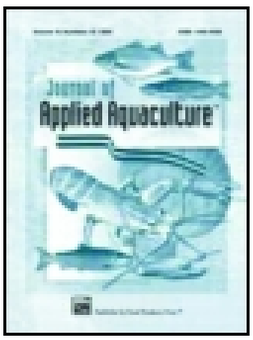

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Effects of partial replacement of dietary fishmeal using plant-protein sources on the growth performance, coloration and liver histology of guppy fry (poecilia reticulata) in outdoor farming conditions

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ABSTRACT

A six-week feeding trial was conducted to evaluate the effect of replacing fish meal (FM) in diets for guppy (*Poecilia reticulata*) fry reared in outdoor farming conditions. Four experimental diets —control (CD) (30% FM), SP (10% sweet potato leaf meal), JS (3% jackfruit seed meal), and SS (10% sesame seed meal)—were fed to guppy fry (1.91 ± 0.23 cm; 0.07 ± 0.14 g). Final body length and weight (3.69 + 0.12 cm; 0.55 + 0.07 g) and hepatosomatic index (3.53 + 1.64) were significantly higher in fish fed the SS diet. The SP diet resulted in low growth, low SGR (4.24 + 0.16), and poor FCR (3.54 + 0.32). Total tissue carotenoid content was significantly higher in the SP and JS treatments. The present study indicates that tested plant ingredients can be used to replace FM in diets for guppy, and sesame seed meal-based feed formulation can be used as a low-cost feed formulation for practical diets for guppy reared in outdoor farming condition.

KEYWORDS

Fish meal; guppy; growth performance

Introduction

The aquaculture industry is one of the fastest-growing industries in the world, and fish meal has long been recognized as a main protein source in fish feed due to its high protein content, well-balanced amino acid profile, high protein digestibility, excellent palatability, vitamins, and growth factors (Tacon 1993). However, fish meal cost is continuously increasing due to the high demand from the escalating aquafeed industry as well as limited supply of fish meal (FAO 2010). A continuous increase of fish meal cost directly influences fish feed cost and subsequently the production cost of any aquaculture operation. Therefore, exploring low-cost feed ingredients to replace the fish meal component has become one of the major priorities in the fish feed industry. Although ornamental fish production is comparatively lower compared to food fish production, the dependence on fish meal as a major protein source

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has resulted in higher feed costs in ornamental feed production. Intense research efforts throughout the last decades have shown that among the commonly used alternative protein sources in fish feed production, plant protein sources appear to be the most appropriate and cheapest alternative protein source to replace fish meal in the diet of fish (Hardy 2010; Tacon and Metian 2008).

A large number of grains, grain by-products, oil seeds, and leaf meals are used as possible alternative plant protein sources in fish feed preparation, and soybean meal (Refstie et al. 2001), canola meal (Thiessen et al. 2004), corn (Regost, Arzel, and Kaushik 1999), cotton seed meal (Bian et al. 2017), sunflower seed meal (Olivera-Novoa, Olivera-Castillo, and Martinez-Palacios 2002), sweet potato leaf meal (Wickramasekaera, Gamage, and Hirimuthugoda 2011), sesame seed meal (Jimoh and Aroyehun 2011), and moringa leaf meal (Makkar and Becker 1996) have showed positive growth performances in fish feeding trials. AftabUddin et al. (2017) have found that antibacterial properties of selected herbal extracts in the diets of *Penaeus monodon* enhance the growth, survival, and disease resistance against *Vibrio harveyi*. Therefore, the use of plant protein sources, which have antibacterial and immunostimulant properties, in feeds is an advantage in the aquaculture industry as use of antibiotics or other preventative chemicals are typically expensive and often have undesirable side effects such as environmental residues and accumulation in cultured organisms (Vaseeharan and Thaya 2014).

Nevertheless, use of plant-derived materials as fish feed ingredients is limited by the presence of a wide variety of antinutritional factors (Tacon and Jackson 1985). These compounds interfere with food utilization and negatively affect growth and other physiological activities of fish. Therefore, focusing on alternative plant ingredients that are nutritionally compatible and readily available for the fish feed industry is vital for the sustainable development of the aquaculture industry. Further, the coloration of fish is one of the most important marketing criteria in the ornamental fish industry. Improving the coloration of ornamental fish is very important in terms of market value, and many previous studies reported that plant protein can be used to enhance the coloration of fish (Ezhil, Jeyanthi, and Narayanan 2008; Gouveia and Rema 2005; Paranamana, Radampola, and Bulugahapitiya 2014, 2015a; Regost, Arzel, and Kaushik 1999; Sinha and Asimi 2007; Wickramasekaera, Gamage, and Hirimuthugoda 2011).

Sri Lanka as an agricultural country produces a large number of plant-based ingredients such as by-products or agricultural waste that may have potential in fish feed preparation. A few examples of such plant-based ingredients are sweet potato leaves, jackfruit seeds, and sesame seed meal, which are underutilized resources available in large quantities during the harvesting period. Information on utilization of these plant-based alternative ingredients in fish

feeds and evaluation of their effects on fish growth performance in practical farming conditions is scarce.

