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Original Article

Effects of Dietary Marshmallow (*Althaea Officinalis* L.) Extract on Growth Performance and Body Composition of Common Carp (*Cyprinus Carpio*)

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ABSTRACT

Objective: This study was conducted to evaluate the effects of different proportions of marshmallow extract (*Althaea officinalis* L.) on growth performance and carcass composition of common carp (*Cyprinus carpio*). **Methods:** Common carp with an average weight of 37.65 ± 4.40 g were fed for two months with a diet supplemented with marshmallow (*A. officinalis*) extract 0.25%, 0.50 and 1%, and with normal diet as controls. The hepatosomatic index (HSI) and viscerosomatic index (VSI) were assessed on days 30 and 60. The growth performance including weight gain, specific growth rate (SGR), feed conversion ratio (FCR), and condition factor (CF) were measured on days 15, 30, 45 and 60. **Results:** Results of the present study showed that specimens fed a diet supplemented with marshmallow extract (0.25%) exhibited dramatically increased growth performance, which was the highest amongst all treatments (P<0.05). The addition of marshmallow extract did not have any effects on hepato-somatic index (HSI), intestine-somatic index (ISI) and condition factor when compared to the control diet. However, administering high levels of marshmallow extract had adverse effects on growth performance of specimens. Our results showed slight changes in body composition of fish fed a diet supplemented with marshmallow extract compared with controls. It can be concluded that marshmallow extract 0.25% can act as a growth stimulator. On the other hand, high levels of marshmallow may have anti-nutritional factors that decrease the utilization of the given feed.

1.INTRODUCTION

The nutritional status of fish is an important factor in their resistance to diseases. In fact, by feeding them a proper diet, not only their health condition improves, but also the probability of diseases decreases. There is a positive correlation between increased resistance to diseases, growth rate and survival. There are many substances that promote growth and help to boost the fish's immune system. Since many plants and their derivatives are known as growth stimulants and/or immunostimulant agents, the use of these compounds has increased in diet of finfish and shellfish in recent decades (Ahmadi et al., 2012; Asadi et al., 2012; Banaee, 2010; Banaee et al., 2011). For example, Abd El-Hakim *et al.* (2010) found out that use of fennel (*Foeniculum vulgare*) 1% improved the fish growth performance. Similar results were observed in shrimps (Olmedo

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Sanchez *et al*., 2009); common carp, *Cyprinus carpio*, (Yilmaz *et al*., 2006); guppy, *Poecilia reticulate*, (Cek *et al*., 2007a); convict cichlid, *Cryptoheros nigrofasciatus*, (Cek *et al*., 2007b); red seabream, *Pagrus major*, (JI *et al*., 2007); olive flounder, *Paralichthys olivaceus*, (Pham *et al*., 2006); the Nile tilapia, *Oreochromis niloticus*, (Salah *et al*., 2008; Metwally, 2009); tilapia, *O. aureus*, (Turan, 2006); rainbow trout, *Oncorhynchus mykiss* (Nya & Austin, 2009; Bohlouli Oskoii *et al*., 2012); and zander, *Sander lucioperca* (Zakes *et al*. 2008) which were fed diets supplemented with plants.

Marshmallow, *Althaea officinalis*, is a member of the Malvaceae family which is indigenous of Iran. Marshmallow flower is commonly used in folk medicine in Iran and Middle East countries. This flower contains a variety of bioflavonoids, vitamins and antioxidant compounds (Sadighara *et al*., 2012). The aqueous extract of *A. officinalis* demonstrated to be potentially helpful in treating lipemia, inflammation, gastric ulcer, and platelet aggregation with no visible adverse effects (Haqe-Sleiman *et al*., 2011). Moreover, extract of marshmallow root has antibacterial, antifungal, anti-inflammatory, anti-mycobacterial, antitussive and antiviral and anti-yeast properties as well as radical scavenger activities (Quotes from Khakdan and Piri, 2013). Marshmallow, *A. officinalis*, is a source of phytochemicals with varying biological activities. This plant contains 6,10,14-trimethyl-2-Pentadecanone (47.31%), Carvacrol (17.65%), 2-Pentadecanone (11.22%), Dodecanoic acid (2.322%), n-Tetradecanoic acid (4.917%), n-Tetradecanol (1.978%), n-Nonanoic acid (1.176%), Thymol (1.073%), Methyl hexadecanoate (1.312%), (Solimani, 2014) which may act as growth stimulators. So that makes marshmallow a possible candidate as a feed additive in practical diets to enhance fish growth. Nevertheless, the presence of anti-nutrients in marshmallow may adversely affect the nutrient utilization that decreases growth performance in fish.

