Iranian Journal of Fisheries Sciences 21(5) 1192-1203 2022
DOI: 10.22092/ijfs.2022.127950

Research Article

Utilization of mallow, *Malva parviflora,* leaves meal for feeding sailfin molly fish *Poecilia latipinna* (Lesueur, 1821)

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Received: June 2022 Accepted: August 2022

Abstract

The present study was carried out to investigate the effect of replacing some conventional feedstuffs (barley and yellow corn) with mallow, *Malva parviflora,* leaves meal on the growth and feeding efficiency of sailfin molly, *Poecilia latipinna*. Five experimental diets containing 0, 5, 10, 15, and 20% of mallow leaves meal M_0, M_5, M_{10}, M_{15}, and M_{20} respectively, were prepared by replacing barley and yellow corn. A total of 90 fish (2.55±0.17 g) were used, distributed equally into fifteen plastic tanks at a rate of 6 fish per tank. The experiment lasted for 60 days, during which fish were fed the experimental diets to satiation twice daily. Results exhibited a gradual significant (p<0.05) increase in the feed intake FI (r=0.935) of the fishes with increasing dietary mallow leave replacement. There were no significant differences (p>0.05) in specific growth rate (SGR), feed conversion ratio (FCR), and protein efficiency ratio of sailfin molly fed control (M_0) diet compared to fish fed on M_5 and M_{10} diets at the end of the experiment. Increasing the involvement of mallow in M_{15} and M_{20} diets led to inhibited SGR, FCR, and PER significantly (p<0.05) compared to the control diet. In conclusion, using mallow dried meal in the diets at a 10% level was proved to be more suitable with no noticeable adverse effects on the growth and feeding efficiency of experimental fish.

Keywords: Feedstuff, Fish nutrition, Growth, Mallow meal, Sailfin molly

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Introduction

Ornamental fish care is one of the favorite and oldest hobbies in the world, because of the aesthetics of these fish and their ability to beautify the place in which they are sited, as well as their ability to reduce stress, the high demand made it an important component of the global fish trade (Sharma and Dhanze, 2018). Production of animals for the aquarium hobbyist trade is a rapidly growing sector of the aquaculture industry and it will continue to become more important as restrictions are placed on collecting animals from the wild (Tlusty, 2002). The sailfin molly *P. latipinna* is a small popular ornamental fish, native to the eastern coastline of North America, introduced into many countries as a biological control agent for preventing malaria by preying on mosquito larvae and through its release by aquarium fish hobbyists (Koutsikos *et al*., 2018; Abu El-Regal and Al-Solami, 2020).

The weeds are an integral part of ecosystems and play an important role in biodiversity, especially as they are an important food source for many animal species (Andersson and Milberg, 1998). Among these weeds the mallow, *Malva parviflora,* which belongs to the widely distributed family Malvaceae, has been introduced to many countries as a garden plant and it is also cultivated in Europe as a salad vegetables or as green manure (Michael *et al*., 2009). It is rich in protein, Gutiérrez *et al*. (2008) recorded a protein content of up to 29.86%. Furthermore, it contains flavonoids, terpenoids, phenol derivatives, vitamins C and E, and fatty acids and has many other healthy beneficial features (Bilen *et al*., 2019). In Iraq, mallow is not cultivated but harvested from the wild and usually consumed boiled. However, mallow is used by some locals as a food because of it nutritive value. The Current research was carried out to evaluate the potential use of mallow leaves meal as a feed ingredient and its effect on the growth and feeding efficiency of sailfin molly.

Materials and methods

Diets preparation

Fresh mallow *M. parviflora* leaves and dietary ingredients (fishmeal, soybean meal, barley, yellow corn, wheat bran, carboxymethyl cellulose binder (CMC) and vitamins and minerals mixture) were purchased from the local market in Thi Qar province, Iraq. Mallow leaves are washed with sufficient water, drained, dried at room temperature for 48 hours, and finely ground via an electric grinder. Before the diet preparation, an analysis of the proximate composition of mallow (Table 1) was conducted according to the method of the AOAC (2005). Amino acid profile of mallow, barley, and corn was determined at the laboratories of the Directorate of Chemistry and Materials Research, Ministry of Science and Technology, Baghdad, Iraq, using the High Performance Liquid Chromatography HPLC system (Shimadzu, Japan) after acid hydrolysis of samples (Dziągwa-Becker *et al*., 2015), except for Tryptophan which was determined spectrophotometrically after alkali hydrolysis (AOAC, 2005).

