



- RESEARCH ARTICLE -

The Effect of Leonardite as Feed Additive on Growth of Goldfish

(*Carassius auratus* L.)

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Abstract

This study was conducted to evaluate the effect of Leonardite supplementation on goldfish, *Carassius auratus* growth performance and survival rate. The Goldfish (mean body weight 4.77 ± 0.43 g) were fed with experimental diets prepared with Leonardite supplementation (0, 2.5 and 5 g kg^{-1}) for 55 days. The weight gain (3.633 ± 0.192 g), FCR (0.830 ± 0.04), SGR (1.003 ± 0.077) and PER (4.325 ± 0.228) were observed higher in goldfish fed with 5 g kg^{-1} leonardite than fed with 2.5 g kg^{-1} leonardite and control groups significantly ($P < 0.01$). Survival at the end of the experiment was high (about 84.44-77.77%) and no significant differences were noted among treatment groups ($P > 0.05$). In conclusion, leonardite inclusion in the diet at 5 g kg^{-1} was shown to have a positive effect on goldfish growth. This suggests that leonardite can be considered as a new feed additive, which improves goldfish's growth performance.

Keywords:

Goldfish, *Carassius auratus*, Leonardite, Feed Additive

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Introduction

The organic compounds of humic substances result from the decomposition of dead plants and animals and contain acidic functional groups, besides being important regulators of biogeochemical processes, such as global nutrients and carbon cycle (Wood et al., 2003; Matsuo & Val, 2007). Humic acid could be defined as an organic substance derived from the decomposition of organic matter and having a long molecular chain high in its molecular weight. Humic acid is insoluble in strong acids and has a pH below 2 and it could be soluble in alkaline media. In this substance, the proportion of oxygen represents 33-36%, and nitrogen represents 4% (Islam et al.,

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2005). There are limited numbers of researches such as Abdel-Mageed (2012) and Taklimi et al. (2012), which displayed that Humic acid enhance growth through modifying partitioning of nutrient metabolism. Humic acid has an essential role in animal productivity due to its chemical compositions such as proteins, vitamins, digestive enzyme, water solubility and many other antibacterial substances and immune stimulating agents. Also, Humic acid has the ability to alter the intestinal microflora by increasing the counts of beneficial bacteria (Schepetkin et al., 2003). Besides that, the uses of humic substances have been recorded in farm animals' feeds which can increase growth in goat and broiler (Degirmencioglu, 2014; Kocabagli et al., 2002). Although encouraging findings concerning the use of Humic acid substances as a prophylactic tool for intestinal health have been reported, the inclusion of leonardite as a feed additive to promote growth, has not been extensively investigated. Several studies have revealed discordant findings in vivo probably due to the wide range of doses tested and the wide variability in the composition of humic-based products (Kaevska et al., 2016; Trckova et al., 2015, 2017).

Leonardite is a microbial-derived product mainly composed of humic acids, which are derived from the decomposition of organic matter, usually exploited for the fertilization of soil. Humic acids also protect intestine mucosa, with recognized anti-inflammatory, antiphlogistic, adsorptive and antitoxic proprieties (Islam et al., 2005; Aksu & Bozkurt, 2009). Natural humic substances have shown antioxidant properties that could sustain the animals during the stressful period of weaning. They have also shown antimicrobial activity against pathogens leading to a decreased incidence of diarrhea and better growth performance also modulating the animal's metabolism (Wang et al., 2008; Aeschbacher et al., 2012).

Humic acids have been used for growth stimulation in plants (Chen et al., 2000), farm animals (Kocabagli et al., 2002; Degirmencioglu, 2014;) and fish species (Costa et al., 2016; Yilmaz et al., 2018; Rasidi et al., 2019). However, in fish species, there are limited studies to our knowledge so far reporting improved growth performance by dietary inclusion of humic acids substances/leonardite in fish species. In the present study we intended to ascertain whether leonardite included in the diet enhance the growth performance of the Goldfish.