In the present study, Common carp, *Cyprinus carpio* were selected as experimental species. Common carp is one of the most important species within the cyprinid fish which are cultured in some areas of Iran due to its rapid growth, good survival in the culture conditions and climatic conditions of Iran (Jalali and Barzegar, 2005). Subsequently, developing a practical diet for common carp is necessary. Therefore, the present study was conducted to evaluate the application of marshmallow extract as a feed additive in fish diets and its impact on growth performance and whole body composition of *Cyprinus carpio*.

2. MATERIALS AND METHODS

Common carp were purchased from a private farm (Carp Farm, Shush, Khuzestan province, Iran) and were transported to the aquaculture laboratory, Natural Resources Faculty, Behbahan Khatam Alanbia University of Technology, Iran. The specimens were fed a commercial diet for 2 weeks to be acclimated to the conditions, and to recover from the stress of transportation.

2.1. Fish diet preparation

The formulated fish food was prepared in the laboratory using powder of commercial food obtained from Beyza Feed Mill, Shiraz, Iran (Table 1). To enrich the normal diet, the 0.25, 0.50 and 1 percent of *A. officinalis* extracts were mixed with 1 kg powder feed. Each supplemented diet was mixed with distilled water (1mL/g) until obtaining a homogenous mixture. This mixture was passed through a meat grinder, producing extruded string shapes, which were dried in an oven at 55°C for 12 h and then broken to produce pellets approximately 10 mm long. The pellets were packed and stored at -20°C in a freezer until be used. The control diet was prepared to use the same process, although no supplement was added.

Table 1.
Composition of commercial diet

Nutrients	Value
Gross energy (Kcal/Kg)	3500
Crude protein (%)	35-37
Crude lipid (%)	9-11
Crude fiber (%)	5%
Moisture (%)	<10
Ash (%)	<10
TVN (mg/100gr)	<45

TVN: Total volatile nitrogen

2.2. The final experiment

One hundred eighty common carp (with the average weight and length of 37.65 ± 4.40 g and 14.15 ± 0.8 cm) were distributed randomly into 4 groups, each containing 15 fish, and fed for 2 months with diets supplemented with marshmallow extract 0.0, 0.25, 0.50 and 1%. The fish were fed twice a day and no more than 2% of their body weight with the aforementioned diet. Fish growth was measured every two weeks and the body composition was assessed at the end of the experimental period.

2.3. Growth performance

The growth parameters such as weight gain (WG), specific growth rate (SGR), feed conversion ratio (FCR) and condition factor (CF) were calculated using the following formulas, on days 15, 30, 45 and 60.

$$\text{Weight gain (\%)} = \frac{\text{Final weight} - \text{Initial weight}}{\text{Initial weight}} \times 100$$

$$\text{Specific growth rate (SGR\%)} = \frac{\text{Ln (final body weight)} - \text{Ln (initial body weight)}}{\text{Experimental periods}} \times 100$$

$$\text{Feed conversion ratio (FCR)} = \frac{\text{Feed intake (g)}}{\text{Wet weight gain (g)}}$$

$$\text{Condition factor (CF)} = \frac{\text{Weight (g)}}{(\text{Length (cm)})^3} \times 100$$

2.4. Body composition

At the end of the experiment, 6 fish in each group were sacrificed and dried in an oven at 105 °C for 24 h before the whole body was crushed for body composition analysis according to AOAC guidelines (Association of Official Analytical Chemists, 2000).

