Accessibility the fry of *Mugil cephalus* to the extract of *Camellia sinensis* concerning growth performance and haematology indices

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Abstract

Nowadays, Researchers are looking for approaches to eliminate or minimize the application the antibiotics in aquaculture industry. The purpose of this study was to determine accessibility the fry of *Mugil cephalus* to *Camellia sinensis* concerning the growth performance and haematology parameters. One hundred and fifty fry of *M. cephalus* with an average weight of 2.15 ± 0.08 were collected from Chabahar coastal waters and transferred to the experimental site, under the optimal conditions. This research was based on 3 treatments, one control in triplicates, 0, 50, 100 and 200 ppm of *C. sinensis*. The main index should be measures in this study were included the growth factors containing mean weight, daily growth, and haematological factors, ultimately.

Overall, the results of this study showed that adding 200 mg kg^{-1} of GTE (Green Tea Extract) of diet resulted in a significant increase in final weight and daily growth indices (DGI), especially during the first 4 weeks of fry *M. cephalus* rearing, compared with the control group. Increasing the green tea extract to diet caused no significant increase in the number of RBC compared to the control. The number of white RBC increased in the treatment containing 200 mg kg^{-1} of green tea extract. The highest amount of hemoglobin (Hb) was measured in the group of 200 mg kg^{-1} GTE. The lowest amount of hematocrit (Hct) was observed in the treatment containing 100 mg kg^{-1} GTE and its highest was measured in treatment containing 200 mg kg^{-1} GTE.

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Introduction

Antibiotics are generally used in the diet of fish to prevent and control any bacterial infection. Improper use of antibiotics can lead to adverse effects in aquatic organisms and ultimate consumers, as well as resistance to pathogenic bacteria (Alderman & Hastings 1998; Bansemir, Blume, Schröder & Lindequist 2006). In addition, oral treatment may result in killing or inhibition of the microbial flora of digestive tract, which is useful for fish (Sugita, Shibuya, Shimooka & Deguchi 1996). Therefore, alternative antibiotic strategies have been proposed for fish culture such as the use of probiotics (Kesarcodi-Watson, Miner, Nicolas, Asmani & Robert 2016) and medicinal herbs (Abdel-Tawwab, Ahmad, Seden & Sakr 2010). Today, herbal medicine is commonly used as dry or herbal extracts worldwide, even in industrialized countries that are technologically and scientifically advanced in the use of synthetic drugs, due to the increasing tendency to use medicines and complications from chemical pollution and synthetic medicines, people in those countries now turn to herbal medicine, and nature (Ardó, Yin, Xu, Váradi, Szigeti, Jeney & Jeney 2008). For thousands of years in China, medicinal herbs have been used as immune-stimulants for human being. These plants contain different types of polysaccharides, alkaloids, or flavonoids (Xu, Han, Li, Chen, Wang, Zhao & Chen 2013). Oxidized and non-fermented green tea, which contains various components that have anti-tumor, anti-inflammatory, anti-oxidant, anti-proliferative, anti-bacterial, antiviral, and anti-parasitic properties (Saeed, Naveed, Arif, Kakar, Manzoor, El-Hack, Alagawany, Tiwari, Khandia & Munjal 2017), can be used to increase fish growth and control bacterial infections of *Aeromonas hydrophyla* and possible candidate as a feed additive (Harikrishnan, Balasundaram & Heo 2011). Cathechins and Flavins are also considered as active ingredients in green tea microbiology. Reports of laboratory animals and humans show that taking green tea (without adding sugar) reduces tooth decay. Frequent consumption of green tea can significantly reduce dental caries, even in the presence of sugar. Polyphenols in green tea inhibit the growth of oral and dental bacteria such as *Streptococcus salivarius* and *S. mutans* (Yamamoto & Ogawa 2002). Green Tea extract in doses of 50, 100 and 200 mg kg^{-1} could reduce blood glucose in diabetic animals. Comparing the results at the end of the sixth week showed that the dose of 100 mg kg^{-1} was the most appropriate dose and did not have a significant effect on the reduction of blood glucose levels in the different doses used in this study. It also prevented weight loss due to diabetes (Mehdizade, Hosseini, Ebrahiminia, Elahi, Hosseini, Azizi, Sadeghzade, Ghahfarrokhi & Masoudi 2009). In different fish populations, the immunity study of bony fishes is so crucial due to the great important source of food (John, Chandran, Aruna & Anbarasu 2002). The fish of mullet has long been of human interest, as ancient Romans and Egyptians developed mullet as their own food

