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RESEARCH ARTICLE

Dietary propolis supplementation on growth and body composition of the African catfish *Clarias gariepinus* (Burchell, 1822)

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Abstract

A preliminary study was conducted to evaluate the effect of dietary propolis extract supplementation on African catfish, *Clarias gariepinus* growth performance, survival rate and body composition. The African catfish (mean body weight 0.45±0.05 g) were fed with experimental diets prepared by using propolis extract supplementation (0, 5 and 10 g kg^{-1}) for 90 days. The weight gain (12.93±0.48 g), FCR (1.39±0.05) and PER (1.44±0.05) were observed higher in catfish fed with 5 g kg^{-1} propolis extract than fed with 10 g kg^{-1} propolis extract significantly ($P<0.05$) but no statistical difference was found with the control group fish ($P>0.05$). Whole-body crude protein was significantly higher in catfish fed with 5 g kg^{-1} propolis extract, than in those fed with control and 10 g kg^{-1} propolis extract ($P<0.05$). Based on the results of the present study, it is suggested that feeding fish with 5 g kg^{-1} of propolis extracts for 90 days has no adverse effect on survival and enhanced the better weight gain and protein in the composition of the African catfish.

Keywords: African catfish, *Clarias gariepinus*, propolis, growth promoter

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Introduction

Aquaculture is a sector that generates revenue based on the application of different technologies for the potential development of high demand aquatic species for human consumption. In the last decade, improvement of aquaculture industry has generally expanded, with the goal that its annual development rate has been more than other industries (FAO 2016). The rapid development of aquaculture system and growing demand of fish leads to the intensification of the culture practices, overdrawing stressors for fish and thus magnifying the risk of diseases. Until now, chemotherapy is the only option for prevention and

treatment of aquaculture disease outbreaks. But the use of chemical drugs has several inherited negative impacts on the environment as well as humans. Many countries have forbidden the use of certain chemotherapeutics and also refuse to import aquaculture products treated with antibiotics and chemicals (Syahidah *et al.* 2015). Consequently, there is an increased need for substituting these chemical compounds with natural products for their application in aquaculture to minimize the risk and side effects that synthetic products may have when used, and attention is given towards eco-friendly and sustainable methods of aquaculture disease management operation in recent years (Mioso *et al.* 2014; Reverter *et al.* 2014).

In aquaculture, natural products or herbal extracts have been reported to have various properties like anti-stress, growth promotion, appetite stimulation, immune system enhancement, broodstock maturation, aphrodisiac and antipathogenic. Furthermore, phytotherapies are cost effective, environment friendly and more eco-friendly than synthetic molecules and are less likely to elicit drug resistance due to the high diversity of plant extract molecules (Citarasu 2010; Chakraborty and Hancz 2011; Olusola *et al.* 2013). There are various reports of use of natural products in aquaculture industry as nutritional supplements (Turan *et al.* 2007; Soltani *et al.* 2017; Acar 2018; Turan 2018), growth promotion (Turan *et al.* 2012; Turan and Yigitarslan 2016) and immunostimulants (Deng *et al.* 2011; Jiang *et al.* 2015; Turan and Gezer 2018) Propolis stands out due to its antiseptic, antimicrobial, antioxidant, anti-inflammatory and bacteriostatic properties (Meurer *et al.* 2009; Yonar *et al.* 2011; Bae *et al.* 2012; Dotta *et al.* 2015).

Propolis is an adhesive, dark yellow to brown colored balsam that smells like resin. It is collected from buds, leaves, and similar parts of trees and plants, such as pine, oak, eucalyptus, poplar, chestnut, etc. by bees, and mixed with wax (Valle 2000). Antioxidative, cytostatic, anti-mutagenic, and immunomodulatory properties of propolis are based on its rich variety of chemical compounds, such as polyphenols (flavonoid aglycones, phenolic acids and their esters, phenolic aldehydes, alcohols, and ketones), sesquiterpene quinones, coumarins, steroids, amino acids, and inorganic compounds (Bankova *et al.* 2000). It is known that flavonoids show antioxidant characteristics for the oxidants in the cell membrane, such as ascorbate (Havsteen 2002). Another compound in the structure of propolis, caffeic acid phenethyl ester, blocks the production of reactive oxygen types (Hosnuter *et al.* 2004). The antimicrobial and antioxidant activity of the compounds of propolis improves intestinal health, digestionand absorption, enhancing growth performance (Denli *et al.* 2005; Seven 2008; Deng *et al.* 2011; Bae *et al.* 2012). Moreover, essential minerals (Fe, Al, Mn) and vitamins (B_1, C, E) present in propolis improve digestive cofactors and enzyme activity, properties that can help the absorption and digestion of nutrients with a subsequent increase in weight in fish (Abd-El-Rhman 2009).