2.5. Moisture content

Samples were weighed before putting them in an oven for 24 h at 105 °C, and then were reweighed to estimate the moisture content. The dry samples were crushed to a fine powder and were stored in a dissector for determination of protein, fat and ash content.

$$\text{Moisture content (\%)} = \frac{\text{Sample weight after drying}}{\text{Sample weight before drying}} \times 100$$

2.6. Crude protein

Crude protein was determined using a Kjeldahl method and by measuring the total nitrogen content of the sample multiplied by the empirical factor 6.25. This method includes digestion by sulphuric acid, distillation and titration.

$$\text{Protein (\%)} = \text{Nitrogen Levels (\%)} \times 6.25$$

2.7. Crude lipid (diethyl ether extract)

Crude lipid content was determined by weighing the filter paper containing the dried sample and then transferring it to Soxhlet apparatus using diethyl ether at 60-80 °C for 12 h. The sample with filter paper was dried and reweighed; the difference between sample weights indicates the total lipid content in the sample.

$$\text{Fat (\%)} = \frac{\text{Sample weight after fat extraction}}{\text{Sample weight before fat extraction}} \times 100$$

2.8. Ash content

Ash content was determined by weighing the crucible containing the dried sample and transferring it to an electric furnace at 550 °C for 8 h. The crucible containing sample was reweighed and the difference between sample weights indicated the ash content.

$$\text{Ash (\%)} = \frac{\text{Sample weight after oven drying}}{\text{Sample weight before oven drying}} \times 100$$

2.9.Statistical analysis

Using one-way ANOVA, a significant difference was found in the biochemical parameters of specimens treated with different concentrations of *Althaea officinalis* extracts. The data were examined for normality (Kolmogorov-Smirnov test). The significant means were compared by Duncan's test and a $p < 0.05$ was considered statistically significant. Statistical analyses were performed using SPSS IBM, 19. Data are presented as mean ± SD.

3. RESULTS

No significant changes were observed in determining hepato-somatic index (HSI) and intestinal-somatic index in fish fed diets containing *A. officinalis* extract when compared with control group. The contents of the digestive system of fish as satiety index indicated that there was no significant difference between amount of feed fed by experimental fish and control group (Figure 1-2).

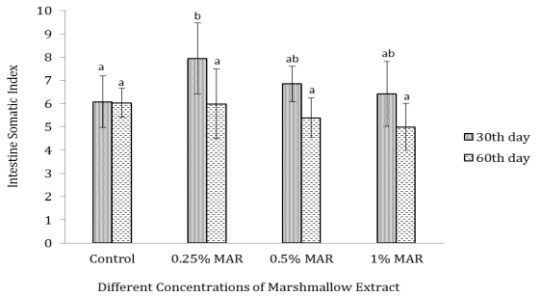

Figure 1. Intestine somatic index of common carp fed with supplemented diet with *Althaea officinalis* extracts. Significant differences between treatment and control groups were represented by alphabets (one-way ANOVA, $p<0.05$). Values represent mean ± S.D.

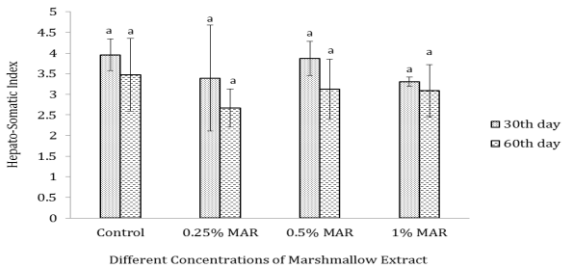

Figure 2. Hepato-somatic index of common carp fed with supplemented diet with *Althaea officinalis* extracts. Significant differences between treatment and control groups were represented by alphabets (one-way ANOVA, $p<0.05$). Values represent mean ± S.D.