Table 1: Proximate composition (% of dry matter) of mallow leaves.

	Moisture	Protein	Ether extract	Carbohydrate	Ash
Proximate composition	6.09±0.39	19.26±1.07	5.41±0.34	58.46±2.17	16.87±0.99

Five isonitrogenous (protein 33.45±0.75%) and isocaloric (energy 452.68±2.65 Kcal/100g) pelleted diets (treatments) were formulated, a control diet without incorporation of mallow leaves, and four test diets M_5, M_{10}, M_{15}, and M_{20} by replacement of barley and yellow corn by mallow leaves at 5, 10, 15, 20%, respectively. Feed ingredients and proximate composition (% of dry matter DM) of the experimental diets are presented in Table 2.

Table 2: Ingredients (%) and proximate composition (% of dry matter) of the experimental diets.

	Control diet	Mallow leaves substitution diets			
Ingredients (%)	M_0	M_5	M_{10}	M_{15}	M_{20}
Fish meal	20	20	20	20	20
Soybean meal	30	30	30	30	30
Barley	10	5	5	0	0
Yellow corn	10	10	5	5	0
Wheat bran	26	26	26	26	26
Mallow	0	5	10	15	20
Binder CMC	2	2	2	2	2
Vitamin and mineral premix	2	2	2	2	2
Proximate composition (%)					
Moisture	9.22	9.03	8.79	8.60	8.37
Protein	32.50	32.98	33.44	33.93	34.38
Ether extract	9.26	9.43	9.45	9.62	9.65
Carbohydrate	47.02	45.72	44.52	43.21	42.02
Ash	7.22	7.87	8.58	9.24	9.95
Gross energy (Kcal/100 g)	455.82	454.64	452.49	451.31	449.16
P:E ratio (mg protein/Kcal)	71.29	72.55	73.90	75.18	76.55

Experimental fish

A total of 90 *P. latipinna* fish weighing 2.55±0.17 g was obtained from a Fish Farming Station, Marine Science Center, Basrah University, Basrah, Iraq. The fish are randomly distributed into 15 (20 liter) plastic tanks at a rate of 6 fish per tank with three tanks (replicates) for each dietary treatment. The fish were acclimatized in laboratory conditions for one week before starting the feeding trials, during this, the fish were fed control diet.

Feeding trial

The feeding trial was conducted at the laboratories of the Fisheries and Marine Resources Department, College of Agriculture, University of Basrah, Iraq. During the feeding trial fish were fed experimental diets to satiation twice daily for 60 days. Water temperature (ºC), dissolved oxygen (mg/L), salinity (PSU), and pH were measured weekly before feeding. At the end of the feeding trial, fish in each tank were weighed individually. The growth performance was measured by the determination of weight gain (WG) and specific growth rate (SGR). While, feeding efficiency was measured by the determination of feed intake (FI), feed conversion ratio (FCR), and protein efficiency ratio (PER) as follows:

Weight gain (g) = Final weight (g) - Initial weight (g)

Specific growth rate SGR (%/day) = ((ln final weight (g) - ln initial weight (g)) × 100) ÷ days of rearing

Feed intake (%) = (feed consumed (g) ÷ body weight (g)) x 100

Feed conversion ratio (FCE) = feed consumed (g) ÷ weight gained (g)

Protein efficiency ratio (PER) = weight gained (g) ÷ protein consumed (g)

Statistical analysis

Results were expressed as mean+ SD. The data were analyzed by One-Way Analyses of Variance (ANOVA) followed by Duncan’s New Multiple Range Test. All statistics were carried out using the SPSS package (version 22.0) for Windows. Differences were considered significant at $p<0.05$.

Results

The water quality parameters during the feeding trial are presented in Table 3. The temperature (24.37-25.53°C), dissolved oxygen (7.69-8.03 mg/L), salinity (1.59-1.83 PSU), and pH (6.64-7.92) indicate that values fall within acceptable limits for fish growth. Values of pH in tanks water were significantly ($p<0.05$) inversely associated (r=-0.922) with the percentage of dietary mallow leaves (Fig. 1).

Table 3: Water quality parameters in tanks during 60 day feeding trial period.

