protein digestibility or other nutritive values might influence the utilization of the nutrients. The significantly higher PER value in the Betta Bio-Gold group indicated a higher dietary protein quality compared with the EBD and the Sakura groups. The high dietary protein level with good protein quality in the Betta Bio-Gold diet caused higher FSL in the fish; and with a higher FR, the fish could maintain their CF values and, hence, resulted in a higher protein deposition in the Betta Bio-Gold group compared with the others, as shown in Tables 1 and 2. Significantly lower FCR values, showing better feed utilization, were observed in the EBD and the Betta Bio-Gold groups compared with the Sakura group. Significantly lower feed consumption (FR values), protein utilization (PER values), and protein deposition in the white muscle were observed in the EBD group compared with the Betta Bio-Gold. Therefore, the similarly high growth (WG values) observed in the EBD could be due to its higher lipid deposition as shown by the higher lipid content in carcass, compared with the Betta Bio-Gold group (Table 4). However, no differences in the lipase specific activities among the three groups were observed (Fig. 1e), which is a usual phenomenon as lipase including amylase and pepsin are not key enzymes for fish growth [27]. High nutritive values in the formulation of the EBD might provide sufficient nutrients for fish and decrease over-feeding. Similar findings have also been observed in some aquatic animals where the decreased FR is associated with growth caused by suitable dietary treatments [2, 27]. Moreover, pre-cooking of the EBD by microwave irradiation can cause denaturation of the dietary protein and improve feed palatability, so that the ingesting time of the fish based on direct observation were observed to be EBD group < Betta Bio-Gold group < Sakura group.

The highest VSI indicates viscerosomatic energy storage and viscerosomatic mass growth processes [29]. The phenomenon leads to somatic growth promotion, locomotion activity reduction, and energy conservation [30, 31]. Based on our direct observations, these changes were supported by the feeding and the swimming behaviors of the fish in the current study, as the EBD and the Sakura groups were more active in movement and display than the Betta Bio-Gold.

Physiological changes in feed utilization can be

traced by specific activities of digestive enzymes. In the present study, these enzymes were not proportionally changed by diet proximate compositions. In the stomach, pepsin activity contributes to the protein digestion. Therefore, it is reasonable that high pepsin specific activity in the Sakura group was due to poor protein quality in the diet. This assumption is supported by the experiments in Caspian brown trout (*Salmo caspius*) [32] and southern catfish (*Silurus meridionalis*) [33] showing a high pepsin activity in starved fish relative to continuously fed fish. However, Rungruangsak-Torrissen et al [22] did not observe high pepsin activity in starved fish, and the enzyme activity was not related to fish growth. In the current study, the fish was at a feeding stage with a low dietary protein quality in the Sakura diet; therefore, the secreted high activity pepsin would probably attempt to access most of the proteins available to the fish.

Trypsin cleaves peptide chains at the C-terminal end of arginine and lysine residues, contributing 40–50% of the protein digestion in alimentary tract of fish [34]. It activates itself and other pancreatic zymogens such as chymotrypsin. Both trypsin and chymotrypsin play a major role in intestinal protein digestion and fish growth [22, 27]. In our study, the specific activity levels of both enzymes corresponded with fish growth (WG values) among the three dietary groups, showing similar levels between the EBD and the Betta Bio-Gold groups, which were higher than the Sakura group.

The specific activities of amylase and lipase did not differ among the three dietary treatments, while the A/T ratio changed in the same trend as observed in pepsin. This suggests that the fish can physiologically adapt by changing their digestive A/T ratio in response to dietary treatments. Generally, A/T ratio can be used as a marker indicating fish feeding habits [12]. Increased A/T ratio in the fish fed with Sakura diet indicated proportionally dominant utilization of carbohydrate over protein. Since protein played a major role in improving growth and feed utilization of Siamese fighting fish, as observed by the significant changes in three proteolytic enzymes, the lower protein content in the Sakura diet resulted in inferior growth and feed utilization of the fish, compared with the EBD and Betta Bio-Gold diet.

Skin and fin coloration are commercially most important traits for male Siamese fighting fish [9]. In the current study, the proposed EBD maintained skin coloration in terms of L^*, a^*, C^*, and H^*, as also did the two commercial diets. Generally, skin redness in Siamese fighting fish is regulated by carotenoids [35], and also probably by melanines, pterediums, and purines, as in other ornamental fishes [36]. Since carotenoids in fish are synthesized *de novo*, findings from the current study suggested that the proposed EBD has sufficient amounts of pigments for fish coloration, without any supplementation by synthetic pigments as used in commercial diets. This is reasonable since whole egg (white and yolk) contains lutein and zeaxanthin at 288 and 279 μg/100 g on dry matter basis, respectively [37]. In addition, the use of commercially available egg enriched with astaxanthin for preparation of EBD might enhance coloration more than this study did. High content of lipids from whole egg in the EBD (25.2% on dry weight basis) can also improve carotenoid bioavailability in fish. However, cooking by heat treatment including the variation from hen's feeding regimens and rearing conditions may cause some changes to the carotenoid contents in the eggs [38].

Protein synthesis capacity (RNA concentration) and protein turnover rate (RNA/protein ratio) in white muscle have been used as indirect indices for fish condition and growth [9, 25, 39]. Significantly increased RNA/protein ratio in the EBD group, with unchanged RNA concentration and decreased protein concentration, indicated a high capacity of the fish to grow by protein, depending on the dietary protein quality. Similar phenomenon was observed in Atlantic mackerel (*Scomber scombrus*) where reduced concentration of muscle protein was found in fish with higher growth rate due to higher lipid than protein depositions [39]. For carcass proximate compositions, significantly increased lipid content (1.59% of fresh weight) and decreased ash content (4.71% of fresh weight) were observed in the fish fed the proposed diet, in accordance with the proximate composition of the EBD. These values are normal and within the ranges of carcass lipid (1.26–5.77%) and ash (3.33–5.59%) contents from previous investigations [2, 9, 40].

CONCLUSION

Based on our investigations, male Siamese fighting fish fed with the proposed egg-based diet exhibited valuable characteristics in growth, feed utilization, digestive enzyme activities, muscle quality, and carcass composition without any effects on skin coloration. Therefore, the homemade egg-based diet could be considered as a beneficial choice for feeding male Siamese fighting fish in aquaria as well as in fish farms. The preparation protocol for the egg-based diet was simple and used only a few ingredients. In addition, this feed formulation was behaviorally attractive and palatable for the fish due to the addition of squid liver powder. Practically, the farmers or fish culturists can prepare the diet using kitchen appliances such as microwave oven or food steamer. The prepared diet, which was well accepted by the fish, could be used freshly on a daily basis or stored in a refrigerator; and no freeze-drying was required. In addition, pelleting with gelatin as a binder followed by drying may improve bioavailability and shelf life of this proposed diet.

Acknowledgements: This research was partially supported by Prince of Songkla University and Ministry of Higher Education, Science, Research and Innovation under the Reinventing University Project (Grant No. REV64039). We highly appreciated Miss Alisa Noothong for supporting fish rearing and some laboratory assays. The authors would like to thank Assoc. Prof. Dr. Seppo Karrila, Publication Clinic, Research and Development Office, Prince of Songkla University, for advice in manuscript preparation.

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