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The effects of *Isatis tinctoria* extract on pigmentation and growth of *Pseudotropheus acei* and diseases resistance against *Aeromonas hydrophila*[#]

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ABSTRACT

This study was conducted with aim to investigate the effects of *Isatis tinctoria* leaves extract on growth performance, pigmentation and protection against *Aeromonas hydrophila* of *Pseudotropheus acei*. Five diets were prepared by adding four different ratios fish woad extraction (Woad: W1: 1%, W1.5: 1.5 %, W2: 2 %, W2.5: 2.5% and C: control diet 0 %). Three replicates were conducted for each treatment. One hundred twenty fish and 15 tanks (80 x 30 x 80 cm) were used in recirculating system. In the end of 90 days, there were no differences in terms of weight gain, FCR and SGR of *Pseudotropheus acei* feed with diets added with different levels of *Isatis tinctoria* extraction compare to control group. *Isatis tinctoria* extract was not found effect on survival rates in *Pseudotropheus acei* after *A. hydrophila infection*. However, there were no differences on pigmentation of *Pseudotropheus acei*. *Isatis tinctoria* extract administration did not provide protection against *Aeromonas hydrophila* infection.

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Introduction

Ornamental fish culture is a most well-known interest on worldwide recently. One of the most attractive features of ornamental fish is arguably their brilliant display of colors (Kop and Durmaz, 2007). Pigmentation of fish demonstrates its quality and wellness. Especially, colors of male fishes become brighter (Alpbaz and Hossucu, 1996). The lively colors in the fish as well as fish species is one of the considerable determinants of the marketing of fish (Hatlen et al., 1997). Producing colored fish is an important issue for producers (Hekimoglu, 2005). It is known that many types of diet are marketed for the coloration of fish in different countries recently (Brine Shrimp Direct, 2005). The fish can't synthesis color substances by themselves, take had to from their diet (Sefc et al., 2014). There is a strong relation between the color of the fish and the carotenoids in the fish nourishment (Hatlen et al., 1997). Both synthetic and natural carotenes are used in coloration of fish. But, the synthetic carotenoids causes some health problems, there is an increasing demand for the elimination of the use of synthetic carotenes (Ambati et al., 2018; Mohammed and Mohd, 2011). Natural carotenoids are derived from plants while synthetic ones are mainly by products from oil distillation (Ambati et al., 2018; Carbonell et al., 2014). Due to the toxic effect of synthetic carotenoid pigments, there is a tendency to use natural carotenoids instead of synthetic carotenoids (Ambati et al., 2018; Dufosse et al., 2005; Yang et al., 2013). Plant-based b-carotene has been shown to be promising when compared to synthetic carotene in terms of antioxidant properties (Murthy et al., 2005). In addition, synthetic carotenoids added to feeds for increase the feed quality by 20-25% (Yesilayer et al., 2008).

Isatis tinctoria, a natural dye plant, is represent with approximately 50 species (Appel and Al-Shehbaz, 2003), which is a genus of Brassicaceae (Davis, 1965). It is dye plant the oldest known source of indigo (Sales et al., 2006). It produces a substance in its leaves called isatan B which, when exposed to the air, forms blue (Tansı, 1998). It is use not only as a dye plant but also a plant which pharmacological effect (Hamburger, 2002).

The chemical compounds found in the leaves of the *Isatis* species have antibacterial, anticancer, antiviral (Bown, 1995). Its leaves, roots and seeds contain indole-derived compounds which are mainly anti-inflammatory and anti-tumoral (Frechard et al., 2001; Hamburger, 2002; Oberthur et al., 2005).

Cichlids are prevalent freshwater aquarium fish (Alderton, 2008) and among the about 4000 species and varities merchantable around the world. *Pseudotropheus acei* which the yellow tail cichlid, is prominent because of its bright colors (Axelrod et al., 2007). This study was carried out with aim to investigate the effects of *Isatis tinctoria* extract on growth performance, pigmentation and protection against *Aeromonas hydrophila* of *Pseudotropheus acei*.