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for decades. These fish have tight, fatty and low-bone meat and are very important in most countries. (El-Gayar 2003). This fish is an omnivorous and Iranians are interested in it. *Mugill cephallus* is one of the most valuable species in aquaculture, and due to its high quality, it is the priority of aquaculture in most countries (Yilmaz 2003; Tacon, Metian, Turchini & De Silva 2009). These fish are widely distributed in tropical and subtropical waters and adapted to a wide range of temperatures, salinity and nutritional conditions (Whitfield, Panfili & Durand 2012).

Due to the aforementioned reasons and according to the study of Kakoolaki, Akbary, Zorriehzahra, Salehi, Sepahdari, Afsharnasab, Mehrabi and Jadgal (2016) conducted on accessibility of the fry stage of *M. cephallus* to *C. sinensis*, this species was selected for the study to determine the effect of herbal plant extract of *Camellia sinensis* on haematological and growth factors.

Materials and methods

Fish

Fry fish of *M. cephalus* (Average weight 2.13 ± 0.38 g), One hundred and fifty were naturally caught with the help of coastal fishermen of Chabahar area in south of Iran. Before the experiment would be started, they adapted with the new conditions (temperature 27 ^{o}C, oxygen 6.5 ppm and salinity of 34 ppt) of the rearing tanks for 15 days. Fish were randomly gathered and spread into the 15 rearing tanks with 10 fish per each.

Trial strategy and green tea extract

This study was accomplished in Off-shore Chabahar Research Center for Fisheries Science, Iran. The research was considered as four working groups with three repetitions named control as group 1, groups with 50, 100 and 200 mg L^{-1} of *C. sinensis* presented as treatments 2, 3 and 4. The *C. sinensis* extract was purchased from Barij Essence Pharmaceutical Company, Iran.

The fish were fed to satiation thrice per day at 06:00, 11:00 and 18:00 with Beiza trade fish feed with gross energy level of 4.30 cal g^{-1} during 2 months.

Haematological Analysis

At the end of the experiment, three fishes were randomly selected from each treatment and its control to measure the alteration of the parameters. Haematological factors including White Blood Cell count (WBC), Red Blood Cell count (RBC) and relative factors including Heamatocrit (Hct) and Haemoglobin levels (Hb) were measured. To do this, the fish were first anaesthetized with clove powder at a concentration of 30 mg L^{-1}, and then dried to prevent water and mucus entering the blood sample. Blood was directly taken from the heart. A part of the blood (for counting red and white blood cells) entered microtubes containing 50 µl heparin (an anticoagulant) and another part dispensed in to the non-heparin microtubes and centrifuged at 10000 g for 5-10 min in a Hct centrifuge to measure Hct. Hemoglobin was measure following the approach of cyanomethemoglobin using the method of Akbary, Kakoolaki, Salehi, Sepahdari,

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Mehrabi and Jadgal (2016) and other factors containing mean cell hemoglobin concentration (MCHC), mean cell haemoglobin (MCH) and mean cell volume (MCV) were measured following Dacie and Lewis (Dacie and Lewis 2001).

