



## Effect of Red Beet Juice (*Beta vulgaris*) on Pigmentation of Fillet and Growth Performance of Rainbow trout (*Oncorhynchus mykiss*)

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**ABSTRACT:** The limitation in the use of synthetic pigments in food industries and aquaculture due to the related health issues is one of the main reasons to substitute natural pigments as food additives. Hence, a study was conducted to evaluate the effect of red beet juice (*Beta vulgaris* L.) as a natural pigment additive on fillet pigmentation and growth performance of rainbow trout. Four practical diets containing 0 (control basal diet), 2, 4 and 6% red beet juice were formulated and fed to triplicate groups of fish. 480 rainbow trout ( $50 \pm 4$  g) were random distributed between 12 fiber glass tanks of 500 L capacity. During a 60 days, fishes received 4 and 6% red beet juice in diet, were affected by higher pigmentation compared to other treatments ( $P < 0.05$ ). However, the rate of the growth of fish group fed with 6% red beet juice were negatively affected significantly. The results indicated that the inclusion of 2 and 4% red beet juice was more reliable for suitable coloration and growth in which 4% were found the most reliable amongst studied ratios.

**Keywords:** Growth Performance, Pigmentation, Rainbow trout, Red beet juice (*Beta vulgaris* L.).

### INTRODUCTION

Most aquatic organisms in confined area have received less pigmentation due to limitation of natural pigment. Therefore, inclusion of pigmentation sources in their diet coloration is needed (Goldman and Navazio, 2003).

Food scientists have mostly focused on the utilization of natural pigments such as red beet, Swiss chard and yellow beet in food industries (Strack *et al.*, 2003). Amongst different species, red beet has less sugar and carbohydrate and also sources of vitamin B, C and act as antioxidant in comparison with sweet beet (Adams, *et al.*, 1976). One of the most interesting characteristics of red beet is its pH stability that ranges at lower than 7 or neutral (Stintzing *et al.*, 2002).

One of the key factors for market acceptability of rainbow trout is color appearance of fillet. Pigmentation can be produced from synthetic carotenoid sources (astaxanthin) that are commonly used for pigmentation of salmonids (Barboso, *et al.*, 1999). There are different sources responsible for typical color of salmonid skin and fillet for example, synthetic astaxanthin, natural red pepper and marigold flower (Buyukcapar, 2005). But due to health concern about the use of synthetic pigments yet alternative natural sources like red beet must be studied. Red beet root is major commercially exploit

betalain crop and it is approved by European Union E162 (Sinclair, 1998).

Color changes hue and their pattern for suitable adaptation of aquatic animals to their surrounding environments are crucial point, the changes subjected to variation of pigment quantity while a fast color change is related to hormone regulation (Boeuf and Le Bail 1999, Oshima 2001). There is lacuna of using red beet for pigmentation of fish. The present study was design to assess the relative pigmentation efficiency of red beet on rainbow trout and also its effect on growth performances.

### MATERIAL AND METHODS

#### A. Fish maintenance

Rainbow trouts were obtained from Dornab rainbow farming (Guilan, Iran) and brought to wet -laboratory of Faculty of Natural Resources (Guilan university, Iran). Initially, all fishes were stocked in one -ton tank for 48 hr. without feeding. Prior to experiment, 480 young fishes with initial average weight of  $50 \pm 4$ g were randomly stock in 12, 500 L circular fiber glass tanks. Fishes were fed at satiation three times a day (8.00, 13.00 and 17.00). The tanks were supplied by constant water flow (ground water) and aeration. Water quality such as temperature, DO<sub>2</sub>, PH and ammonia were  $17.2 \pm 1.8^\circ\text{C}$ ,  $8.9 \pm 0.64$  mg/l,  $7.4 \pm 0.14$  and  $<0/01$  mg/l which maintained through experiment.

### B. Diet design

Food was supplied by Biomar (France). Initially, a total 500 g of red beetroots were washed, hand-peeled and cutted to small pieces. Pieces of red beet were cooked for juice extract, then the extracted juices were sprayed on practical basal diet in proper proportion.

### C. Chemical analysis

Chemical composition of red bead and practical diet are presented in Table 1.

Their composition were determined according to the procedure prescribed by A.o.A.C. (1995) at nutritional lab of faculty of natural resources. Dry matter was determined by drying sample to constant weigh at 105°C in over dryer, Nitrogen was measured by the semi-kjeldahl method and crude protein value calculated from nitrogen (N) multiplied by Soxhlet method through ether extraction for 8 hrs and total ash content was determine by combustion at 550 °C at muffle furnace for 8 hrs.