No fish died during the experiment. The fish readily accepted all diets and consumed them the first few minutes after being fed, which minimized leaching marshmallow extract from the feed into the water. Details of growth performance of fish fed commercial diets without marshmallow extract (group A) and diets containing marshmallow extract 0.25, 0.50 and 1% (groups B, C and D, respectively) are presented in Table 2. Average live weight of fish fed diets supplemented with marshmallow 0.50 and 1% was significantly lower than control group on day 60

Table 2.
Growth performance of common carp fed with supplemented diet with *Althaea officinalis* extracts

Growth parameters	Treatment	1st day	15th day	30th day	45th day	60th day
Final weight (g)	Control	40.89±3.64^{a}	51.42±8.86^{a}	53.88±6.77^{a}	56.66±9.09^{a}	70.89±7.40^{b}
	0.25% MAR	38.57±3.56^{a}	56.59±11.37^{a}	62.60±10.99^{a}	64.50±13.19^{a}	64.18±11.97^{ab}
	0.5% MAR	38.12±3.12^{a}	58.88±10.38^{a}	58.33±11.67^{a}	58.46±12.95^{a}	56.27±5.53^{a}
	1% MAR	39.21±4.08^{a}	55.08±6.67^{a}	56.17±6.69^{a}	55.99±4.69^{a}	59.03±5.27^{a}
Weight gain (WG%)	Control		25.41±15.73^{a}	33.27±25.30^{a}	38.72±20.52^{a}	74.40±22.65^{b}
	0.25% MAR		48.25±34.83^{a}	64.34±37.86^{b}	66.38±25.84^{b}	68.37±40.23^{ab}
	0.5% MAR		56.12±34.80^{a}	53.48±30.08^{ab}	54.22±35.99^{ab}	48.07±14.72^{a}
	1% MAR		41.39±18.10^{a}	43.84±15.45^{ab}	44.08±18.37^{ab}	51.30±12.94^{ab}
Specific growth rate (SGR%)	Control		1.46±0.82^{a}	0.91±0.59^{a}	0.71±0.32^{a}	0.91±0.21^{b}
	0.25% MAR		2.46±1.54^{a}	1.58±0.73^{b}	1.10±0.36^{b}	0.83±0.36^{ab}
	0.5% MAR		2.82±1.47^{a}	1.37±0.66^{ab}	0.92±0.47^{ab}	0.65±0.16^{a}
	1% MAR		2.26±0.90^{a}	1.20±0.36^{ab}	0.80±0.27^{ab}	0.68±0.14^{a}
Feed conversion ratio (FCR)	Control		1.69±1.07^{a}	3.32±2.32^{b}	3.94±2.30^{b}	2.30±0.60^{a}
	0.25% MAR		1.27±1.17^{a}	1.58±0.91^{a}	2.27±1.31^{a}	3.09±1.31^{ab}
	0.5% MAR		0.85±0.60^{a}	2.01±1.21^{ab}	3.03±1.33^{ab}	3.83±1.01^{b}
	1% MAR		1.17±1.31^{a}	1.93±0.86^{ab}	3.06±0.96^{b}	3.39±0.66^{b}
Condition factor (CF)	Control	1.32±0.27^{a}	1.43±0.20^{a}	1.47±0.24^{a}	1.66±0.19^{a}	1.74±0.33^{a}
	0.25% MAR	1.39±0.15^{a}	1.49±0.12^{a}	1.48±0.14^{a}	1.64±0.14^{a}	1.70±0.11^{a}
	0.5% MAR	1.30±0.13^{a}	1.44±0.12^{a}	1.52±0.16^{a}	1.57±0.16^{a}	1.63±0.19^{a}
	1% MAR	1.38±0.21^{a}	1.39±0.18^{a}	1.48±0.14^{a}	1.64±0.15^{a}	1.66±0.20^{a}

Effects of different concentrations of *Althaea officinalis* extract as supplement (0, 0.25, 0.5 and 1 % per 1 kg food) on growth performance determined in common carp after 15, 30, 45 and 60 days. Effects of different concentrations of *Althaea officinalis* extract on growth index were analyzed using a one-way ANOVA. Significant differences between treatment and control groups were represented by alphabets ($p<0.05$). Values represent mean ± S.D.