Sweet potato (*Ipomoea batatas*) leaves, jackfruit (*Artocarpus heterophyllus*) seeds, and sesame (*Sesamum indicum*) seed cake were selected as low-cost plant-based ingredients to replace fish meal as they were previously tested as alternative ingredients in feeds for guppy fish reared in small glass tanks with low stocking densities under aquarium conditions (Paranamana, Radampola, and Bulugahapitiya 2014, 2015a; Wickramasekaera, Gamage, and Hirimuthugoda 2011). Some formulations of those experiments showed better growth performance under experimental conditions. However, those formulations were not verified under practical commercial farming conditions to evaluate their effects on growth performance and survival of guppy fish cultured in commercial farms. Thus, the feed formulations selected for this study are based on the previous studies done under experimental aquarium conditions (Paranamana, Radampola, and Bulugahapitiya 2014, 2015a; Wickramasekaera, Gamage, and Hirimuthugoda 2011), and best feed formulations that showed higher growth performances were selected for the present study. Therefore, the objective of the present study was to investigate the effects of partial replacement of dietary fish meal using sweet potato leaf meal, jackfruit seed meal, and sesame seed meal on feed utilization, growth performance, coloration, and liver histology of guppy fry reared in outdoor farming systems.

Materials and methods

Feed preparation

Sweet potato leaves and jackfruit seeds were collected from rural areas in the Galle district, and oil-extracted sesame seed meal was purchased from oil-extraction industries in the Jaffna district. Other feed ingredients were purchased from local markets in Sri Lanka. The ingredients were dried in an oven at 45°C for 24 hours, ground, and sieved to obtain fine powders of the ingredients.

In the control diet (CD), fish meal (FM-30%) is the main protein source, and in other three diets FM was replaced using sweet potato leaf meal (SP), jackfruit seed meal (JS), or sesame seed meal (SS) respectively. Formulations and proximate compositions of the experimental diets are presented in Table 1. All ingredients were mixed thoroughly by adding warm (60°C) water (~20% of feed dry weight) to all ingredients to form a dough, which was then steamed for about 5 minutes and pelleted using a multipurpose mixing pellet machine (H.L B 20-A, Guangdong Panyu Henglian Food Processing Machine Factory) at the fish nutrition laboratory in Udawalawa carp breeding center, Sri Lanka. Feed pellets were air dried for 2–3 days, packed in sealed polythene bags, and

Table 1. Feed formulations (g/100 g dry matter) and proximate composition (g/100 g) of experimental diets.

	Experimental diets			
Ingredients	CD	SP	JS	SS
Fish meal[a]	30	20	27	8
Soybean meal	14.5	15.5	14.5	24
Coconut meal	25	25	25	30
Wheat flour	10	8	10	12
Rice bran	14.5	15.5	14.5	10
Vitamin & mineral mixture[b]	3	3	3	3
Soy oil	3	3	3	3
Sweet potato leaf meal	–	10	–	–
Jackfruit seed meal	–	–	3	–
Sesame seed meal	–	–	–	10
Proximate analysis (% dry weight)				
Moisture	13.2	12.2	9.9	11.5
Crude protein	35.7	32.0	34.0	29.5
Crude lipid	4.7	4.4	4.5	5.1
Ash	13.5	11.6	13.1	11.3
[c]NFE	32.2	36.4	37.7	42.0
[d]GE (K cal/g)	313.9	313.6	327.6	332.1
[e]P:E Ratio	113.73	102.04	103.79	88.83

Note. CD = control diet, SP = sweet potato leaf meal diet, JS = jackfruit seed meal diet, SS = sesame seed meal diet).
[a]Sea shell fish meal.
[b]Starter premix B.
[c]NFE = Nitrogen Free Extracts, calculated as 100 – (%Moisture+%Protein+%Lipid+%Ash+%Fiber) (Thompson et al. 2006).
[d]GE = Gross Energy content calculated as Atwater standard method (FAO 2003).
[e]P:E Ratio = Protein to energy ratio in mg protein/K cal GE.

stored in a freezer at −20°C until use. Proximate analyses of the experimental diets were performed using standard methods (Association of Official Analytical Chemists [AOAC] 1990). Fatty acid composition of the ingredients and the experimental diets was analyzed using gas chromatography equipped with a flame ionization detector.

Experimental system and management

The experiment was conducted at Karandeniya ornamental fish farm, Sri Lanka over a 6-week period from June to August 2017. Thirty-day-old male guppy fry (*Poecilia reticulata*, Red blonde tuxedo variety) (1.91 ± 0.23 cm and 0.07 ± 0.14 g) were randomly distributed among 12 outdoor cement tanks filled with well water (216 L; 120 × 90 × 40 cm) with 70 fish fry per tank (3 fry/L). The tanks were covered with a net hanging from above, and a natural photoperiod was used. The diets were randomly assigned to tanks in triplicate, and fish were hand-fed to apparent satiation three times per day at 8 a.m., 12 noon, and 4 p.m. until the fish reached a final total body length of about 3–3.5 cm, which is a standard commercial size for the export market in Sri Lanka. The daily feed intake of fish in each tank was recorded. Tanks were cleaned daily by siphoning out waste materials and refilled with well water before feeding. Water temperature was measured daily with a standardized mercury thermometer (^{0}C), and

pH, NO_2^- (mg/L), NO_3^- (mg/L), and NH_4^+/NH_3 (mg/L) were measured once per week using standard aqua test kits (ZOOLEK).