Materials and Methods

Extraction of Isatis tinctoria Leaves

The plants were supply from the Faculty of Agricultural of Isparta University of Applied Sciences. Rosette leaves were collected from cultivate field and dried. 50 g of the leaves were washed and homogenized by homogenizer. Then, at each stage, 150 ml of ethyl alcohol was filtered by blotting paper and collected in a flask. Ethyl alcohol was removed from the flask by rotary evaporator and the extract was obtained (Baydar and Kineci, 2009).

Experimental Diets

The experimental diets were isonitrogenous (39% crude protein) and isoenergetic (4500 kcal). Five diets were prepared by adding four different ratios of *Isatis tinctoria* leaves extraction (W1: 1%, W1.5: 1.5 %, W2: 2 %, W2.5: 2.5% and C: control diet 0% *Isatis tinctoria* leaves extraction. The composition of the diets is shown in Table 1. The feed ingredients were supplied from a local fish feed manufacturer.

All ingredients were ground into small particles (0.5 mm) in a mill. Dietary ingredients were mixed in a mixer. Micro ingredients were first mixed and then slowly added to the macro ingredients to ensure a homogenous mixture. Water was added to obtain a 30% moisture level. Diets were passed through a mincer with a 1 mm sieve. The pellets were fan-dried and stored frozen at –20°C until used (Dufosse et al., 2005).

Table 1 Ingredients and chemical composition of experimental diets (%)

Ingredients	C	W-1	W-1.5	W-2	W-2.5
Isatis tinctoria	0.00	1.00	1.50	2.00	2.50
Fish meal	35.00	35.00	35.00	35.00	35.00
Soybean meal	30.00	30.00	30.00	30.00	30.00
Corn starch	3.00	2.00	1.50	1.00	0.50
Wheat	20.00	20.00	20.00	20.00	20.00
Fish oil	9.00	9.00	9.00	9.00	9.00
Vitamin mix[1]	1.00	1.00	1.00	1.00	1.00
Mineral mix[2]	1.00	1.00	1.00	1.00	1.00
Pellet binder	1.00	1.00	1.00	1.00	1.00
Chemical composition					
Crude lipid (%)	12.36	12.19	12.19	12.19	12.20
Crude protein (%)	40.55	39.47	39.72	39.97	39.15
Crude ash (%)	10.08	9.72	9.73	9.74	9.75
Energy (kcall/kg	4585	4559	4557	4556	4554
Crude fiber (%)	2.55	2.59	2.59	2.59	2.59
Indigo content	0	0.13	0.20	0.26	0.33

W: Woad Vitamin premix.[1]; per kg, 4,000,000 IU vitamin A, 480,000 IU vitamin D3, 40,000 mg vitamin E, 2400 mg vitamin K3, 4,000 mg vitamin B1, 6,000 mg vitamin B2, 40,000 mg niacin, 10,000 mg calcium D-pantothenate, 4,000 mg vitamin B6, 10 mg vitamin B12, 100 mg D-biotin, 1,200 mg folic acid, 40,000 mg vitamin C and 60,000 mg inositol. Mineral premix.[2]; per kg 23,750 mg Mn, 75,000 mg Zn, 5,000 mg Zn, 2,000 mg Co, 2,750 mg I, 100 mg Se, 200,000 mg Mg.

Rearing Conditions and Design

Fish (*Pseudotropheus acei*) were obtained from aquaculture laboratory of the Egirdir Fisheries Faculty, Isparta University of Applied Science, Turkey. Eight fish (mean weight 2.5g) were randomly stocked to each tank. Three replicates were conducted for each treatment. One hundred twenty fish and 15 tanks were used in total. Recirculating aquaculture system composed from 15 tanks (80 × 30 × 80 cm) was used for the experiment. Shelters were placed to the tanks and the water was well-aerated and filtered. The water temperature was maintained at a mean of 27±1°C. The tanks were climate with aquarium thermostat heaters. The dissolved oxygen rate ranged from 5 to 7 mg/L^{-1}. The experimental groups were fed by hand, *ad libitum*, twice daily (8:30 and 20:30). The fish were fed experiment diets for 90 days. The tanks were cleaned twice a week, and the residual feed and feces were siphoned out.