**Table 1: Chemical composition of red beet and practical diet on dry matter basic (±SD).**

Composition(%)	Chemical	
	Red beet	Practical basal
Crude Protein	3.3±0.45	40.3±1.2
Lipid	1.1±0.11	18.3±2.1
Fiber	19.8±1.3	2.3±0.13
Total Ash	4.6±0.81	8.2±0.69
N.F.E	15.7±0.99	11.3±1.4

### D. Color analysis

At end of trial, three fish from each tank (9 from each treatment) were randomly selected and anesthetized by ms222 (120 mg/ml) for xerography purpose. Analysis of digital pictures of fillet surface sample are possible by L\*, a\* and b\* Hunter color parameters and color spectrum pattern (Hunt, R. w. cr, 1991). For better description, we used Adobe Photoshop with transformation in to color parameters base on the L\*, a\* and b\* values (Quantities analysis). Each fish (samples) from treatment was photographed in 9 different fillet location (Picture). The value, represent by L\*, ranged from pure black (0) to pure while (100), a\* from red (indicates a<sup>+</sup>) to green (indicated a<sup>-</sup>) and b\* indicated from yellow (b<sup>+</sup>) to blue (b<sup>-</sup>) for use measurement of light intensity (Cai, 2005).

### E. Growth performances

Fish in each tank were batch weight every week, and weight.

For the growth performances the following parameters were estimated:

1. Weight gain (WG) = final weight (g) – initial weight (g)

2. Food conversation ratio (FCR) =  $\frac{\text{dryfoodoffered (g)}}{\text{wet weight gain (g)}}$

3. Protein efficiency ratio (PER) –  $\frac{\text{weight gain (g)}}{\text{protein consumed (g)}}$

4. Survival rate =  $\frac{\text{final no.of fishes}}{\text{initial no.of fishes}} \times 100$

### F. Statistical analysis

Raw data were subjected to a One-way analysis of variance (ANOVA) to test for statistical difference between treatments Duncan multiple range test and critical ranges were used to test differences among the individual means. The differences were regarded as significant (P<0.05).

## RESULTS

### A. Effect of studied diets on growth performance

The effect of different red beet juice percentages in studied fish diets on growth performances were presented in Table 2. From the results, the highest weight gain protein efficiency ratio and survival with lower food conversion ratio were obtained using 4% red beet in diet (p< 0.05). However, further increase in red beet to 6% level, decreased the performances significantly compared to other treatments.

Different letters in the same column are significantly different (p<0.05) mean ± SEM.

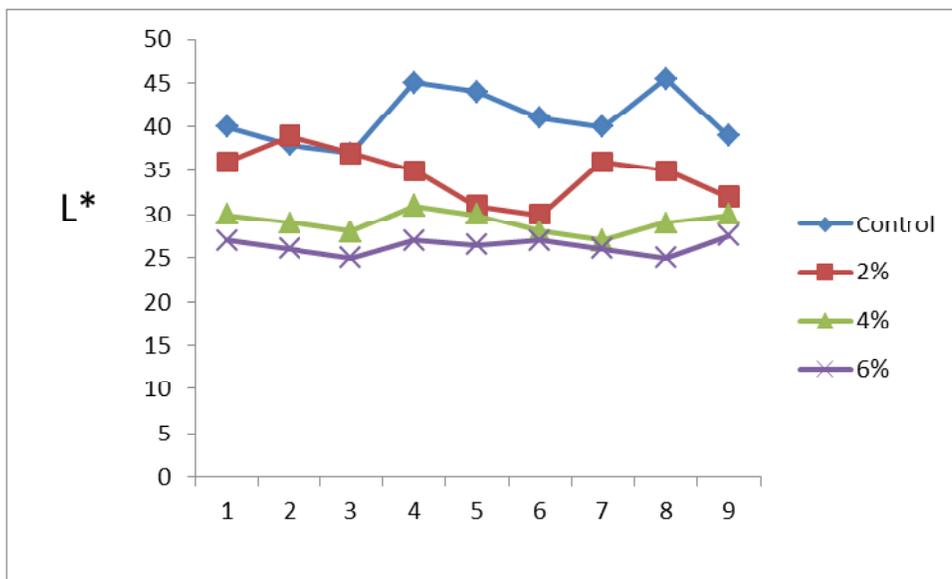
**Table 2. Growth performances of rainbow trout fed with red beet for 60 days (mean ± S. E.).**

Diet Red beet juice(%)	Growth performances			
	WG <sup>1</sup> (g)	FCR <sup>2</sup>	PER <sup>3</sup>	SR <sup>4</sup> (%)
0	125.2 ± 2.10 <sup>a</sup>	1.34 ± 0.066 <sup>a</sup>	0.80 ± 0.021 <sup>a</sup>	95 ± 3 <sup>a</sup>
2	127.6 ± 1.80 <sup>a</sup>	1.30 ± 0.045 <sup>a</sup>	0.86 ± 0.014 <sup>a</sup>	98 ± 2 <sup>a</sup>
4	127.9 ± 2.33 <sup>a</sup>	1.29 ± 0.078 <sup>a</sup>	0.88 ± 0.026 <sup>a</sup>	95 ± 3 <sup>a</sup>
6	75.50 ± 3.90 <sup>b</sup>	1.96 ± 0.0068 <sup>b</sup>	0.76 ± 0.05 <sup>b</sup>	50 ± 2 <sup>b</sup>