Total length (cm) and total weight (g) of 10 fish were measured initially, and then 10 fish from each tank were measured at 2-week intervals during the experimental period. Tanks were observed daily for any mortality. At the end of the growth trial, six fish per tank were anaesthetized with ethyl-4-amino-benzoate (Tibaldi et al. 2006). Fish were sacrificed and skin and fins were removed to estimate total carotenoid content of fish tissues. Liver weight and visceral weight were taken to estimate the hepatosomatic index (HSI) and viscerosomatic index (VSI).

Mortality and daily feed consumption were recorded, and other parameters such as specific growth rate (SGR), condition factor (K), % survival, feed conversion ratio (FCR), and protein efficiency ratio (PER) were calculated according to standard equations:

$$\%\mathrm{SGR} = \frac{\ln[\mathrm{Finalbodyweight(g)}] - \ln[\mathrm{Initialbodyweight(g)}]}{\mathrm{Time(days)}} \times 100$$

$$\mathrm{K}\left(\mathrm{gcm}^{-3}\right) = \frac{\mathrm{Bodyweight(g)}}{[\mathrm{Totalbodylength(cm)}]^3} \times 100$$

$$HSI = \frac{Liver\ weight(g)}{Body\ weight(g)} \times 100$$

$$VSI = \frac{Viscera\ weight(g)}{Body\ weight(g)} \times 100$$

$$FCR = \frac{Total\ feed\ intake(g)}{Body\ weight\ gain(g)}$$

$$PER = \frac{Weight\ gain(g)}{Total\ protein\ intake(g)}$$

The pigment extraction of experimental diets and fish tissue samples was conducted according to the Torrissen and Naevdal (1988) modified method. Total carotenoid content (μg g^{-1}) was determined using the following equation:

Total carotenoid content (TCC) (μg g^{-1}) = [Absorption at 476 nm wave length × 10] / 0.25 ×

Sample weight (g)]; [where, 10 = dilution factor; 0.25 = extinction coefficient]

Histological analyses

At the end of the each growth trial, two to six fish per tank were euthanized by overdose of benzocaine; they were dissected, and the liver was removed and fixed in Bouin's solution. Tissue samples were dehydrated, cleared, and subjected to wax impregnation. Processed fish tissues were embedded in paraffin wax and sectioned using a rotary microtome (Thermo Scientific, UK) at 5 μm thickness. Tissue sections were stained with haemotoxylin and eosin and examined under a photomicroscope (U-CBS Olympus digital microscope, Japan) to assess any histological changes in the liver (Humason 1979; Wuertz 2005).

Statistical analysis

All statistical analyses were performed using SPSS version 17.0 and data presented as Mean ± *SD*. A randomized block design model was used to observe the differences in final total length, final body weight, condition factor, HSI, VSI, daily feed consumption, and liver histology data. Percentage SGR, PER, survival rate, FCR, total carotenoid content of diets, and fish tissues were analyzed using one-way ANOVA. Duncan's multiple range tests in post hoc analysis were used to determine the significant differences ($P < 0.05$) between treatments.

Results

Feed formulations, proximate composition, and protein to energy ratios of experimental diets are shown in Table 1. The dietary protein and lipid levels ranged from 29.5% to 35.7% and 4.4% to 5.1% respectively, and gross energy content of diets ranged from 313.6 to 332.1 Kcal/g. The fatty acid composition of experimental diets varied with the inclusion of plant protein ingredients in the diets (Table 2). Total saturated fatty acids (33.3%–37.1%) and monounsaturated fatty acids (MUFA) (24.4%–27.1%) were nearly similar in all diets, and oleic acid was the major contributor to MUFA levels in all four diets. Polyunsaturated fatty acids (PUFA) levels were high in all experimental diets (36.0%–39.4%) mainly due to the high content (25.8%–32.3%) of linoleic acid (18:2 n-6). Comparatively lower amounts of docosahexaenoic acid (2.0%–7.3%) and linolenic acid (1.%5–2.4%) were present in all diets. Total n-3 fatty acids were considerably lower in the SS diet compared to other three diets, but the proportion of n-6 fatty acids in the SS diet was higher (32%). Therefore, the SS diet showed a notably lower n-3/n-6 ratio compared to the other three diets. The n-3/n-6 ratio was highest in the control diet mostly due to the increase level of n-3 PUFA; the SP and JS diets also showed a higher n-3/n-6 ratio compared to the SS diet.

Table 2. Fatty acid composition (as % of total fatty acids) of experimental diets.