Measurement of Fish Pigmentation

Pigmentation parameters of fish were measured by Minolta CR-300 Chroma Meter (Minolta Camera Co. Ltd., Asaka, Japan) on tail and body after fish were put to sleep with anesthetic. The Chroma Meter was set to take absolute measurements in the $L^* a^* b^*$ measuring mode (CIE 1976) using D65 illuminant. L^* is the lightness variable (where white = 100 L^* and black = 0 L^*), a^* is the red chromaticity coordinates where a^* stands for red, and $-a^*$ stands for green, and b^* is the yellow chromaticity coordinates where b^* stands for yellow, and $-b^*$ stands for blue (Kara and Baydar, 2013).

Bacterial Challenge

After 90 days of feeding, a challenge test was performed on each experimental group with *A. hydrophila*. The pathogen was grown for 24 hours at 25°C in Tryptic

Soy Agar. The number of bacteria was standardized at 600 nm absorbance. The challenge test was done in 3 replicates where 20 fish from each group (10 fish/replicate). Fish were infected with *A. hydrophila* resulting in a dose 3.3×0^6 cfu ml^{-1} (LD_{60} dose) by immersion bath at 30 min. The relative percent survival (RPS) was calculated according to Amend (1981).

$$RPS=\frac{(1\text{-}\%\ \text{mortality in experiment group})}{\%\ \text{mortality in control}}\times 100$$

All the data were analyzed with analysis of variance (ANOVA) using SAS Statistical Package Program. Means were compared using the LSD (Least Significant Difference) test.

Results

Growth parameters

The results on growth performance of *P. acei* were given in Table 2. There was significant differences in final mean weight between control and 1.5 % groups (P<0.05). However, no statistical difference was found between the groups in terms of weight gain, specific growth rate and FCR values (P>0.05)

Pigmentation

Body pigmentation of *Pseudotropheus acei*, which have blue color (-b), fed diets added *Isatis tinctoria* extract no difference compared to the control (P>0.05). Similarly, also in pigmentation of tail (+b: yellow) were no determined differences in between groups (P>0.05).

Challenge Test

As a result of challenge tests, dietary administration of %1 *Isatis tinctoria* leaves extraction reduced fish mortality and showed better RPS (33%) than other groups. However, the mortality rate in other *Isatis tinctoria* extraction groups was similar to control (Table 4).

Discussion

In the present study, there were no differences in terms of growth performance, FCR and of *Pseudotropheus acei* fed with diets added with different levels of *Isatis tinctoria* extraction compare to control group. There are a few studies on effects of *Isatis* plant on fish. Song et al. (2018) reported that *Takifugu obscurus* fish fed with diet supplemented *Isatis* root polysaccharide (2.0%) showed better final weight and feed conversion ratio than the control treatment. Li et al. (2011) reported that weight gain and SGR increased in grass carp (*Ctenopharyngodon idellus*) fed with the diets (1, 2 and 4 %) including mixture of *Astragalus membranaceus, Allium sativum, Dendranthema morifolium, Frutellaria crataegi and Isatis indigotica*. But, in the present study is different from the previous studies in terms of extraction of *Isatis* leaves. In the present study, *Isatis tinctoria extract* did not affect on survival rates in *Pseudotropheus acei* after experimental *A. hydrophila* infection. However, Yuan et al. (2007) reported that the herbal plants mixture including *Isatis tinctoria* extract were modulate some immune parameters (phagocytosis, respiratory burst activity) in carp, *Cyprinus carpio.* Similarly, Li et al. (2011) indicated that resistance against to *A. hydrophila* of grass carp fed with Chinese herbal mixture, containing *Isatis indigotica.* In another studies, *Euphorbia hirta* leaf extract (Pratheepa and Sukumaran, 2014), *Lawsonia inermis* methanol extract (Soltanian and Fereidouni, 2016) provided protection against *A. hydrophila* infection in carp. 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), fed with two Chinese medicine herbs (*Astragalus membranaceus* and *Lonicera japonica*) (Ardo et al., 2008) and fed with *Solanum trilobatum (*Divyagnaneswari et al., 2008); in victoria labeo (*Labeo victorianus*) fed with *Urtica dioica* (Ngugi, et al., 2015).