A significant increase was observed in the average weight gain of fish fed a diet enriched with marshmallow extract (0.25%) when compared with control group on days 30 and 45. At the end of the experiment, fish fed marshmallow extract (1%) showed the lowest relative weight. Specific growth rate (SGR) significantly increased in the group treated with marshmallow 0.25% when compared with control group on days 30 and 45, whereas, among the supplemented groups no significant difference was observed. At the end of the experiment, a significant decrease was observed in SGR of fish fed a diet supplemented with marshmallow extract 0.50 and 1%. There was no significant difference in condition factor between all groups. Feed conversion ratio (FCR) decreased significantly in marshmallow (0.25%) treated group when compared with control group on days 30 and 45, whereas, in the supplemented groups no significant difference was observed. FCR was significantly higher in fish fed marshmallow extract 0.50 and 1% than in control group. The data of protein

content, moisture, fat and whole body ash are presented in Table 3.

No significant changes were observed in moisture levels in all groups. Although, the controls revealed the highest level of crude protein, there was not any significant difference between each treatment group and the control. Compared to controls, lipid levels for the dried body were the lowest after feeding the fish marshmallow extract (0.50%). In carcass dried fish, the whole body ash was lower after administering marshmallow extract (0.25%) compared with control groups.

Table 3.

Biochemical composition of carcass of common carp fed with supplemented diet with *Althaea officinalis* extracts

Treatments	Biochemical composition of carcass			
	Moisture (%)	Protein (%)	Lipid (%)	Ash (%)
control	66.81±2.73^{a}	62.65±0.71^{a}	26.10±0.29^{bc}	4.53±0.05^{b}
0.25% MAR	67.47±1.42^{a}	59.85±2.79^{a}	26.66±1.23^{c}	3.96±0.27^{a}
0.50% MAR	66.68±7.22^{a}	62.34±4.10^{a}	24.49±1.45^{a}	4.92±0.41^{b}
1% MAR	69.13±2.24^{a}	60.99±2.88^{a}	25.28±0.47^{ab}	4.67±0.42^{b}

Effects of different concentrations of *Althaea officinalis* extract as supplement (0, 0.25, 0.5 and 1 % per 1 kg food) on Biochemical composition of carcass of common carp after 60 days. Effects of different concentrations of *Althaea officinalis* extract on biochemical composition were analyzed using a one-way ANOVA. Significant differences between treatment and control groups were represented by alphabets (p<0.05). Values represent mean ± S.D.

The present study was based on the hypothesis that marshmallow extract has beneficial effects on the growth performance. This hypothesis was tested by feeding common carp diets containing marshmallow extract with different concentrations including 0.0, 0.25, 0.50 and 1%. The carp were fed at the rate of 2% of body weight. In this study, no mortality was recorded in marshmallow supplemented groups throughout the experiment. The results of the present study showed that marshmallow extract supplementation had no effects on feed intake. Platel *et al*. (2002) stated the favorable effects of medicinal plants on digestion and a stimulating effect on bile secretion and the activity of pancreatic enzymes. Moreover, adding plants extracts can affect the fish's food finding ability by stimulating their sense of smell and encouraging them to eat more than normal (Adams, 2005). Some compounds in medicinal plants extracts including bioflavonoides can act as growth stimulators and increase growth rate due to having estrogenic properties (kocour *et al*., 2005). Under these conditions, the addition of marshmallow extract to diet did not significantly increase whole body weight compared with fish fed a normal diet. The results of this study demonstrated that marshmallow extract (0.25%) significantly improved the fish's weight gain, whereas this value of fish fed marshmallow (1%) significantly decreased when compared with control group. The