	Experimental diet			
Name of fatty acid	CD	SP	JS	SS
C6:0 Hexanoic acid	ND	0.1	ND	ND
C8:0 Octanoic acid	0.4	0.7	0.5	0.5
C10:0 Decanoic acid	0.5	0.7	0.6	0.7
C12:0 Lauric acid	6.1	7.7	8.6	9.5
C14:0 Myristic Acid	3.7	4.3	5.2	4.9
C15:0 Pentadecanoic acid	0.3	0.2	0.2	0.1
C16:0 Palmitic acid	16.6	15.4	16.4	14.5
C17:0 Heptadecanoic acid	0.4	0.3	0.4	0.2
C18:0 Stearic acid	4.7	4.5	4.7	4.0
C20:0 Arachidic acid	0.3	0.3	0.3	0.3
C22:0 Behenic acid	0.3	0.3	0.2	0.3
∑Saturated	33.3	34.5	37.1	35.0
C16:1 Palmitoleic acid	1.1	0.9	0.9	0.5
C17:1 10-Heptadecenoic acid	0.2	0.2	0.2	0.1
C18:1 Oleic acid	23.2	22.7	22.8	26.1
C20:1 11-Ecosenoic acid	0.7	0.5	0.6	0.3
C22:1 Erucic acid	0.2	0.1	0.8	0.1
∑MUFA	25.4	24.4	25.3	27.1
C18:2 Linoleic acid (ω-6)	27.2	28.8	25.8	32.3
C18:3 n-3 Linolenic acid (ω-3)	1.6	2.4	1.5	1.6
C20:2 11–14 Eicosadienoic acid (ω-6)	0.1	0.2	0.2	0.1
C20:3 n-6 Homogamma Linolenic acid (ω-6)	ND	ND	0.4	ND
C20:3 n-3 11–14-17 Eicosatrienoic acid (ω-3)	0.8	0.6	0.5	0.2
C20:5 Eicosapentenoic acid (EPA) (ω-3)	1.6	1.2	0.7	0.4
C22:2 Docosadienoic acid (ω-6)	ND	0.1	ND	ND
C22:5 n-3 Docosapentenoic acid (ω-3)	0.5	0.4	0.5	0.2
C22:6 n-3 Docosahexenoic acid (DHA) (ω-3)	7.3	5.7	6.4	2.0
∑PUFA	39.1	39.4	36.0	36.8
∑Saturated	33.3	34.5	37.1	35.0
∑MUFA	25.4	24.4	25.3	27.1
∑PUFA	39.1	39.4	36.0	36.8
∑ n-3	11.8	10.3	9.6	4.4
∑ n-6	27.3	29.1	26.4	32.4
n-3/n-6	0.43	0.35	0.36	0.14

Note. CD = control diet, SP = sweet potato leaf meal diet, JS = jackfruit seed meal diet, SS = sesame seed meal diet).
ND = not detected.
MUFA = monounsaturated fatty acids.
PUFA = polyunsaturated fatty acids.

All fish showed a high survival rate (99.5%–100%), and no pathological conditions were observed during the study period. Final total length, final total weight, and SGR were significantly different among the treatments (Table 3). Fish fed with the sesame seed meal (SS) diet showed the significantly highest growth performance, and fish fed with SP diet exhibited the lowest growth performance. However, the condition factor (K) was nearly 1 and was not significantly different among treatments (Table 3). Hepatosomatic index (HSI) and viscerosomatic index (VSI) varied between different treatments in the present study. VSI was highest in fish in the CD treatment, where FM was the major protein source; hepatosomatic index was significantly lower in those fish. In contrast, fish fed the SS (sesame seed meal) diet, which had a higher dietary lipid level, exhibited lower VSI and higher HSI.

Table 3. Mean ± *SD* of growth parameters of guppy fry, TCC of diets and fish tissues, and liver histology parameters.

	Experimental treatment			
Parameter	CD	SP	JS	SS
Initial total length (cm)	1.91 ± 0.23	1.91 ± 0.23	1.91 ± 0.23	1.91 ± 0.23
Initial weight (g)	0.07 ± 0.14	0.07 ± 0.14	0.07 ± 0.14	0.07 ± 0.14
Final total length (cm)	3.52 ±0.20^{b}	3.40 ±0.14^{a}	3.54 ±0.19^{b}	3.69 ± 0.12 ^{c}
Final total body weight (g)	0.48 ±0.09^{b}	0.42 ± 0.05 ^{a}	0.49 ±0.09^{b}	0.55 ± 0.07 ^{c}
SGR	4.61 ± 0.13^{b}	4.24 ± 0.16^{a}	4.63 ± 0.31^{b}	4.90 ± 0.08^{b}
K (g cm^{-3})	1.11 ± 0.11^{a}	1.07 ± 0.13^{a}	1.11 ± 0.09^{a}	1.09 ± 0.12^{a}
HIS	1.65 ± 0.80^{a}	2.46 ± 1.51^{ab}	2.61 ± 1.50^{ab}	3.53 ± 1.64^{b}
VSI	32.72 ± 4.79^{b}	24.55 ± 1.69^{a}	24.02 ± 2.44^{a}	25.93 ± 3.91^{a}
% Survival	100.0 ± 0^{a}	100.0 ± 0^{a}	99.52 ± 0.83^{a}	99.52 ± 0.83^{a}
Daily feed consumption (%BWt/day)	11.70 ± 3.84^{a}	14.92 ± 5.76^{b}	11.65 ± 3.98^{a}	11.74 ± 4.39^{a}
FCR	2.84 ± 0.14^{a}	3.54 ± 0.32^{b}	2.83 ± 0.41^{a}	2.46 ± 0.08^{a}
PER	0.11 ± 0.01 ^{a}	0.10 ± 0.01^{a}	0.12 ± 0.02^{a}	0.15 ± 0.01^{b}
Total carotenoid content				
TCC (diet)	3.97 ± 0.53^{a}	24.18 ± 3.17^{b}	2.97 ± 1.11^{a}	3.80 ± 0.64^{a}
TCC (fish)	1.71 ± 0.12^{a}	2.84 ± 0.37 ^{c}	3.25 ± 0.55 ^{c}	2.21 ± 0.35^{b}
Histological parameters				
N_i (per 1.13 mm^2 area)	108.80 ± 10.38^{a}	149.40 ± 19.43^{b}	212.60 ± 22.26 ^{c}	237.20 ± 28.47^{d}
D_i (x 10^3 µm)	5.45 ± 2.04^{d}	4.64 ± 1.66 ^{c}	3.17 ± 1.11^{a}	3.70 ± 1.10^{b}

Note. CD = control diet, SP = sweet potato leaf meal diet, JS = jackfruit seed meal diet, SS = sesame seed meal diet). N_i = Number of hepatic lipid vacuoles and D_i = diameter of hepatic vacuoles.
Different superscripts within rows indicate significant differences between means among treatments ($P < 0.05$).