To calculate the relative parameters of RBC the following formula were used:

MCV (fL) = [(Hct; %) × 10]/(RBC; 10^6 per mm^3)

MCH (pg $cell^{-1}$) = [(Hb; g/=dL) × 10]/(RBC; 10^6 per mm^3)

MCHC %= [(Hb, g/dL) × 100]/ (Hct, %)

Growth parameters

The mid (4-week) and ultimate weights (8-week) of 9 fish from each treatment, control and their repetitions were used to determine the growth factor (Kakoolaki, Akbary, Zorriehzahra, Salehi, Sepahdari, Afsharnasab, Mehrabi & Jadgal 2016). This calculation was accomplished as follows:

WG (g)= (BWf –BWi)/t (Ozorio, Kopecka-Pilarczyk, Peixoto, Lochmann, Santos, Santos, Weber, Calheiros, Ferraz-Arruda & Vaz-Pires 2016). Where, WG, BWf, BWi and t are weight gain, initial and final individual body weights (g) and time of duration as 4 and 8 weeks (for half and end of experiment), respectively. For more information, 3 samples from each tank were taken and got the average value from 9 samples for each treatment and control.

$$DGI\ (\%) = \frac{[(WG \times 100)/(Wi+Wf)/2]}{t}$$ (Abdel-Tawwab et al. 2010).

Where, DGI, Wi and Wf were primary body weight and ultimate body weight means, respectively.

Statistical analysis

Firstly, the Shapiro-Wilk test was used to conclude whether the population was normally distributed. The effect of C. sinensis extract in different concentrations on mean value of growth factors and hematological indices were analyzed with the one-way ANOVA test following the pos-hoc multi-comparison Bonferroni's one. The data analysis was carried out using IBM SPPSS V21.0 software (IBM Cooperation, Chicago, IL, USA) and significant at α=0.05.

Results

The general aspect of growth performance is presented in Table 1. The mean weight of fry, *M. cephalus* at the mid time of the study was shown a significant difference ($p< 0.05$) between treatments of 100 and 200 mg L^{-1} *C. sinensis* supplemented diets compared with control and the group of 50 mg L^{-1} of *C. sinensis.* The greatest weight of *M. cephalus* fry (12.33 ± 0.47 g) belongs to the fish fed with 200 mg L^{-1} of *C. sinensis,* which followed by 100 mg L^{-1} of *C. sinensis.* This pattern was observed at the end of the experiment, so that the maximum mean weight was 26.88 ± 1.48 g and has a significant difference ($p< 0.05$) with that of other groups. Weight gain at the mid time was shown the greatest value in the group of 200 mg L^{-1} of *C. sinensis* with 0.36 ± 0.01. This pattern was repeated at the end of the study so that the weight gain at this time was

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the maximum value (0.44 ± 0.02) in the group of 200 mg L^{-1} of *C. sinensis.* The greatest DGI percent at the first month was 0.04 ± 0.00% with significant difference ($p< 0.05$) compared with the control and 50 mg L^{-1} of *C. sinensis* in the feed of *M. cephalus.* The DGI percent was shown 0.01 ± 0.00 % with no significant difference ($p> 0.05$) compared with the control and the treatments.

Table 1 Mean values of immunological parameters in different concentrations of *C. sinensis* (*n=9*)

	Control	**50 mg L^{-1}**	**100 mg L^{-1}**	**200 mg L^{-1}**	***P* value**
Weight of Mid study (g)	8.00 ± 0.24[a]	8.10 ± 0.24[a]	10.82 ± 0.33[b]	12.33 ± 0.47[c]	0.000
Final weight (g)	18.21 ± 0.57 [a]	19.20 ± 0.48 [a]	22.14 ± 0.52 [b]	26.88 ± 1.48 [c]	0.000
Weight gain 4 weeks (g)	0.20 ± 0.00 [a]	0.21 ± 0.01 [a]	0.30 ± 0.01 [b]	0.36 ± 0.01 [c]	0.000
Weight gain 8 weeks (g)	0.28 ± 0.00 [a]	0.30 ± 0.00 [a]	0.35 ± 0.00 [b]	0.44 ± 0.02 [c]	0.000
DGI mid study %	0.03 ± 0.00 [a]	0.03 ± 0.00 [a]	0.04 ± 0.00 [b]	0.04 ± 0.00 [b]	0.000
DGI final %	0.01 ± 0.00 [a]	0.01 ± 0.00 [a]	0.01 ± 0.00 [a]	0.01 ± 0.00 [a]	0.000

In each row, similar scripts show no significant difference (α= .05) between the groups.

