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The Effects of Dietary Administration with High Level Red Pepper (*Capsicum annuum*) on Growth Performance, Coloration, Histology and Protection Against *Aeromonas sobria* in Yellow Tail Cichlid, *Pseudotropheus acei*

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Research Article

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Abstract

The present study was conducted to determine the effects of high level red pepper supplementation to diet on the growth performance, coloration, histology, intestinal microflora and protection against *Aeromonas sobria* in yellow tail cichlid *(Pseudotropheus acei)*. Two isonitrogenous (37% crude protein) and isocaloric (3831 kcall/kg Gross energy) experimental diets were prepared by adding of 15% pepper meal to control diet. The feeding trial was conducted in triplicate for 90 day in aquariums (80 L). At the beginning of the experiment, twenty fish (initial weight 0.06 g) were stocked into each aquarium. The end of the experiment**,** fish fed red pepper supplemented to diets did not have any marked effect on the weight gain, feed conversion ratio, specific growth rate and survival. However, tail of fish fed diet containing red pepper was showed increase significantly in pigmentation. High level red pepper addition to diet caused no pathological findings in any organs. Red pepper didn't observed significant differences for protection to *A. sobria* infection. However, *Aeromonas* and *Pseudomonas* counts in red pepper group were significantly lower than control groups.

Keywords: Pseudotropheusacei, red pepper, growth performance, coloration, histology, disease resistance, *Aeromonas sobria*

Sarı Kuyruk Çiklit balığı, *Pseudotropheus acei*, Yemlerine Yüksek Seviyede Kırmızı Biber İlavesinin Büyüme Performansı, Renklenme, Histoloji ve *Aeromonas sobria*'ya Karşı Korunma Üzerine Etkileri

Özet

Bu çalışma, sarı kuyruk ciklit, *Pseudotropheus acei*, balıklarının yemlerine yüksek seviyede kırmızı biber ilavesinin büyüme performansı, renklenme, histoloji, bağırsak mikroflorası ve *Aeromonas sobria*'ya karşı hastalık direnci üzerine etkilerini belirlemek için yürütülmüştür. Kontrol yemine % 15 oranında biber unu eklenerek İki izonitrojenik (% 37 ham protein) ve izokalorik (3831kcall / kg toplam enerji) deneme yemleri hazırlanmıştır. Besleme denemesi, akvaryumlarda (80 L) üç tekerrür olacak şekilde 90 gün beslenerek yürütülmüştür. Denemenin başında, her akvaryuma yirmi balık (başlangıç ağırlığı 0.06 g) stoklanmıştır. Deneme sonunda,yemlere kırmızı biber ilavesi ile beslenen balıkların ağırlık kazancı, yem dönüşüm oranı, spesifik büyüme oranı ve yaşama oranı üzerine önemli bir etkisi olmamıştır. Ancak, kırmızı biber içeren yemlerle beslenen balıkların kuyruğunda pigmentasyonun önemli derecede arttığı görülmüştür. Yemlere yüksek seviyede kırmızı biber eklenmesi hiçbir organda patolojik bir bulguya neden olmamıştır. Kırmızı biber, *A. sobria* enfeksiyonuna karşı korumada önemli bir farklılık göstermemiştir. Kırmızı biber grubunda *Aeromonas* ve *Pseudomonas* sayıları kontrol gruplarına göre anlamlı derecede düşükt bulunmuştur.

Anahtar kelimeler: Pseudotropheus acei, kırmızı biber, büyüme performansı, renklenme, histoloji, hastalık drenci, *Aeromonas sobria*

INTRODUCTION

Ornamental fish keeping is one of the most popular hobbies in the world today. The growing interest in aquarium fishes has resulted in steady increase in aquarium fish trade globally. The cichlid fish (*Cichlasoma severum* sp., Heckel 1840) is one of the most preferred species in the world. *Pseudotropheus acei*, also known as yellow tail cichlid of Lake Malawi in Africa, is popular among ornamental fish hobbyists, due to its vibrant coloring (Smith, 2000).

Coloration in the aquarium fish is one of major factors which determine the price of fish in the market. In addition, pigmentation of aquatic fish may also directly indicate its healthiness and quality. Fish can't synthesize pigment substances by themselves, and must obtain from their diet. Hence, there is a direct relationship between coloration of fish and carotenoids in the diet (Halten et al.,1997).