Parameters	**Control diet**	**Mallow leaves substitution diets**			
	M_0	**M_5**	**M_{10}**	**M_{15}**	**M_{20}**
Temperature (°C)	25.53±1.33	24.58±1.67	25.49±1.09	24.37±2.01	24.91±1.11
Oxygen (mg/L)	7.89±0.32	8.03±0.56	7.80±0.19	7.69±0.48	7.90±0.91
Salinity (PSU)	1.83±0.30	1.59±0.28	1.61±0.21	1.64±0.31	1.77±0.35
pH	7.73±0.29	7.92±0.42	7.47±0.38	7.15±0.41	6.64±0.39

Table 4 demonstrates amino acid profiles of mallow meal, barley and yellow corn which are used as ingredients in experimental feed preparation. It could be observed that amino acid composition was very close among the three plant feedstuffs. All the 10 essential and 8 nonessential amino acids were represented in almost comparable contents.

The effect of mallow leaves as a dietary replacement for barley and corn on the growth performance of sailfin molly was investigated and presented in Table 5. The results revealed an optimal growth performance in fish groups fed control M_0, M_5, and M_{10} diets with the mallow leave at 0, 5, and 10% with no significant difference ($p>0.05$) among them.

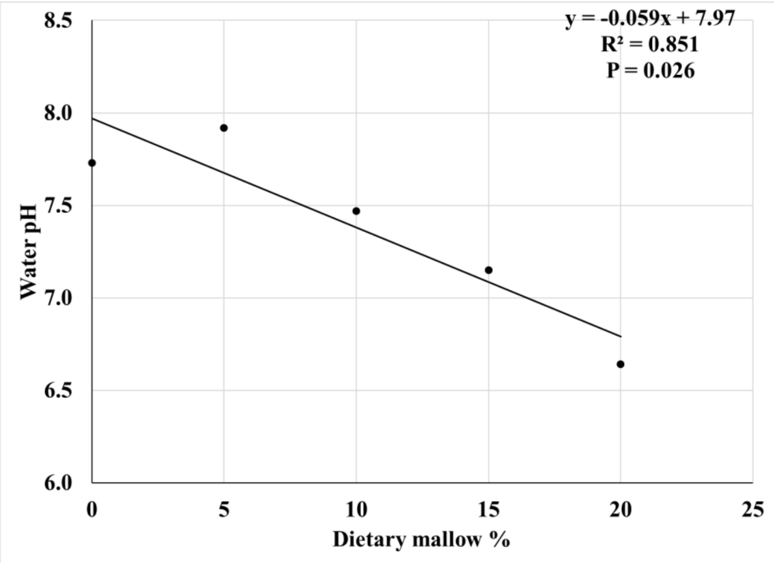

Figure 1: The relationship between the percentage involving dietary mallow and water pH.

Table 4: Amino acid profile (g/100 g protein) of mallow leaves, barley, and corn.

Amino acid	Mallow	Barley	Yellow corn
Alanine	2.36	3.58	6.84
Arginine	4.87	4.62	4.70
Aspartic	8.92	5.98	6.43
Cystine	1.14	2.07	1.59
Glutamic	8.12	9.98	7.48
Glycine	3.72	3.41	3.88
Histidine	2.16	2.08	2.74
Isoleucine	3.12	3.62	3.07
Leucine	6.63	6.58	9.98
Lysine	4.02	3.58	2.77
Methionine	1.31	1.82	1.78
Phenylalanine	4.48	5.38	4.32
Proline	4.63	9.88	7.75
Serine	3.01	3.89	4.33
Threonine	2.91	3.21	3.48
Tryptophan	0.88	1.87	0.75
Tyrosine	2.88	2.69	3.71
Valine	4.62	4.58	4.60

Increasing the involvement of mallow leaves by up to 15 and 20% in M_{15} and M_{20} diets respectively led to inhibited growth performance significantly (p<0.05) compared to the control diet. In general, there is a noticeable decrease in SGR with the increase of mallow leaves quantity in the diets.

Table 6 shows the feed intake and feed efficiency in sailfin molly fed with mallow replacement diets. Results exhibited a gradual significant (p<0.05) increase in the FI (r=0.935) of the fishes with increasing in dietary mallow leave replacement (Fig. 2), this increase was higher significantly (p<0.05) in M_{15} and M_{20} diets compare to other dietary treatments including M_0 control diet.

Table 5: Growth performance of sailfin molly fed with mallow replacement diets during 60 days feeding trial period.