Average daily feed consumption (%BWt/day) during the study period ranged between 11.7% and 14.9% (Table 3). The significantly highest feed intake (14.92 ± 5.76%BWt/day) and poorest FCR (3.54 ± 0.32) were observed in fish fed with the diet including sweet potato leaf meal. The FCR of fish in the other three diets was around 2.4–2.8. However, PER of fish fed the SS diet was significantly highest (0.15 ± 0.01) compared to that of fish fed other three diets.

The total carotenoid content (TCC) of the sweet potato leaf meal diet (24.18 ± 3.17 µg/g) showed the highest TCC level, nearly 5 times higher than the TCC of other three diets (Table 3). Although dietary carotenoid concentration was not different among the CD, JS, and SS diets, the tissue carotenoid levels were significantly different among those treatments (Table 3). Although the JS diet showed lower total dietary carotenoid content compared to the other diets, higher total carotenoid content in fish tissues was observed in the JS treatment, and the fish were brightly colored. Similarly, fish in the SP treatment showed bright colors and higher total carotenoid content in tissues, and dietary total carotenoid content was also higher in that diet. These differences were clearly observed in live fish, and those with higher TCC in fish tissues showed brighter colors compared to fish in the other treatments (Figure 1).

Clear morphological differences of liver histology were notable in fish fed with diets containing alternative plant ingredients when compared with that of fish fed the control diet. Liver of fish fed the CD was characterized by larger lipid vacuoles, which were uniformly interspersed among liver cells at lowest

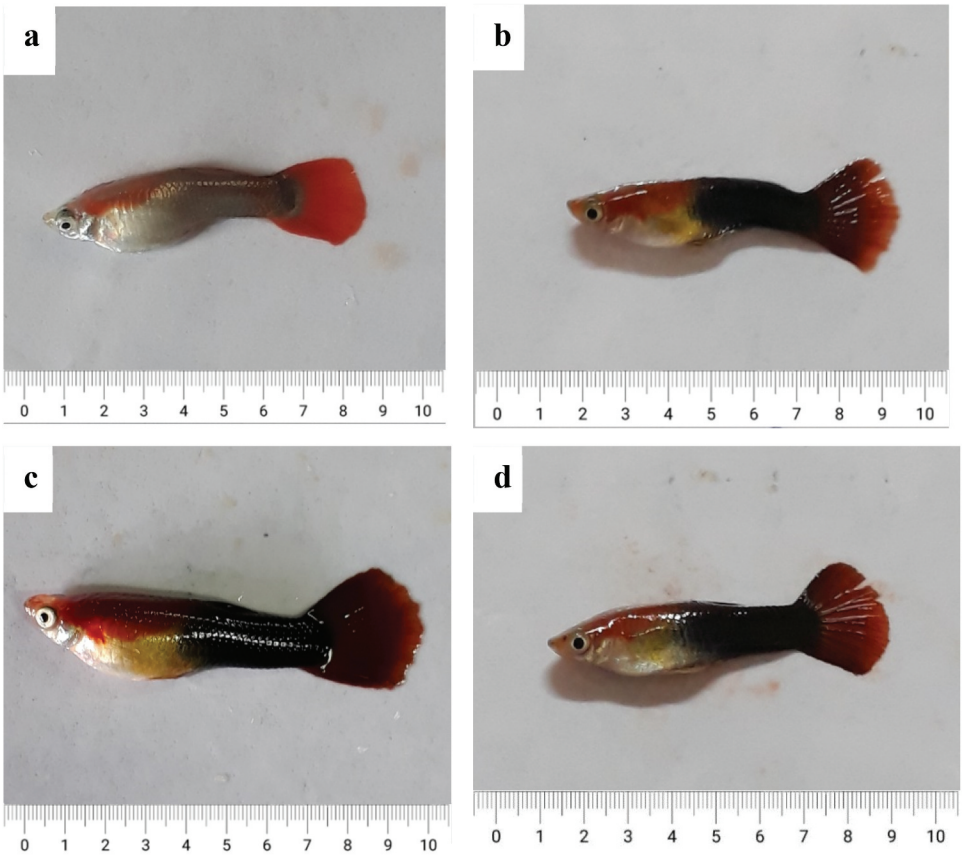

Figure 1. Guppy fish at 42 days of experimental period. Fish were fed with (a) control diet, (b) sweet potato leaf meal diet, (c) jackfruit seed meal diet, (d) sesame seed meal diet.

density (Figure 2). However, liver of fish in the JS and SS treatments showed comparatively smaller lipid vacuoles of different sizes, which were randomly distributed among liver cells at a higher density. The level of vacuolization was high (237.20 ± 28.47) in the SS treatment, followed by the JS (212.60 ± 22.26), SP (149.40 ± 19.43), and CD (108.80 ± 10.38) treatments. Statistical differences were observed in number of hepatic lipid vacuoles (Ni) and diameter of hepatic lipid vacuoles (di) of fish in different dietary treatments (Table 3). A significant negative relationship was also observed between hepatic lipid vacuole diameter (mm) and number of hepatic lipid vacuoles (Figure 3); there was a clear separation between different treatments.