Aeromonas species is the most common bacterial disease in freshwater aquarium fish (Lewbart, 2001). Motile Aeromonas Septicemia (MAS) is associated with infections caused by *A. hydrophila, A. sobria, A. veronii, and A. caviae. A. hydrophila* is the predominant causative agent of MAS. These pathogens exist worldwide in fresh and brackish waters and occasionally in salt water and they have a diverse host range. Motile *Aeromonas* spp. is considered as opportunistic pathogens and could easily found in organically rich waters. Thus, stress and poor water quality play a key role in occurrence (Öztürk and Altınok, 2014).

Red pepper is plant sources which are cheap, abundant in market and carotenoids rich. Red pepper contains the xanthophylls, capsanthin and capsorbin which make up the largest pigments percentage (Curl, 1962, Gregory et al., 1987, Rizk and Tolba, 2002). In the study, one of the most promising alternatives fish pigmentation is red pepper (Harpaz and Padowicz 2007; Kop et al., 2010¸ Lee et al., 2010¸ Yılmaz and Ergün 2011).

The aim of this study is to determine the effect of red pepper on growth and coloration, resistance against *A. sobria* and histology in yellow tail cichlid, *P. acei*.

MATERIALS and METHODS

Experimental diets and feeding trial

Experiment diets were prepared by adding of %15 red pepper to control diet (without red pepper). Diet ingredients and nutrient compositions used in the experiment are shown in Table 1. Diets were isonitrogenous (37% crude protein) and isocaloric (3831kcall/kg Gross energy). Red pepper was obtained from local market. Red pepper flour is prepared by drying and grinding at room temperature of red pepper. Flour and other ingredients were ground to a small particle size in a mill. All ingredients homogenous were mixed. Water was added to obtain a 25% moisture level. Diets were passed through a mincer with a 1.5 mm sieve. Then, diets were dried for 24 h in room temperature. After drying, the diets were broken into 1.5 mm granules. All granule diets were stored at +4°C until used. Protein content was determined by the Kjeldahl method, fat by the chloroform-methanol extraction method (Bligh and Dyer, 1956), and ash and moisture by standard methods (AOAC, 1990).

Table 1. Diet ingredients and nutrient compositions of diet used in the experiment

	Control	**15 % red pepper**
Fish meal	35	35
soybean meal	20	20
Wheat flour	35	20
Red pepper	0	15
Oil	7	7
Vitamin [1]	2	2
Mineral [2]	1	1
Chemical analysis		
Crude protein (%)	37.13	37.21
Crude fiber (%)	1.81	2.36
Crude fat (%)	10.91	11.95
Crude ash (%)	9.61	9.76
Total carotenoid	0.00	120.30
Gross Energy (kcal/kg)	3831	3831

[1]Vitamin premix contained the following per kilogram; 4 000 000 IU vitamin A, vitamin D3 480 000 IU, 2400 mg vitamin E, 2400 mg vitamin K3, 4000 mg vitamin B1, 6000 mg vitamin B2, 4000 mg Niacin, 10 000 mg Cal. D. Pantothenate, 4000 vitamin B6, 10 mg vitamin B12, 100 mg D-Biotin, 1200 mg folic acid, 40 000 mg vitamin C, 60 000 mg inositol.[2] Mineral premix contained the following per kilogram; 23 750 mg manganese, 75 000 mg zinc, copper 5000 mg, cobalt 2000 mg, iodine 2750 mg, selenium 100 mg, magnesium 200 000 mg.

Fish and experimental conditions

The feeding trial was conducted in aquarium (80L), for 90 days. Twenty fish (0.06 g weight) were stocked in separate aquarium as 3 replicates for each experimental feed. Experimental groups were fed by hand, *ad libitum* twice daily. The aquariums were cleaned by siphoning out the residual feed and feces. The water temperature ranged between 23 - 25 ^{0}C. The dissolved oxygen rate ranged from 5 to 7 mg L^{-1}.

Colour assessment

Tail and skin colour assessment was performed by minolta chroma meter CR-300 calibrated towards a white standard; L: lightness, 100 L: white, 0 L: black, a: red, -a: green, b: yellow, -b: blue (Sharma, 2003). Skin and tail color was measured under anesthesia (40 mg L clow oil) from fish in each aquarium.