Growth performance	Mallow leaves substitution diets				
	M_0	M_5	M_{10}	M_{15}	M_{20}
Initial weight (g)	2.56±0.16^a	2.48±0.20^a	2.64±0.17^a	2.51±0.18^a	2.58±0.22^a
Final weight (g)	4.32±0.10^a	4.21±0.25^a	4.26±0.17^a	3.83±0.17^b	3.82±0.24^b
Weight gain (g)	1.76±0.08^a	1.74±0.07^a	1.62±0.05^a	1.32±0.10^b	1.24±0.07^b
Specific growth rate (%/day)	0.87±0.07^a	0.89±0.04^a	0.82±0.06^{ab}	0.71±0.07^{bc}	0.66±0.05^c

Values in a row with the different superscript letters indicate a significant difference (p<0.05).

Table 6: Feed intake and efficiency in sailfin molly fed with mallow replacement diets during 60 day feeding trial period.

	Mallow leaves substitution diets				
	M_0	**M_5**	**M_{10}**	**M_{15}**	**M_{20}**
FI (%)	$1.79{\pm}0.29^b$	$1.76{\pm}0.23^b$	$1.91{\pm}0.30^b$	$2.17{\pm}0.21^a$	$2.51{\pm}0.37^a$
FCR	$2.09{\pm}0.18^a$	$2.01{\pm}0.35^a$	$2.44{\pm}0.43^a$	$3.13{\pm}0.05^b$	$3.86{\pm}0.42^b$
PER	$1.48{\pm}0.13^a$	$1.51{\pm}0.28^a$	$1.25{\pm}0.20^{ab}$	$0.95{\pm}0.02^{bc}$	$0.76{\pm}0.09^c$

Values in a row with the different superscript letters indicate a significant difference (p<0.05).

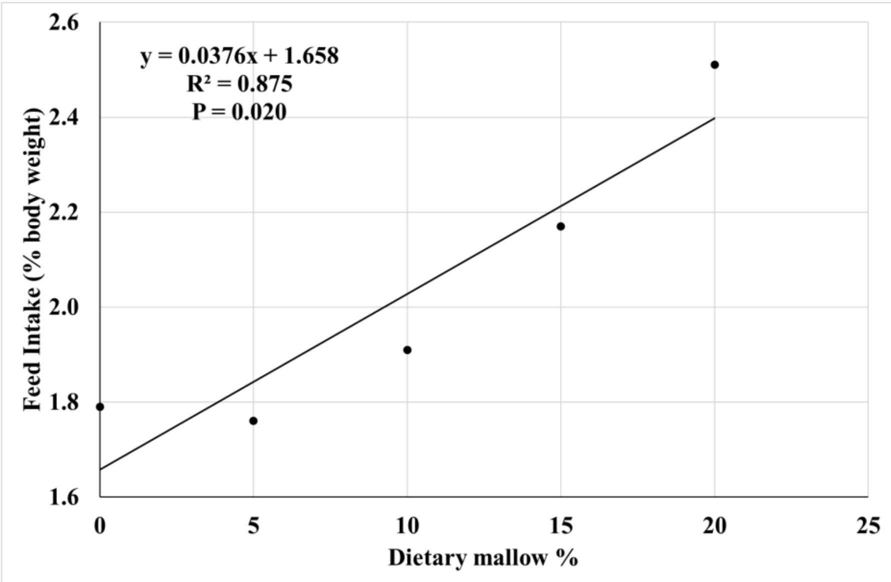

Figure 2: The relationship between the percentage of dietary mallow and feed intake.

Among dietary treatments, neither FCR nor PER in M_5 and M_{10} diets were not significantly (p>0.05) different compared to the control diet. Otherwise, both the M_{15} and M_{20} diets recorded with the worst values for FCR and PER with significantly (p<0.05) differences compared to the control diet.

Discussion

The current study indicated no significant differences when substituting barley and yellow corn as feed ingredients with mallow leaves meal up to 10% on growth performance and feeding efficiency of cultured sailfin molly. Similar results were obtained with the Nile tilapia *Oreochromis niloticus* fingerlings, which showed reduced growth when fed on 20% dried mallow plant as an alternative protein source for soybean meal compared to those fed on 10% (Abd Elhamid *et al*., 2004).

The superiority of M_{10} over M_{15} and M_{20} mallow leaves substitution diets may be partially due to the decrease in the pH value of the tank water, as it was noticed from the results that the pH value of tank water in the M_{20} treatment was sharply decreased during the experiment (as low as 6.64). The increase in water acidity could be ascribed to the effect of the diet containing mallow leaves because mallow is considered a medium phenolic compounds content at a rate up to 468.43 mg GAE (Gallic acid equivalents)/100 g db (Vasco *et al*., 2008; Eli Mireya *et al*., 2021). Although phenols are known as weak acids, they are more acidic than alcohol in an aqueous medium (Ouellette and Rawn, 2015). Similar results were obtained with the silver catfish larvae which showed reduced growth when exposed to lower pH (5.5-7.0) compared to those maintained in higher pH (8.0 and 8.5) levels (Lopes *et al*., 2001). In addition, the common carp *Cyprinus carpio* recorded greater weight, length, and biomass when it was exposed to pH values of 7.5 and 8.0 compared to the 6-7 pH range (Heydarnejad, 2012).