increased growth in a diet supplemented with marshmallow (0.25%) may be attributed to the influence of marshmallow on improving the nutrient digestibility, increasing the efficiency of nutrient absorption and utilization of feed. Increase in body weight of carp fed with supplemented diet with a mixture of Astragalus root (*Radix astragalin*) and Chinese angelica root (*R. angelicae sinensis*) were reported by Jian and Wu. (2004). Ji *et al.* (2007) observed an increase in weight gain of Japanese flounder (*Paralichthys olivaceus*), fed with herbal derivatives. In contrast, no significant changes were observed in weigh body of tilapia fed diets supplemented with garlic 0.5 and 1% for 4 weeks (Ndong and Fall, 2007). However, Pierce *et al.* (2008) reported decrease in WG in rainbow trout fed with plant meal diet. For the specific growth rate (SGR), the results for marshmallow 0.25% were higher than the corresponding 0.50 and 1% dose. The usefulness of marshmallow may be attributed to its phytochemical substances including vitamin E, antioxidant, flavonoids, essential fatty acids, etc. that may have played an important role in growth enhancement. Similarly, SGR in rainbow trout fed different concentrations of ginger was significantly higher than control group (Nya and Austin, 2009). Increase in SGR was reported in fishes after feeding with prickly chaff-flower (Rao *et al.*, 2006), mango kernel (Sahu *et al.*, 2007) and alfalfa (Olvera-Novoa *et al.*, 1990). The inclusion of marshmallow extract (0.25 %) in diet improved the feed conversion ratio (FCR), although FCR significantly increased for specimens fed marshmallow 0.50 and 1% on day 60. When fish

are fed a diet containing marshmallow extract 0.25%, growth may be increased to reduce feed conversion ratio. Therefore, when both feed conversion ratio and body weight gain were considered for gaining maximum profit, the inclusion of marshmallow extract (0.25 percent) may be recommended. Some compounds in medicinal plants extracts including bioflavonoids can induce effects on growth performance and on the general health of fish (Yilmaz *et al.*, 2006). Similar results are found in cichlid, *Cryptoheros nigrofasciatus*, (Cek *et al.*, 2007b); red seabream, *Pagrus major* (Ji *et al.*, 2007); and rainbow trout (Bohlouli Oskoii *et al.*, 2012) which were fed diets supplemented with medicinal plants extracts. In all groups, condition factor (CF) did not significantly change. CF reflects physiological status and welfare of the fish. Our results showed that administration of marshmallow did not affect the relationship between weight and length of fish. According to results, administration of high concentrations of marshmallow extract (1% per kg feed) had negative impacts on growth performance of common carp. Results of the present study suggest that high levels of marshmallow may have an anti-nutritional factor that decreases the utilization of the given feed. However, using mango seed extract (Sahu *et al.,* 2007), tea extract (Cho *et al.*, 2007), and nutmeg extract, *Myristica fragrans*, (Sivaram *et al.*, 2004) had no effects on varied growth parameters of these fish. The present study showed slight changes in body composition in fish fed a diet supplemented with marshmallow extract compared with controls. Similar results were observed in fish fed with alfalfa (15 and 20%), soybean meal (30 and 60%) and cottonseed meal (30 and 60%), (Ali *et al.*, 2003; Toko *et al.*, 2008). Glencross *et al.* (2004) found an increase in crude protein levels in rainbow trout fed with 12.5% yellow lupin meal. Increased body composition of tilapia was observed after feeding them diets enriched with 5 and 10% alfalfa meal (Ali *et al.*, 2003). In contrast, decreased body moisture, crude protein, crude lipid and ash in rainbow trout fed diets supplemented with hazelnut meal were reported by Bilgin *et al.* (2007).

CONCLUSION

In conclusion, growth performance of common carp was improved following the administration of diets supplemented with marshmallow extract (0.25%). In other words, low concentrations of marshmallow in diet stimulated growth rate in common carp.

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