Histopathological examination

All visceral organ specimens were collected and fixed in 10% neutral formalin during the necropsy. After routine processing by automatic tissue processing equipment (Leica ASP300S, Leica Microsystem, Nussloch, Germany), samples embedded in paraffin and 5µm sections were taken by a Leica RM 2155 rotary microtome (Leica Microsystem, Nussloch, Germany). Than sections stained with hematoxylin and eosin (H&E) and examined under the 40X objective of an Olympus CX41 light microscope. Morphometric evaluation and microphotography was performed using the Database Manual Cell Sens Life Science Imaging Software System (Olympus Corporation, Tokyo, Japan).

Bacterial challenge

Pathogen *A. sobria* strain was isolated from in yellow tail cichlid during the previous study. *A. sobria*, was grown for 24 hr at 25°C in Tryptic Soy Agar. After incubation, the cells were harvested by centrifugation (2,000 × *g*), washed with PBS and re-suspended in the same buffer. The number of bacteria was standardized at 600 nm absorbance. After 90 days of feeding, 10 fish (0.4g) from experimental group and control group fish were challenged with *A. sobria* resulting in a dose of 10^7 cfu ml^{-1} by immersion bath at 1 h. Mortality recorded for a period of 15 days. Cumulative mortalities were calculated on the last day of the trial. *A. sobria* were reisolated from the kidney, liver and spleen of freshly dead fish after the mortalities.

Intersinal microfloa

Collections of samples for intestinal microflora determination of yellow tail were done at the end of the experiment. Eight fish randomly collected from each groups were used for total bacteria, *Aeromonas* and *Pseudomonas* counts in fish intestine. Individual fish was scooped out of the holding vessel, euthanized by MS222. The external areas of each fish were then thoroughly disinfected with 70 % ethyl alcohol before the intestine of fish were aseptically dissected and separately homogenized in 9 volumes of sterile peptone water. Each of the homogenates were serially diluted (up to 10^{-7}) and each diluted sample (100 µl) was spread in duplicate on Plate count agar for total bacteria count, *Aeromonas* Medium Base for *Aeromonas* count and *Pseudomonas* medium for *Pseudomonas* count. Then plates were incubated at 30 °C for 48 h for total bacteria count and 25 °C for 48 h for *Aeromonas* and *Pseudomonas* counts. The colonies were counted and the colony forming units (CFU) per gram of intestinal content were calculated.

Statistical analyses

Trial result of fish fed red pepper in diet are presented as mean±SE and subjected to independent-samples t-test for determining significant differences between treatment means (SPSS, 2000).

RESULTS

Growth parameters and FCR displayed no significant differences with addition of red pepper (Table 2). Growth performance and FCR with the addition of red pepper to the diet did not show any significant difference ($P > 0,05$).when compared to the control group (Table 2). No mortality was associated with experimental treatments.

Table 2. Growth performance, FCR, specific growth rate, weight gain and survival rate of *P. acei* fed diets containing red pepper

	Control	Red pepper	P
Initial mean weight (g)	0.064±0.005	0.067±0.004	0.328
Final mean weight (g	0.401±0.002	0.403±0.001	1.00
Weight gain	0.337±0.005	0.336±0.003	0.12
SGR(% day^{-1})	1.627±0.067	1.595±0.049	0.189
FCR	1.80±0.025	1.79±0.007	0.267
Total length	$3.08±0.11^{a}$	$3.38±0.83^{b}$	0.027
Condition factor	1.32±0.05	1.11±0.04	0.123
Survival rate (%)	100	100	

[a-b]: Values in the line having the same superscript are not significantly different (P > 0.05).

Skin and tail color measurements of fish fed diets containing red pepper is shown in Table 3. and figure 1. The end of the experiment, L* (lightness), b* (yellow color) and -b* (blue color) values on tail and skin of fish with addition red pepper to diet did not show any statistically significant difference. The a* (red color) values on tail of fish fed red pepper were significantly higher than those of the control. The a* values only showed statistically significant different on the body coloration of fish.

Figure 1. fish fed with control and pepper.
Left: fish fed with control, right: fish fed with pepper

Table 3. Skin and tail color measurements of fish fed diets containing pepper for 90days.

Tail		Control	Red pepper
	L	72,39±1,41	73,96±3,23
	a	$1,43±0,17^{a}$	$6,26±0,29^{b}$
	b	11,82±0,84	13,05±1,27
	L	54,59±1,69	52,06±2,42
Skin	a	$2,00±0,27^{a}$	$3,61±0,37^{b}$
	b	-2,00±0,32	-3,32±0,77

[a-b]: Values in the line having the same superscript are not significantly different (P > 0.05).