The use of plant-derived materials to replace conventional feed ingredients can be limited because they may contain a wide range of anti-nutritional agents, even if these materials have been tested and proven successful in growth trials (Mandal and Ghosh, 2010). Accordingly, the reason that fish-fed dietary mallow leaves at more than 10% (M_{15} and M_{20}) in the current study showed increased feed intake and decreased FCR, and PER could be the decreasing of carbohydrate digestibility, which may lead to reduce the digestible energy content of the ingested ration, which coincided with Mirzakhani *et al*. (2020) finding on Siberian sturgeon *Acipenser baerii*. Consequently, mallow's phenols may weaken and inhibit the digestion process, polyphenols (vegetable tannins) as Charlton *et al*. (2002) emphasized that can have a different harmful effect, including the isolation of iron and the inhibition of digestive enzymes. Furthermore, Haslam (1981) indicated that several types of compounds found in crop residues may affect the digestion process, including simple phenolic compounds. Phenolic compounds may also limit the digestion of carbohydrates (Ajayi *et al*., 2021) and protein (Wong and Cheung, 2001). Additionally, Jiang *et al*. (2017) found that phenolic compounds potently inhibited α-amylase and α-glucosidase activity, having potential for prevention of hyperglycemia. This is consistent with the findings of Kang'ombe *et al*. (2007) who found that increase or decrease in dietary digestible energy content over or below the optimum level may have resulted in reduced growth rates, FCR, and PER.

Moreover, Gutiérrez *et al*. (2008) indicated that the mallow plant has a high neutral and acid detergent fiber content of 26.33 and 21.41 respectively, which could be another reason for lowering carbohydrate digestibility, and leads to increasing FI and reducing FCR and PER (Sun *et al*., 2019). Vidal *et al*. (2017) mentioned that the crude fiber decreases the apparent digestibility of dry matter, and consequently decreases the ingredients nutritional value. On other hand, Hill *et al*. (1988) found that bass, *Microrooterus dolomeiui*, consume more feed at low pH levels, which is consistent with the results of the current study. The results showed that sailfin molly has an increased rate of feed intake but without achieving any weight gain when it consumed the experimental mallow substitution diets M_{15} and M_{20}, Shuangyao *et al*. (2018) suggested that slightly acidic environment may stimulate appetites and dietary intake by the fish.

The amino acid profile of mallow meal was very close to that of barley and corn which supports its usage as a substitute for each or both of them in fish feed especially when it is combined with other ingredients. Vegetables and many other feedstuffs of plant origin are known to be deficient in some amino acids which could be considered limiting amino acids, thus, feed manufacturers always combine ingredients from different sources to overcome this potential problem (Dimina *et al*., 2022), and this was in line with the current study.

However, mallow could have a medical aspect rather than a nutritional one as Shale *et al*. (1999) confirmed that mallow is used to treat bruises and fractures in the extremities. While its extract can positively affect the growth performance and immune responses of rainbow trout (Rashidian *et al*., 2020). Pharmacological studies indicate that the mallow possesses antibacterial properties against widely recognized pathogens such as *Bacillus subtilis*, *Staphylococcus aureaus*, *Escherichia coli*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, and *Salmonella typhi* (Tadeg *et al*., 2005). In addition, Gutiérrez (2012) demonstrated that mallow is anti-diabetic and antifungal, and possesses some natural antioxidants represented by flavonoids and phenolics. Furthermore, Prebiotics can be found naturally in vegetables, due to their content of fiber and complex polysaccharides, and have been shown to have effects on the microflora population in the fish intestine, which consequently enhance digestion and promote fish health and growth (Wee *et al*., 2022). This could explain part of the positive effect of mallow leaves when added at a 10% rate in the diets of sailfin molly which have been observed during the present experiments.

It could be concluded from the results of the current study that it is possible to use mallow *M. parviflora* dried leaves meal as a feed ingredient at a rate of up to 10%, with no adverse effects on the growth and feeding efficiency of experimental sailfin molly.

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