Table 2 Growth performance of *P. acei* (Mean ± SE)

Growth performance	Control	W1	W1.5	W2	W2.5
Initial mean weight (g)	2.19±0.02	2.16±0.03	2.14±0.03	2.14±0.05	2.07±0.06
Final mean weight (g)	3.48±0.06	3.07±0.10	2.81±0.27	2.95±0.13	2.95±0.28
Weight gain (g)	1.29±0.07	0.90±0.11	0.67±0.27	0.82±0.14	0.87±0.33
Specific growth rate (SGR; %)	0.53±0.03	0.39±0.04	0.30±0.11	0.37±0.06	0.39±0.14
Feed Conversion Ratio (FCR)	3.29±0.21	4.65±0.75	3.84±0.82	3.58±0.35	4.14±0.51

In the same line, values with different superscript letters are significantly different (P<0.05), W: Woad

Table 3 Tail and body pigmentation of *P. acei* (Mean ± SE)

		Control	W1	W1.5	W2	W2.5
Body	*L*	51.00±1.84	53.27±1.12	50.02±1.51	54.69±1.59	50.03±1.63
	a	-1.06±0.11	-1.16±0.16	-1.04±0.22	-0.75±0.15	-0.97±0.19
	b	-2.88±0.41	-2.48±0.34	-3.26±0.46	-2.22±0.45	-2.90±0.44
Tail	*L*	71.91±3.11	73.95±0.88	73.51±1.48	75.81±0.95	76.15±0.88
	a	2.10±0.33	2.18±0.31	2.82±0.30	2.65±0.36	3.03±0.32
	b	20.22±1.59	22.69±1.76	23.30±1.24	23.14±1.26	21.28±1.47

L= 100 white; L= 0 black; a = red; -a= green; b= yellow; -b= blue.

Table 4 Resistance of *P. acei* fed with different concentrations of *Isatis tinctoria* extraction to *A. hydrophila*

	Control	W1	W1.5	W2	W2.5
Fish number	20	20	20	20	20
Dead fish	12	8	11	10	11
Mortality (%)	60	40	55	50	55
RPS	-	33	8.33	16.66	8.33

In the present study, there was no differences pigmentation of *Pseudotropheus acei* fed with diets added with different levels of *Isatis tinctoria* exract compare to control group. There were no studies related to use of *Isatis tinctoria* plant in fish pigmentation.

In conclusion, *Isatis tinctoria* extract administration did not provide protection against *Aeromonas hydrophila* infection. The addition to diets of *Isatis tinctoria* exract was not effected on growth and pigmentation of *Pseudotropheus acei.*

References

Alderton D. 2008. Encyclopedia of Aquarium and Pond Fish. DK Publ., New York. 400 pp.

Alpbaz A, Hossucu H. 1996. Inland Aquaculture. Ege University Faculty of Fisheries, İzmir. 221pp.

Ambati RR, Gogisetty D, Aswathanarayana RG, Ravi S, Bikkina PN, Bo L, Yuepeng S. 2018. Industrial potential of carotenoid pigments from microalgae: Current trends and future prospects. Critical reviews in food science and nutrition 59:1-22. DOI: https://doi.org/10.1080/10408398.2018.1432561.

Amend DF. 1981. Potency testing of fish vaccines. Fish Biologics: Serodiagnostics and Vaccines 447-454.

Appel O, Al-Shehbaz IA. 2003. Cruciferae. in Flowering Plants· Dicotyledons Springer, TFH Publ. Berlin, USA, pp. 75-174.

Ardó L, Yin G, Xu P, Váradi L, Szigeti G, Jeney Z, Jeney G. 2008. Chinese herbs (*Astragalus membranaceus* and *Lonicera japonica*) and boron enhance the non-specific immune response of *Nile tilapia* (*Oreochromis niloticus*) and resistance against *Aeromonas hydrophila*. Aquaculture 275(1-4): 26-33.