Histopathological examination of the hepatopancreases revealed that while moderate lipidosis observed in control group, pepper addition decreased the lipidosis (Figure. 1). At the histopathological examination of the ovaries, there were no differences observed between the groups (Figure.2). No marked differences were also noticed between the groups. Red pepper addition on fish diet caused no pathological findings in any organs. Only decrease in lipidosis was seen in hepatopancreases of red pepper added group.

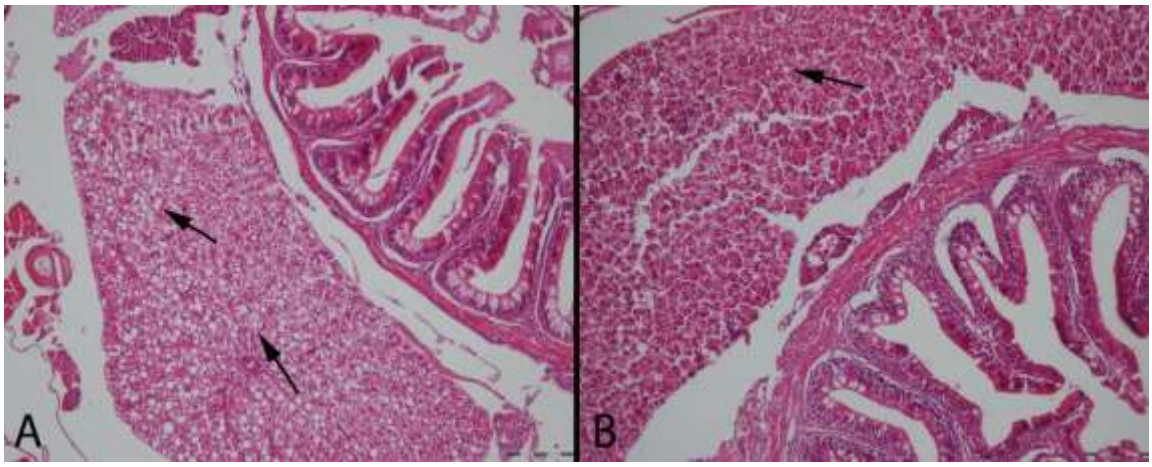

Figure1. Microscopical appearance of the hepatopancreases between the groups. (A) Moderate lipidosis in control group and (B) only slight lipidosis in pepper group. HE, Bars= 100µm.

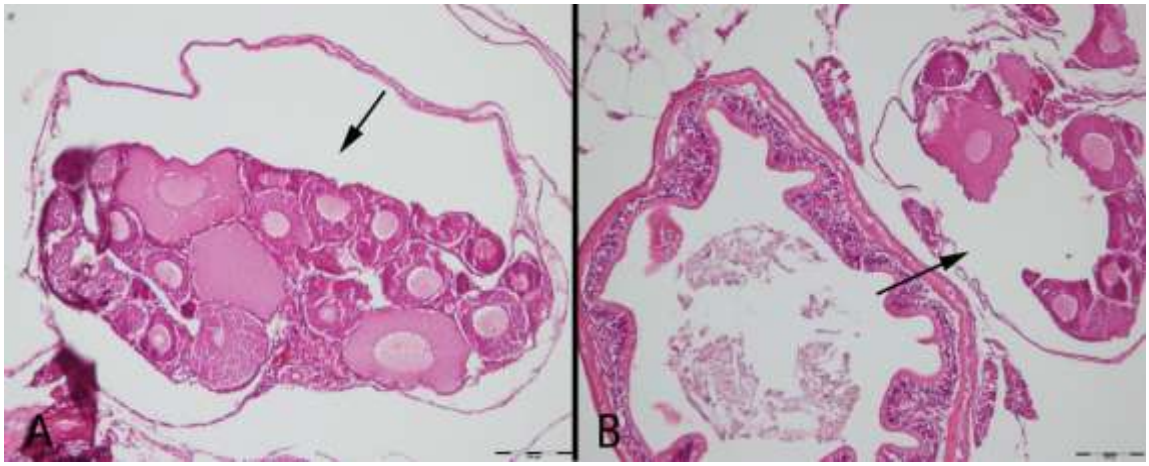

Figure 2. Histopathological appearance of the ovaries (A) control and (B) Pepper group. Similar activity and appearance between the groups (arrows), HE, Bars= 100µm.