Although previous studies have mostly investigated on the effects of propolis supplement on fish diets (Meurer *et al.* 2009; Abd-El-Rhman 2009; Deng *et al.* 2011; Yonar *et al.* 2011; Jiang *et al.* 2015; Soltani *et al.* 2017; Acar 2018), there is a lack of data on the use of propolis extract in diets for the growth of African catfish, *Clarias gariepinus*. In the present study we intended to ascertain whether propolis extract included in the diet enhance the growth performance and body composition of the African catfish.

Materials and Methods

A sum of 765 African catfish with the mean weight of 0.45±0.05 g were placed in nine glass aquarium (100 L, triplicate for every treatment). The aquaria were outfitted with air circulation and provided with persistently flowing water (2L min^{-1}), and controlled temperature (25±1°C). The photoperiod was kept up as 12-h light: 12-h dark. African catfish were fed with trout diets (Aquamaks, Turkey: 45% protein, 18% lipid). Proximate composition of the trial diet is determined by analysis (AOAC 1990). Propolis extract (Shiffa Home) was supplied by Aksu Vital (Turkey). In the preparation of experimental diet, propolis extract were mixed with a pulverized trout diet in which, water (450 mL kg^{-1}) was added and extruded through a food grinder with a 2 mm diameter diet plate (Lee *et al.* 2004). Two diets containing propolis extract with amounts of 5 (P5) and 10 (P10) g kg^{-1} of diet and a control diet (P0) (without propolis extract) 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 90 days.

The average water temperature was 25±1^{o}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 $N\text{-}NH_4$/l, and nitrite nitrogen was not higher than 0.03 mg $N\text{-}NO_2$/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 groups during the experiment. Each fish was individually weighed and measured (total length) to the nearest 0.01 g and 0.01 cm, respectively. After 90 days catfish were collected, weighed and counted refrigerated for further analyses. WG (Weight Gain) (g) = Final weight-Initial weight; ADG (Average Daily Growth Rate) (g/day) = Growth/Experimental duration; SGR (Specific Growth Rate) (%)= $[(\ln W^2 - \ln W^1) \setminus (T^2 - T^1)] \times 100$, where W^1, and W^2 are mean body weight at times when the first and second samples were taken (T^1 and T^2); 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. In the beginning of investigation, 50 fish

arbitrarily were treated with an overdose of phenoxyethanol (1.5 mg l^{-1}) solution, and stored at -20°C for body proximate composition. Toward the end of the experiment, 10 fish from each dose group (n=30 fish\per dose) were analyzed for final whole body proximate composition (AOAC 1990). 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 were significant.

Results

The effects of different concentrations of dietary propolis extract on growth and survival of on the African catfish for 90 days are shown in Table 1.

Table 1. The effects of different concentrations of dietary propolis on growth and survival of African catfish (*C. gariepinus*)

	Diets[a]		
	0	5	10
Inıtial Weight (g)	0.45±0.05[a]	0.44±0.02[a]	0.45±0.03[a]
Final Weight (g)	12.81±0.19[b]	13.37±0.49[b]	11.19±0.27[a]
Weight gain (g)	12.35±0.14[b]	12.93±0.48[b]	10.74±0.30[a]
SGR	3.72±0.11[a]	3.79±0.07[a]	3.57±1.11[a]
ADG	0.105±0.005[a]	0.101±0.011[a]	0.116±0.005[a]
FCR	1.45±0.01[a]	1.39±0.05[a]	1.68±0.04[b]
PER	1.37±0.01[b]	1.44±0.05[b]	1.19±0.03[a]
Survival (%)	76.08±4.62	81.56±5.01	85.88±3.52

*Values (mean ± S.E. of triplicate) with different superscripts in each line indicate significant differences (P<0.05).