Axelrod GS, Axelrod HR, Burgess WE. 2007. Dr. Axelrod's Atlas of Freshwater Aquarium Fishes. TFH Publ., USA. 1160 pp.

Baydar H, Kineci S. 2009. Scent composition of essential oil, concrete, absolute and hydrosol from lavandin (*Lavandula* x *intermedia* Emeric ex Loisel.). Journal of Essential Oil Bearing Plants 12(2): 131-136.

Bown D. 1995. Encyclopaedia of herbs and their uses. Dorling Kindersley, London.

Brine Shrimp Direct. 2005. Natural Rose: Natural Astaxanthin as a Pigment Source for Ornamental Fish and Animals.

Carbonell CJM, Buniowska M, Barba FJ, Esteve MJ, Frígola A. 2014. Analytical methods for determining bioavailability and bioaccessibility of bioactive compounds from fruits and vegetables: A Review. Comprehensive Reviews in Food Science and Food Safety 13(2): 155-171. DOI: https://doi.org/10.1111/1541-4337.12049.

Davis PH. 1965. Cruciferae. In: Davis PH (ed.) Flora of Turkey and the East Aegean Islands,. Edinburgh: Edinburgh University Press pp: 1-567

Divyagnaneswari M, Christybapita D, Michael RD. 2008. Immunomodulatory activity of Solanum trilobatum leaf extracts in Oreochromis mossambicus. In *Diseases in Asian Aquaculture VI, Fish Health Section* (pp. 221-234). Asian Fisheries Society, Manila, Philippines.

Dufosse L, Galaup PA, Yaron SM, Arad P, Blanc KN, Chidambaram MGA. 2005. Microorganisms and microalgae as sources of pigments for food use: a scientific oddity or an industrial reality? Trends in Food Science and Technology 16 (9): 389–406. DOI: 10.1016/j.tifs.2005.02.006.

Frechard A, Fabre N, Pean C, Montaut S, Favvel M, Rollin P, Fauraste L. 2001. Novel indole-type glucosinolates from woad (*Isatis tinctoria* L.). Tetrahedron Letters 42(51): 9015-9017. DOI: https://doi.org/10.1016/S0040-4039(01)02015-9.

Hamburger M. 2002. *Isatis tinctoria*–From the rediscovery of an ancient medicinal plant towards a novel anti-inflammatory phytopharmaceutical. Phytochemistry Reviews 1(3): 333. DOI: https://doi.org/10.1023/A:1026095608691.

Hatlen B, Arnmesan A, Jobling M, Bjerkeng B. 1997. Carotenoid pigmentation in relation to feed intake, growth and social integration in Arctic char, *Salvelinus alpinus* (L.), from two anadromous strains. Aquacult. Nutr., 3: 189-199. DOI: https://doi.org/10.1046/j.1365-2095.1997.00087.x.

Hekimoglu MA. 2005. A study on growing and coloring of gold fish in colored tanks. Ege Uni. Journal of Fisheries and Aquatic Sciences 22(1):137-141.

Kara N, Baydar H. 2013. Determination of Lavender and Lavandin Cultivars (*Lavandula sp*.) Containing High Quality Essential Oil in Isparta, Turkey. Turkish Journal of Field Crops 18 (1): 58-65.

Kop A, Durmaz Y. 2008. The effect of synthetic and natural pigments on the colour of the cichlids (*Cichlasoma severum* sp., Heckel 1840). Aquaculture International 16(2): 117-122. DOI: https://doi.org/10.1007/s10499-007-9130-1.

Li C, Qz Z, Yang YY, Zhu CK, Li CT, Chen X, Luo F. 2011. Effect of different doses of compound Chinese herbal immunostimulant in feed on the growth and immune function of grass carp (*Ctenopharyngodon idellus*). J Shanghai Ocean University 20: 534-540.

Mohammed MK, Mohd MK. 2011. Production of carotenoids (antioxidants/ colourant) in Spirulina platensis in response to indole acetic acid (IAA). International Journal of Engineering Science and Technology 3: 1-7.