Material and methods

A sum of 135 Goldfish (*C. auratus*) with mean weight of 4.77 ± 0.43 g were placed into three tanks with 45 goldfish (triplicate for every treatment). This study was conducted with fiberglass tank (420 L) in a 2.1 m length, 0.50 m width and 0.40 m depth. The fiberglass tank was divided into three areas of similar size (0.70 m long, 0.50 m wide, and 0.40 m deep), holding 140 L of water to allow replication. The tanks were outfitted with air circulation and provided with persistently flowing water (2 L min^{-1}), and controlled temperature ($25 \pm 1^\circ\text{C}$). The photoperiod was kept up on a 12-h light: 12-h dark goldfish were fed with carp diets (28% protein). Proximate composition of the trial diet is determined by analysis (AOAC 1990). In the preparation of the experimental diet, leonardite was mixed with a pulverized carp diet in which, water (450 mL kg^{-1}) was added and extruded through a food grinder with a 2 mm diameter die plate (Lee et al., 2004). Two diets containing leonardite with amounts of 2.5 (L2.5) and 5 (L5) g kg^{-1} of diet and a control diet (L0) (without leonardite) were prepared. The control diet was also mixed with 450 ml water. All the groups were fed with their respective diet to ca. 4% body weight day^{-1} twice daily for 55 days. The average water temperature was $25 \pm 1^\circ\text{C}$, and the oxygen content of the water was 4.98 ± 0.36 . At pH

7.8±0.5, the ammonia nitrogen content did not exceed 0.1 mg N-NH₄/l, and nitrite nitrogen was not higher than 0.03 mg N-NO₂/l. During the experiment, the mortality was recorded daily and fish in each aquarium were counted and weighed individually at biweekly intervals after anesthetization for 2.5 min in water that contained 0.4 g L⁻¹ tricaine methanesulphonate (TMS) and 0.8 g L⁻¹ sodium bicarbonate as a buffer.

Growth was monitored to determine the growth in each treatment group during the experiment. Each fish was individually weighed and measured (total length) to the nearest 0.01 g and 0.01 cm, respectively. After 55 days goldfish were collected, weighed and counted refrigerated for further analyses. WG (Weight Gain) (g) = Final weight-Initial weight; SGR (Specific Growth Rate) (%) = $[(\ln W^2 - \ln W^1) / (T^2 - T^1)] \times 100$, where W¹, and W² are mean body weight at times when the first and second samples were taken (T¹ and T²); FCR (Food Conversion Ratio) = Dry feed intake (g) / wet weight gain (g); PER (Protein Efficiency Ratio) = Live body weight gained (g) / protein intake (g), and survival rate (SR%) = (final number of fish collected/initial number of fish stocked × 100) were determined. All information was subjected to a one-way analysis of variance to determine if there is a difference in weight gain and body composition among treatments. Duncan test was utilized to compare the means of the treatments when differences occurred.

Results

The effects of different concentrations of dietary leonardite on growth and survival of on the Goldfish (*C. auratus*) for 58 days are shown in Table 1.

Table 1. The effects of different concentrations of Leonardite on growth and survival of Goldfish (*C. auratus*)^{*}

	Diets ^a		
	0	2.5	5
Initial Weight (g)	4.866±0.233 ^a	4.800±0.305 ^a	4.630±0.296 ^a
Final Weight (g)	5.783±0.212 ^a	7.096±0.204 ^b	8.266±0.261 ^c
Weight gain (g)	0.916±0.440 ^a	2.296±0.336 ^b	3.633±0.192 ^c
SGR	0.299±0.021 ^a	0.680±0.114 ^b	1.003±0.077 ^c
FCR	3.287±0.154 ^b	1.373±0.230 ^a	0.830±0.04 ^a
PER	1.091±0.052 ^a	2.734±0.401 ^b	4.325±0.228 ^c
Survival (%)	82.22±2.22	84.44±2.22	77.77±5.87

^{*}Values (mean ± S.E. of triplicate) with different superscripts in each line indicate significant differences (P<0.01; P<0.001). ^aThree experimental diets were formulated to contain graded levels of Leonardite: 0 (L0), 2.5 (L2.5), 5 (L5) g kg⁻¹ Leonardite.