[a]Three experimental diets were formulated to contain graded levels of propolis: 0 (P0), 5 (P5), 10 (P10) g kg^{-1} propolis extract.

Survival at the end of the experiment was high (about 81.56-85.88%) and, the survival of fish fed the P10 diet was higher (85.88%) than that of the other diets (P0 and P5), but no significant differences were noted among other treatments. Moreover, there was no adverse influence of propolis extract on the health status of the catfish in the present study. The best weight gain (12.93±0.48 g) of African catfish fed with P5 diet was significantly (P<0.05) higher than that of fish fed with P10 diet but was not significantly (P>0.05) different from that fish fed with control diet (P0) (Table 1). The dietary inclusion of propolis extract had no significant (P>0.05) impact on specific growth rate (SGR) and average daily growth rate (ADG) of African catfish among the propolis diet (P5 and P10) and control diet (P0). The best FCR (1.39±0.05) and PER (1.44±0.05) were observed in catfish fed with P5 diet were significantly (P<0.05) higher

than that of fish fed with P10 diet but were not significantly (P>0.05) different from that fish fed with control diet (P0) (Table 1).

Whole-body proximate composition of African catfish fed different levels of dietary propolis are shown in Table 2.

Table 2. The effects of different concentrations of dietary propolis on the chemical composition of the whole-body African catfish (*C. gariepinus*) *

Chemical composition (%)	Initial	Diets[a]		
		0	5	10
Moisture	74.41±0.33	74.72±0.81[a]	73.23±0.24[a]	76.03±0.20[b]
Crude protein	18.20±0.84	18.51±0.51[a]	20.72±0.59[b]	18.02±0.29[a]
Crude lipid	6.15±0.17	5.78±1.18[a]	5.49±0.78[a]	5.05±0.08[a]
Ash	1.19±0.02	0.98±0.07[b]	0.56±0.05[a]	0.90±0.06[b]

*Values (mean ± S.E. of triplicate) with different superscripts in each line indicate significant differences (P<0.05).
Body composition data presented on a wet basis.
[a]Three experimental diets were formulated to contain graded levels of propolis: 0 (P0), 5 (P5), 10 (P10) g kg^{-1} propolis extract

Whole-body crude protein was significantly higher in catfish fed P5, than in those fed P0 and P10 (P<0.05). There were no significant differences in whole-body crude lipid among catfish fed P0, P5 and P10 (P>0.05) (Table 2).

Discussion

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

Propolis has been used since 300 B.C. (Ghisalberti 1979) and its use continues to this day due to its multiple biological and various pharmacologic properties (Orsolic *et al.* 2005). In several animal culture, the use of propolis as a dietary supplement has been documented (De la Cruz-Cervantes *et al.* 2018). In lambs, goats, broiler chickens and rabbits propolis increases weight gain and, stimulates rumen microbial growth and increases the digestibility of structural and soluble carbohydrates (Bonomi *et al.* 2002; Junior *et al.* 2004; De la Cruz-Cervantes *et al.* 2018). Products such as propolis have been added to the diet to improve the production performance in various fish species. The effect of propolis on the growth, immune response and whole-body crude protein of the fish has been investigated and summarized in Table 3.