The cost of producing 1 kg of feed of the different experimental diets ranged between Rs 149 to Rs 205 (Table 4). The incidence cost to produce 1 g of fish was significantly lowest in the SS treatment compared to other three treatments. The SS treatment also showed the significantly highest profit index; the CD treatment showed the lowest profit index.

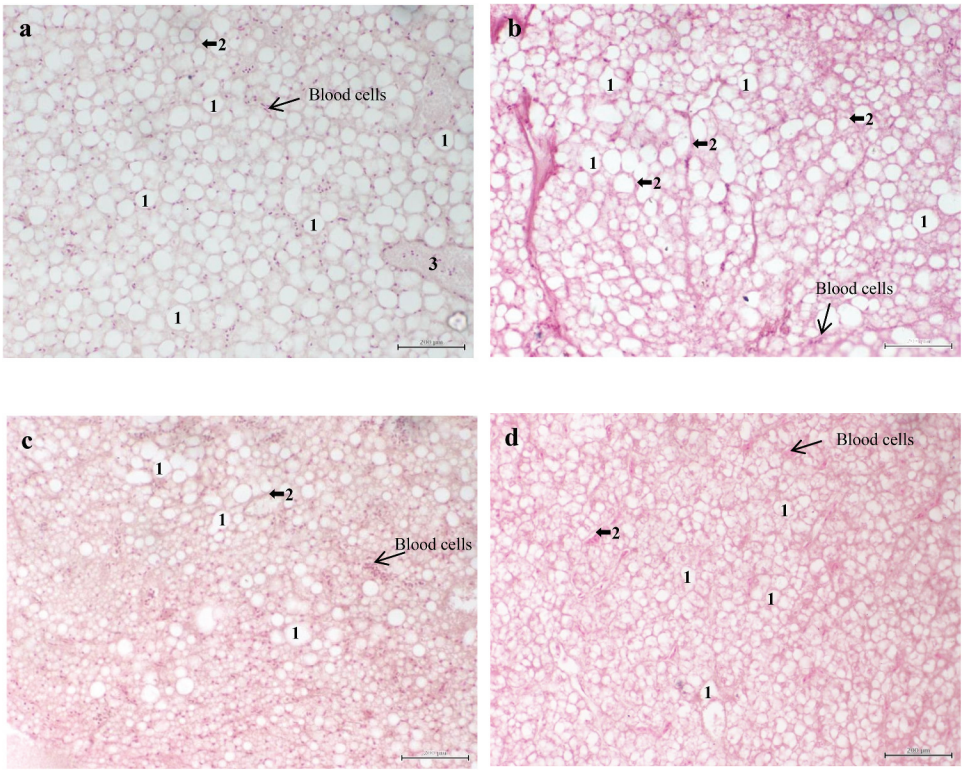

Figure 2. H&E stained liver sections (5 µm thickness) of guppy fish (H&E, 40). (a) CD (b) SP (c) JS (d) SS. Note the aggregation of blood cells (arrows); 1 = hepatic lipid vacuoles; 2 = sinusoid; 3 = blood vessels. Scale bar = 200 µm.

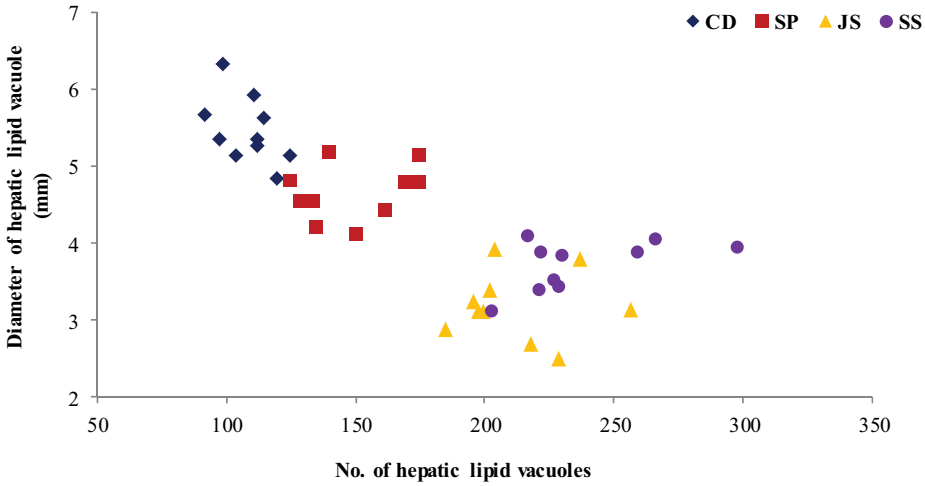

Figure 3. Hepatic lipid vacuole diameters as a function of number of hepatic lipid vacuoles.

Table 4. Cost of producing 1 kg of feed, profit index (Mean ± *SD*) and incidence cost (Mean ± *SD*) for different experimental diets.