The value and trends of the mean of the haematological indices show in Figure 1. The greatest mean value of RBC was 19.1 ± 1.02 × 10^5 mm^3 calculated for group 200 mg L^{-1} with no significant difference ($p> 0.05$) compared with that of the group 100 mg L^{-1} (Fig 1) but control and 50 mg L^{-1}. This pattern was repeated for WBC so that the greatest value (16.9 ± 2.37 × 10^5 mm^3) was observed in group 4. The lowest Hb was shown 10.3 ± 0.72 in control with no significant difference ($p> 0.05$) with those of the groups 50 and 100 mg L^{-1} of *C. sinensis.* The greatest Hb occurred in the group of 200 mg L^{-1}. Similarly, the maximum values for Hct, MCV, MCH and MCHC were 32.3, 167.8, 69.7 and 41.7, respectively (Fig. 1), which observed in the group of 200 mg L^{-1} of *C. sinensis*.

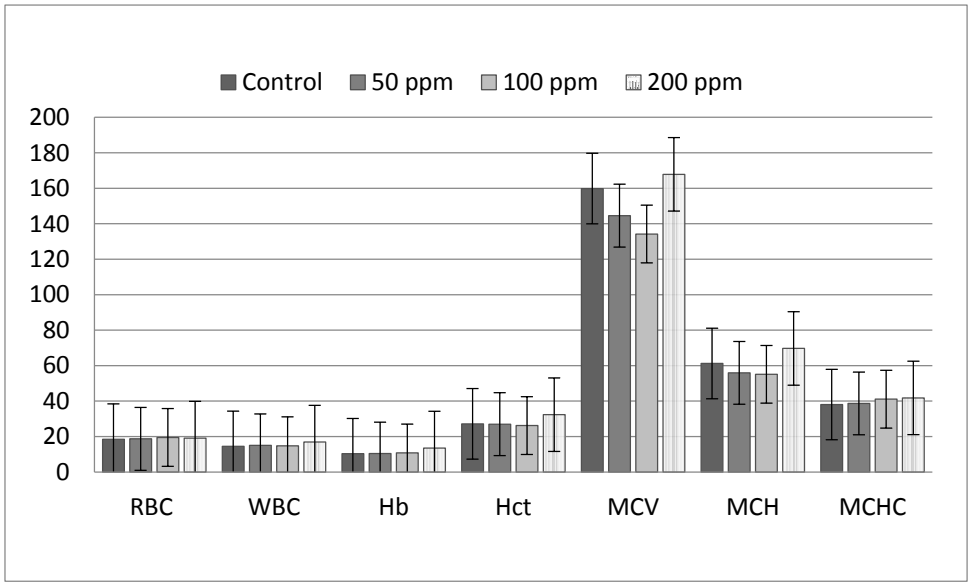


Figure 1 Haematological indices of fry *M. cephalus* fed GTE supplemented diet after 8 weeks of feeding

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Discussion

According to the results presented in table 1, it seems that the diet enriched with 200 mg L^{-1} of *C. sinensis* was more effective to promote weight of fish at mid and end of the experiment. Daily weight gained by fry of *M. cephalus* was the maximum in the group of diet enriched with 200 mg L^{-1} of *C. sinensis.*