Table 3. Reports of the use of propolis in fish and their effects

Type of Propolis	Species	Concentration	Application	Effect	Reference
Crude propolis Ethanolic extract of propolis	*Oreochromis niloticus*	10 g kg^{-1}	Diet	Increase in lysozyme activity, hematocrit	Abd-El-Rhman 2009
Crude propolis	*Anguilla japonica*	2.5- 5 g kg^{-1}	Diet	Increase of the lysozyme activity in serum and mucus	Bae *et al*. 2012
Ethanolic extract of propolis	*Oreochromis mossambicus*	2 g kg^{-1}	Diet	Growth promoter, immunostimulant	Acar 2018
Propolis extract	*Oreochromis niloticus*	2.74 g kg^{-1}	Diet	Growth promoter	Meurer *et al.* 2009
Propolis extract	*Oncorhynchus mykiss*	4 g kg^{-1}	Diet	Growth promoter, hepatoprotective agent and immunostimulant	Deng *et al*. 2011
Water extract of propolis	*Carassius auratus gibelio*	50 mg mL^{-1}	Intraperitoneal	Stimulating the activity of leukocytes	Chu 2006
Ethanolic extract of propolis	*Oncorhynchus mykiss*	1 and 5 g kg^{-1}	Diet	Growth promoter, immunostimulant	Tukmechi *et al*. 2014
Ethanolic extract of propolis	*Oreochromis niloticus*	9 g kg^{-1}	Diet	Growth promoter, immunostimulant	Hamed and Abdel-Tawwab 2017
Ethanolic extract of propolis	*Oreochromis niloticus*	5 g kg-1	Diet	Better Weight gain and whole-body crude protein of the fish	Wafaa *et al*. 2014
Propolis extract	*C. gariepinus*	5 g kg^{-1}	Diet	Better Weight gain and whole-body crude protein of the fish	Present study

The positive estimations of growth parameters and whole-body crude protein obtained in fish fed with propolis may be because of the expanded enzymatic action in the gut; increasing the digestibility and hence nutrients were utilized properly. Several researchers (Denli *et al.* 2005; Seven 2008; Deng *et al.* 2011; Bae *et al.* 2012) stated that the compounds of propolis improves intestinal health, digestionand absorption, enhancing growth performance. Moreover, Abd-El-Rhman (2009) reported that essential minerals (Fe, Al, Mn) and vitamins (B_1, C, E) present in the propolis improve digestive cofactors and enzyme activity, properties that can help the improved absorption and digestion of nutrients with a subsequent increase in weight in fish. Similar results were found by Wafaa *et al.* (2014) who revealed that the inclusion of 5 g kg^{-1} of ethanolic extract of propolis in the diet for Nile tilapia fingerlings significantly improves weight gain, protein efficiency rate (PER), FCR, while also significantly increasing the percentage of dry matter and protein in the composition of the fish.

Consequently, it is suggested that feeding fish with 5 g kg^{-1} of propolis extracts for 90 days has no adverse effect on survival and enhanced the better weight gain and protein in the composition of the African catfish. In the same way, further studies are needed using different doses of propolis 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. Moreover, future research around there should focus on understanding the physiological systems by which dietary propolis extract enhances development in catfish.

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Karabalıklarda *Clarias gariepinus* (Burchell, 1822) büyüme ve vücut kompozisyonu üzerine propolis diyet desteği

Öz

Bu çalışmada, propolis ekstraktı takviyesinin Karabalıklarda (*Clarias gariepinus*) büyüme performansı, yaşama oranı ve vücut kompozisyonu üzerine etkileri araştırıldı. Araştırmada karabalıklar (ortalama 0.45±0.05 g canlı ağırlık) 90 gün süre ile propolis ekstrakt (0, 5 ve 10 g kg^{-1}) destekli deneme diyetleri ile beslendi. Deneme sonunda 5 g kg^{-1} propolis extraktı ile beslenen gruptaki ağırlık kazancı (12.93±0.48 g), Yem Değerlendirme Oranı (1.39±0.05) ve PER (1.44±0.05) değerleri, 10 g kg^{-1} propolis ekstraktı ile beslenen gruba göre istatistiksel anlamda önemli çıkarken ($P<0.05$), kontrol grubu ile benzerlik göstermiştir ($P>0.05$). Tüm vücut protein oranı 5 g kg^{-1} propolis extraktı ile beslenen grupta, kontrol ve 10 g kg^{-1} propolis extrakt gruplarına göre istatistiksel anlamda daha yüksek çıkmıştır ($P<0.05$). Bu çalışmanın sonuçlarına

dayanarak; karabalıklarda 90 gün boyunca 5 g kg^{-1} propolis extraktı ile beslemenin yaşama oranı üzerinde olumsuz bir etkisinin olmadığı, istatistiksel farklılık olmamakla birlikte daha iyi ağırlık artışı sağladığı ve Karabalıklarda tüm vücut protein oranını önemli derecede artırdığı söylenebilir.

Anahtar kelimeler: Karabalık, *Clarias gariepinus*, propolis, büyüme destekleyici

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