Survival at the end of the experiment was high (about 84.44-77.77%) and no significant differences were noted among treatment groups (P>0.05). Moreover, there was no adverse influence of leonardite on the health status of the goldfish in the present study. The best weight gain (3.633±0.192 g) of goldfish fed with L5 diet was significantly (P<0.001) higher than that of fish fed with L2.5 diet and control diet (L0) (Table 1). The best FCR (0.830±0.04) and PER

(4.325 ± 0.228) were observed in goldfish fed with L5 diet were significantly ($P < 0.001$) higher than that of goldfish fed with L2.5 diet and goldfish fed with control diet (L0) (Table 1).

Discussion

The findings of the present work have shown that 5 g kg^{-1} of Leonardite based diet has no adverse effect on survival and enhanced the better weight gain in the goldfish. This is a preliminary report indicated that the Leonardite (5 g kg^{-1}) is a positive dietary additive to induce effective technical and economical propagations for goldfish. To best of our knowledge, no work has been reported using Leonardite as feed additive and growth substance in goldfish culture.

Humic substances as natural growth enhancers are used for their antioxidant, antifungal, detoxifying, and antiseptic properties (Rath et al., 2005). The approach of using humates in animal nutrition as an alternative feed additive has gained increasing importance (Ceylan et al., 2003), especially after banning the use of antibiotics in feed as growth enhancers. Humic acid increased the FI and nutrient digestibility by increasing villus length and subsequently when villus length increases the area for absorption of nutrients increases. The nutrients absorption ultimately improves growth performance. It has ability to maintain gut microflora. It provides a protective layer against the penetration of microbes and other toxic substances and stops their entry to the intestine (Taklimi et al., 2012). A study by Arif et al. (2016) evaluated the effect of Humic acid as a growth promoter on broiler and they proved that HA is a good growth promoter and it also improved nutrient digestibility by maintaining gut microbiota. Humic acid substances such as Leonardite have been added to the diet to improve the production performance in various fish species. The effect of humic acid substances on the growth and immune response of the fish has been investigated and summarized in Table 2.

Table 2. Reports of the use of Leonardite and humic acid substances in fish and their effects.

Type of Humic acid	Species	Concentration	Effect	Reference
Humic acid sodium salt	<i>Oncorhynchus mykiss</i>	6 %	Growth performance, haemato-immunological and physiological responses	Yilmaz et al., 2018
Humic acid substances	<i>Rhamdia quelen</i>	10 mg L^{-1}	Growth promoter	Costa et al., 2016
Leonardite and Humic acid substances	<i>Cyprinus carpio</i>	20 g kg^{-1}	Stimulation of immune system of carps and Growth promoter	Yamin et al., 2017

Humic acid	<i>Lates calcarifer</i>	1600 mg kg ⁻¹	Growth promoter, Improves feed digestibility, protein retention and feed efficiency	Rasidi et al., 2019
Fulvic acid substances	<i>Paramisgurnus dabryanus</i>	2 %	Growth performance and intestinal health	Gao et al., 2017
Humic acid substances	<i>Cyprinus carpio</i>	360 mg kg ⁻¹	Improve growth, feed utilization efficiency, more potent destructive effect in the gill, liver tissues of carp	Sharaf & Tag, 2011
Leonardite	<i>Carassius auratus</i>	5 g kg ⁻¹	Better Weight gain	Present study

Consequently, it is suggested that feeding fish with 5 g kg⁻¹ of leonardite for 55 days has no adverse effect on survival and enhanced the better weight gain of the goldfish. The advantages of dietary HA supplementation as a feed additive are promising. In the same way, further studies are needed using different doses of leonardite in those cases where there were positive effects, increasing the time of application, as well as challenging the fish with the main pathogens that invade the gastrointestinal system in order to obtain significant, reliable results.

Conflict of Interest

The authors decelerate that they have no conflict of interests.

Ethical approval

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed by the authors.

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