	Experimental diet			
Parameter	CD	SP	JS	SS
Total cost (Rs/kg)	204.5	174.6	195.5	149.4
Profit index	45.47 ± 0.58[a]	51.54 ± 0.68 [c]	47.69 ± 0.08[b]	62.49 ± 0.39[d]
Incidence cost (Rs/g)	0.50 ± 0.02[b]	0.52 ± 0.04[b]	0.47 ± 0.06[b]	0.32 ± 0.01[a]

Note. CD = control diet, SP = sweet potato leaf meal diet, JS = jackfruit seed meal diet, SS = sesame seed meal diet).
Total cost (Rs/kg) = Ingredient cost + Transportation cost + Preparation cost.
Market prices (1 kg) for fish meal: Rs.320, soybean meal: Rs.110, coconut meal: Rs.60, rice bran: Rs.45, wheat flour: Rs.90, vitamin-mineral mixture: Rs.600, soybean oil: Rs.400, sweet potato leaves: Rs.10, jackfruit seeds: Rs.20, and sesame seeds cake: Rs.50.
Different superscripts within rows indicate significant differences between treatments ($P < 0.05$).

Discussion

The nutrient levels of the experimental diets were comparable with the required levels of nutrients (protein 30%–40%; lipid 4%–9%) for the growth of guppy (National Research Council (NRC) 1993; Shim and Chua 1986). Freshwater fish require either dietary linoleic acid (18:2 n-6) or linolenic acid (18:3 n-3) or both (National Research Council (NRC) 1993), and all experimental diets contained both fatty acids. Lipid content of the feed ingredient is an important factor in fish feed formulation as lipids supply essential fatty acids (EFA) and serve as transporters for fat-soluble vitamins (Craig and Helfrich 2009). Studies have shown that dietary essential fatty acid profiles derived from plant protein sources can affect the immune response and the gut and liver structures of fish (Bransden et al. 2005; Uran et al. 2009). Further, n-3 highly unsaturated fatty acids are considered essential to the biological structure and normal function of cell membranes of fish (Sargent et al. 1999). Therefore, it is important to evaluate dietary fatty acid composition in diets as it can influence fatty acid composition of fish tissues (Xue et al. 2006). Generally, fish require fatty acids with longer chain length and a higher degree of unsaturation than mammals (Sales and Janssens 2003).

Dietary lipids are important sources of energy, and fatty acids are essential for growth and survival of fish (Pie et al. 2004). Although fish have a low energy demand, they are susceptible to deposition of excessive lipid in the body (Earle 1995). Liver and visceral tissues are major fat deposition tissues in the animal body (De Silva and Anderson 1995). The sesame seed meal diet showed higher dietary lipid levels, and those fish exhibited lower VSI and higher HSI. It might be that the liver acts as a major lipid storage tissue in those fish. However, lower HSI and higher VSI of fish fed the control diet with fish meal as the main protein source indicated that the viscera act as the main lipid storage tissues in those fish. Caballero et al. (1999) and Wang et al. (2005) have also mentioned that dietary lipid content of the diet plays a major role in higher fat deposition in the viscera. Further, they explained that fat storage

tissue is varied depending on the ingredient composition and lipid quality (fatty acid composition) and may have influenced the lipid deposition in different tissues.

Fish growth depends on the types of ingredients and their composition in formulated feed (Glencross, Booth, and Allan 2007). Several authors reported that reduced growth performance of fish fed with plant protein-based diets might be partly due to the absence of feed attractants that improve dietary palatability, imbalances of essential amino acids, and presence of antinutritional factors in plant protein sources (Espe et al. 2006). Previous studies reported that sweet potato leaf meal contained some antinutrients such as saponin, phytic acid, cyanide, and tannin (Paranamana, Radampola, and Bulugahapitiya 2015b). Further, high fiber content and the presence of protease inhibitors in sweet potato leaf meal interferes with proteolytic enzyme activity and nutrient absorption and may negatively influence growth and feed utilization of fish (Eusebio, Coloso, and Mamauag 2004). Therefore, this may explain the low growth performance and poor feed conversion (FCR) in fish fed with diets with 10% sweet potato leaf meal in the present study. However, some studies reported that different processing techniques can reduce antinutrients of plant proteins and show better palatability and growth of fish than fish fed raw plant ingredients (Fagbenro 1999; Francis, Makkar, and Becker 2001). Adewolu (2008) stated that experimental diets that included sweet potato leaf meal were well accepted by *Tilapia zilli* fingerlings and indicated that the inclusion level of sweet potato leaf meal (5%–20%) did not affect the palatability of the diets. He also specified that this might be due to processing techniques (drying and grinding) used during ingredient preparation, which reduced the antinutrients content in sweet potato leaf meal, while increasing its palatability. Daily feed consumption of fish fed the CD, JS, and SS diets did not significantly differ, and feed consumption values were comparable to the values reported previously for market-sized guppy fish (Kithsiri et al. 2010; Paranamana, Radampola, and Bulugahapitiya 2014, 2015a). The condition factor reported in this study (closer to 1) indicates that the fish were in good health and showed isometric growth (Williams 2000); this was not influenced by addition of any plant-based protein sources.

The FCR values of the present study (2.5–3.5) were similar to values previously reported for market-sized guppy fish (Kithsiri et al. 2010) but slightly higher than the values (1.5–2.2) reported by Paranamana, Radampola, and Bulugahapitiya (2014, 2015a). This might be due to the differences in the culture tanks and systems used in different studies. Cement tanks in farm conditions with higher density were used in the present study; small glass tanks in aquarium conditions with lower densities were used in the studies reported by Paranamana, Radampola, and Bulugahapitiya (2014, 2015a). FCR value is largely dependent on both dietary formulation and a feed distribution scheme that needs to be carefully monitored and adapted to the

feeding habits of fish (Borges et al. 2009). However, PER is not a correct measure of protein deposition as weight increase can be due to fat deposition (Borges et al. 2009). The lowest FCR value is mainly due to better feed utilization of artificial feeds, and high-energy diets produce the lowest FCR and highest nutrient retention (Coyle et al. 2004). In the present study, fish fed with the diet containing sesame seed meal (SS), which had a higher dietary energy level (332 K cal/g), showed the lowest FCR (2.46 ± 0.08).