The result expressed in these findings showed that the fish fed with 200 mg L^{-1} of *C. sinensis* has more growth compared to other fry *M. cephalus.* The avoidance of soft egg shell was experimented through a study using *C. sinensis* extract. The findings were confirmed that 0.4% of the extract at 30 min with copper ion supplemented diet could effectively inhibit the growth of fungus and incidence of soft egg shell disease in rainbow trout eyed eggs (Hatakeyama, Hatchery & Koide 2009). According to our study, the final weight (g) of the fry *M. cephalus* showed an increasing pattern so that it showed significant difference ($p< 0.05$) between control and 100 or 200 mg L^{-1} supplemented diet. Unlikely, other researchers (Nootash, Sheikhzadeh, Baradaran, Oushani, Maleki Moghadam, Nofouzi, Monfaredan, Aghebati, Zare & Shabanzadeh 2013) emphasized on that the green tea in each concentration has no any effect on final weight of rainbow trout fingerlings. On the other hand, final weight of *Oreochromis niloticus* increased among the different supplemented diets in green tea-concentration dependent manner but only up to 0.5 g kg^{-1} and decreased after this point (Abdel-Tawwab et al. 2010). The result of DGI percent showed the growth of fry *M. cephalus* was greater in first month of the rearing compared to the second month of the experiment. Against to this finding, Kakoolaki et al. (2016) confirmed no significant difference could be find among the groups of fry *M. cephalus* fed with diets supplemented with 50, 100 and 200 mg L^{-1} of green extract and control group. The value of WBC, RBC, Hct and Hb was increased based on dose dependent manner among the fish fed with diets enriched in 50 up to 200 mg L^{-1} of *C. sinensis* extract. Against, MCV showed no significant difference ($p> 0.05$) between the first 2 groups or the second (Kakoolaki et al. 2016). Our results showed that RBC or MCHC values were not significantly difference ($p> 0.05$) but WBC value, which shows the immunity condition of the fish, was greater in fish fed with the diet enriched with 200 mg L^{-1} of *C. sinensis* extract. Hematocrit and Hb addition to MCV and MCV showed the same pattern with WBC.

It is concluded that *C. sinensis* extract apparently induced immunity level at the level of WBC and rate of RBC related indices in fry *M. cephalus* specifically in fish fed with diet supplemented with 200 mg L^{-1} of green tea extract.

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Conflict of interests

The authors declare that there is no conflict of interest.

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تأثیر عصاره متانولی گیاه دارویی چای سبز (*Camellia sinensis*) بر روی عوامل خونی و رشدی ماهی *کفال* (*Mugil cephalus*) جوان

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چکیده

امروزه، محققان به دنبال روش هایی برای از بین بردن یا کم کردن کاربرد آنتی بیوتیک‌ها هستند. هدف از این مطالعه بررسی اثر عصاره چای سبز (GTE) بر رشد و هماتولوژی ماهی کفال بود. یکصد و پنجاه ماهی کفال با میانگین وزنی ۰/۰۸± ۲/۱۵ از سواحل چابهار جمع آوری و به محل آزمایشگاه منتقل شد. این تحقیق بر اساس ۳ تیمار و یک کنترل در سه تکرار انجام شد. عوامل اصلی در این مطالعه عبارت بودند از شاخص های رشد شامل وزن متوسط و رشد روزانه و عوامل خونی. به طور کلی، نتایج این مطالعه نشان داد که اضافه کردن ۲۰۰ میلی گرم در کیلوگرم عصاره چای سبز به رژیم غذایی موجب افزایش معنی‌داری در وزن نهایی و شاخص رشد روزانه (DGI) می‌شود، به خصوص در طی ۴ هفته اول نگهداری ماهی ها، در مقایسه با گروه کنترل افزودن عصاره چای سبز به رژیم غذایی باعث افزایش قابل توجهی در تعداد گلبول‌های قرمز (RBC) نسبت به گروه کنترل نشد. تعداد RBC ها با افزودن ۲۰۰ میلی گرم در کیلوگرم عصاره چای سبز به غذا افزایش یافت. بیشترین میزان هموگلوبین (Hb) در گروه ۲۰۰ میلی‌گرم بر کیلوگرم GTE اندازه گیری شد. کمترین مقدار هماتوکریت (Hct) در درمان حاوی ۱۰۰ میلی گرم بر کیلوگرم GTE مشاهده شد و بیشترین مقدار آن در درمان ۲۰۰ میلی گرم بر کیلوگرم GTE اندازه‌گیری شد.

کلمات کلیدی: چای سبز، ماهی کفال جوان، رشد، هماتولوژی

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