Cumulative mortalities after challenging with *A. sobria* were 60 and 55 % in fish fed red pepper and control group, respectively. However, no significant differences (p >0.05) were observed among mortalities in groups.

Total bacteria counts in intestinal microflora were found similar between red pepper and control groups. However, *Aeromonas* and *Pseudomonas* counts in red pepper group were significantly lower than control groups (Table 4).

Table 4. Intestinal microflora in yellow tail cichlid fed with red pepper and control (cfu/gx$10^{5)}$

	Control	**Red pepper**	**P**
Total bacterial counts	0,61±0,01[a]	1,90±0,58[b]	0.04
Aeromonas counts	1,15±0,15[a]	0,25±0,05[b]	0.00
Pseudomonas counts	1,15±0,05[a]	0,15±0,05[b]	0.00

[a-b]: Values in the line having the same superscript are not significantly different (P > 0.05).

DISCUSSION

Addition of red pepper meal to diet of *P. acei* did not affect growth performance and FCR. Similarly, addition of red pepper and carrot to diet of cichlid (*Cichlasomaseverum*) did not exhibit any distinctions in FCR and growth (Kop et al., 2010). Sea bream fed with diet containing 3% red pepper did not affect growth and feed utilization (Wassef et al., 2010). In contrast, growth performance was

significantly improved in *O. mossambicus* fed 1.5% sweet pepper (paprika) extracted from *Capsicum annuum* (Yılmaz et al., 2013). Addition of 3.8% and 7.6% red pepper to diet improved growth performance of rainbow trout (Diler et al., 2005). Büyükçapar et al. (2007) informed that addition level of 6.6% or higher red pepper into the rainbow trout diet had negative effects on growth performance.

Tail color of fish increased as visually with addition of 15 % red pepper to diet in the present study. However, the addition of red peppers to diet affected on red color in body coloration of fish. But, this effect was understood from statistically data detected by device. This red color change was not observed as visually in blue areas of fish body. Similar findings were obtained in studies conducted in different species. Yılmaz and Ergün (2011) informed that 2% and 5% red pepper (*C. annuum*) in diet is an appropriate dietary level to ensure good pigmentation in blue streak hap. Harpaz and Padowicz (2007) noted that addition of 60 mg pepper (capsicum) extract per kg to diet is sufficient to obtain good coloration in dwarf cichlid, *M. ramirezi*. Kop et al. (2010) informed that addition of red pepper and carrot to provide 50 mg (5 %) of total pigments kg^{-1} in diet have an impact on coloration of cichlid (*Cichlasoma severum*) diet. Pale chub fed with diet containing 8% paprika could improve skin pigmentation without any adverse effect on growth performance (Lee et al., 2010). Büyükçapar et al. (2007) showed that most appropriate dietary doses of red pepper for pigmentation of rainbow trout are 4.4%

This study showed that high level red pepper addition to fish diet caused no pathological findings in any organs. So far as we know, effect on organs histopathology of red pepper use in fish diet has not been studied. So far as we know, there is no study effect on organs histopathology of red pepper use for fish. Nwaopar et al., (2008) observed that there was no effect on the kidney of rabbit fed red pepper. Again, Nwaopara et al., (2007) show that the excessive consumption of red pepper in rat can cause necrosis of liver hepatocytes and therefore acute hepatitis.

In the present study, red pepper did not effect on survival rates in yellow tail cichlid after experimental *A. sobria* infection. However, the aqueous *Azadirachta indica* leaf extract has been tested against *A. hydrophila* infection in common carp, *Cyprinus carpio* and the results showed that this plant could effectively control *A. hydrophila* infection in *C. carpio* (Harikrishnan et al., 2003). Similar results were reported in *Labeo rohita* fingerlings fed with *Magnifera indica* (Sahu et al., 2007); in tilapia (*Oreochromis niloticus*) fed with dry leaf powder and ethanol extract of *Psidium guajava* leaf (Pachanawan et al., 2008) and fed with two Chinese medicine herbs (*Astragalus membranaceus* and *Lonicera japonica*) (Ardo et al., 2008); in Victoria Labeo (*Labeo victorianus*) fed with stinging nettle (*Urtica dioica*) (Ngugi, et al., 2015).

In this study can be concluded that high level of red pepper in diet is suitable use for increases coloration in tail of fish without adversely affecting growth and health of *P. acei*. However, red pepper administration did not provide protection against *A. sobria* infection.

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