Murthy KC, Vanitha A, Rajesha J, Swamy MM, Sowmya PR, Ravishankar GA. 2005. In vivo antioxidant activity of carotenoids from *Dunaliella salina* a green microalga. Life Sciences 76(12):1381-1390.DOI: https://doi.org/10.116/j.lfs.2004.10.015.

Ngugi CC, Oyoo-Okoth E, Mugo-Bundi J, Orina PS, Chemoiwa EJ, Aloo PA. 2015. Effects of dietary administration of stinging nettle (*Urtica dioica*) on the growth performance, biochemical, hematological and immunological parameters in juvenile and adult victoria labeo (*Labeo victorianus*) challenged with *Aeromonas hydrophila*. Fish & Shellfish Immunology 44(2): 533-541.

Oberthur C, Jaggi R, Hamburger M. 2005. HPLC based activity profiling for 5-lipoxygenase inhibitory activity in *Isatis tinctoria* leaf extracts. Fitoterapia 76 (3): 324-332. DOI: https://doi.org/10.1016/j.fitote.2005.03.002.

Pachanawan A, Phumkhachorn P, Rattanachaikunsopon P. 2008. Potential of Psidium guajava supplemented fish diets in controlling Aeromonas hydrophila infection in tilapia (*Oreochromis niloticus*). Journal of Bioscience and Bioengineering 106(5): 419-424.

Pratheepa V, Sukumaran N. 2014. Effect of *Euphorbia hirta* plant leaf extract on immunostimulant response of *Aeromonas hydrophila* infected Cyprinus carpio. Peer J 2: 671. DOI: https://doi.org/10.7717/peerj.671.

Sahu S, Das BK, Pradhan J, Mohapatra BC, Mishra BK, Sarangi N. 2007. Effect of *Magnifera indica* kernel as a feed additive on immunity and resistance to Aeromonas hydrophila in Labeo rohita fingerlings. Fish & Shellfish Immunology 23(1):109-118. DOI: https://doi.org/10.1016/j.fsi.2006.09.009.

Sales E, Kanhonou R, Baixauli C, Giner A, Cooke D, Gilbert K, Ros R. 2006. Sowing date, transplanting, plant density and nitrogen fertilization affect indigo production from *Isatis* species in a Mediterranean region of Spain. Indust. Crops and Product 23(1): 29-39.

Sefc KM, Brown AC, Clotfelter ED. 2014. Carotenoid-based coloration in cichlid fishes. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology 173:42-51. DOI: https://doi.org/10.1016/j.cbpa.2014.03.006.

Soltanian S, Fereidouni MS. 2016. Effect of Henna (*Lawsonia inermis*) extract on the immunity and survival of common carp, Cyprinus carpio infected with *Aeromonas hydrophila*. International Aquatic Research 8(3): 247-261. DOI: https://doi.org/10.1007/s40071-016-0141-2.

Song L, Guo Z, Wang A. 2018. Effects of *Isatis* root polysaccharide on nonspecific immune responses and nutritive indices in obscure pufferfish, *Takifugu obscurus*. Aquaculture Research 49(2): 603-613.

Tansi S. 1998. The research on plant performance of woad (*Isatis tinctoria* L) under the Cukurova conditions. Journal of Field Crops Central Research Institute 7(1):38-43.

Yang Y, Kim B, Lee JY. 2013. Astaxanthin structure, metabolism, and health benefits. J. Hum. Nutr. Food Sci 1:1-11.

Yesilayer N, Dogan G, Erdem M. 2008. Use of natural carotenoid sources in fish feed. Journal of FisheriesSciences 2(3): 241-251. DOI: 10.3153/jfscom.mug.200709.

Yuan C, Li D, Chen W, Sun F, Wu G, Gong Y, Tang J, Shen M, Han X. 2007. Administration of a herbal immunoregulation mixture enhances some immune parameters in carp (*Cyprinus carpuio*). Fish Physiol. Biochem 33: 93-101. DOI 10.1007/s10695-006-9120-7.