Carotenoid content varied between ingredients and also depends on processing methods of the ingredient; therefore characteristics of the ingredient may have an effect on aquatic animal pigmentation (Linan-Cabello, Paniagua-Michel, and Hopkins 2002; Torrissen and Naevdal 1988). Moreover, carotenoids of feed ingredients should be in digestible form, and a sufficient amount of carotenoids is needed to maintain tissue concentration, pigmentation, and bioconversion to bioactive forms of fish. In the present study, coloration and carotenoid content of fish tissues were significantly higher in fish fed diets that contained plant-based alternative ingredients when compared to the fish meal-based control diet. Therefore, it is apparent that the tested plant ingredients contain sufficient amounts of carotenoids, which can influence the pigmentation and coloration of guppy fish. So it is clear that different characteristics of the feed ingredients in diets influence the pigmentation in fish tissues. Enhancement of fish coloration using different feed ingredients was tested, and positive results were obtained in many previous studies. For example, marigold petal meal (Ezhil, Jeyanthi, and Narayanan 2008) was successfully tested as a feed ingredient in the diet of red sword tail for color enhancement. *Chlorella vulgaris* has been used in previous research as the most effective natural pigmented source for the improvement of skin color of ornamental fish (Ezhil, Jeyanthi, and Narayanan 2008; Gouveia and Rema 2005). *Spirulina*, China rose petals, marigold petals, and *lactobacilli* were assessed to find the most effective natural carotenoid source in goldfish by Sinha and Asimi (2007).

It seems that the quality and quantity of lipid supplied from fish meal and other alternative ingredients (SP, JS, and SS) are different and behave differently when deposited in the liver. In the present study, hepatic lipid vacuolization seemed to be altered by alternative plant protein inclusion. Rusell et al. (2001) observed that when corn starch was replaced with pea seed meal in European sea bass diets, those fish showed a lower vacuolated liver than the fish fed with the control diet. Similarly, Pereira et al. (2002) observed that rainbow trout fed with brassica by-products showed a lower lipid vacuolization than fish in the control treatment. More lipids in the livers could be associated with a slower rate of glucose uptake resulting in glucose being converted into lipids (Rusell et al. (2001). Glencross et al. (2004) also showed that rainbow trout fed with 50% yellow lupine as an alternative protein source had a significant decrease in lipid vacuolization. Therefore, the rate of glucose

uptake might be different in the four dietary treatments where differences in vacuolization were observed in the liver cells in the present study. Spisni et al. (1998) previously identified this type of lipid deposition in vacuoles as a pathological process—fatty degeneration or steatosis that can be used as an indicator of hepatic disturbances in fat metabolism. Spisni et al. (1998) also described steatosis as a liver alteration, due to excessive dietary intake of lipid, which saturates the physiological capability of the liver, leading to accumulation of lipid vacuoles. Hence, liver tissues of fish fed the CD, SP, JS, and SS diets might be affected by this type of liver alteration and showed different accumulation patterns of lipid vacuoles in liver cells. The control treatment showed fewer larger vacuoles in the liver; and the SS treatment showed a higher number of smaller vacuoles in the liver. There is an alternation in the lipid deposition that was clearly influenced by the ingredients in the feed.

The economic viability of the experimental diets indicated that the feed cost was reduced when plant-based protein sources were included in the diet. Among the four diets, the sesame seed meal diet was cheaper compared to other diets and had the higher profit index and the lower incidence cost. Low-cost feeds decrease the operating cost of the farm and ultimately increases the revenues earned by fish farmers. Therefore, finding suitable, low-cost alternative protein sources to replace expensive fish meal protein in the diet is a timely needed aspect that will ultimately affect the sustainability of aquaculture operations (Kader et al. 2010; Naylor et al. 2009).

Conclusion

In the present study, the results indicated that all three alternative plant protein sources have potential as fish feed ingredients to formulate fish feeds for guppy reared in commercial field conditions. Fish fed a 10% sesame seed meal diet showed the best growth performance, feed utilization, and survival. The best coloration was observed in fish fed sweet potato leaf meal (10%) and jackfruit seed meal (3%) included in the diets when compared with the other two diets. Therefore, sesame seed meal-based feed formulation can be used as a low-cost feed formulation for practical diets for guppy reared in outdoor farming conditions. Further studies are also needed to determine the maximum possible inclusion levels of these plant feed ingredients in fish feed, to evaluate its suitability, and also to investigate the effect of these plant protein sources on the growth of different fish species.

Highlights

- Fish meal can be replaced with 3%–10% of tested ingredients without having growth retardation.

- The best growth performance of guppy was obtained from sesame seed meal-included diet.
- 10% sweet potato and 3% jackfruit seed meal diets gave better coloration of guppy.
- Liver morphology was altered in fish fed plant protein sources.

Disclosure statement

No potential conflict of interest was reported by the authors.

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