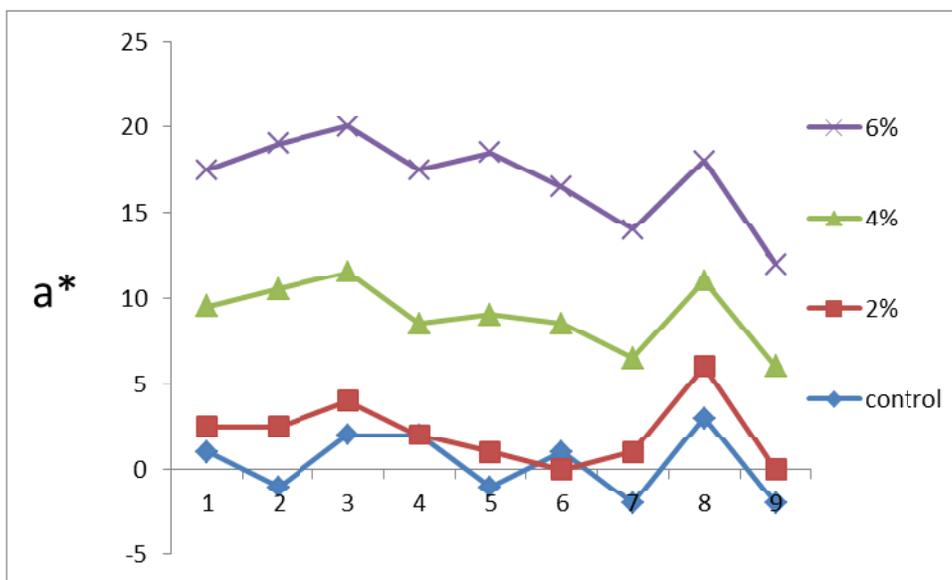
*B. Effect of experimental diet on the fish fillet*

The intensity of color of the studied fish fillets were presented as low, high and higher in Figs. 1, 2 and 3. The Figures show the pigment intensity toward redness in a\* zone (Pink fillet color) when fish fed

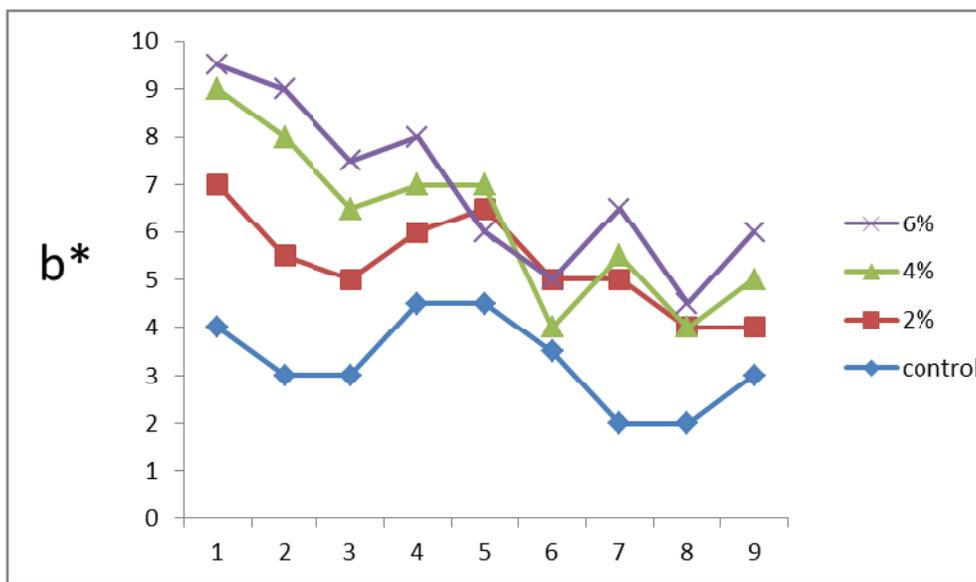
with 4% and 6% red beet in their diet that were significant compared to other treatments (p < 0.05). The group of fish whom received control basal diet (without red beet) and 2% red beet in their food were exhibit low color intensity in L\* zone (toward white).



**Fig. 1.** Influence of red beet (%) on pigmentation of fillet of rainbow trout based on L\* value (mean ± SE).



**Fig. 2.** Influence of red beet (%) on pigmentation of rainbow trout fillet based on a\* value (mean ± SE).



**Fig. 3.** Influence of red beet (%) on pigmentation of rainbow trout fillet base on b\* value (mean  $\pm$  SE).



**Fig. 4.** Effect of red beet on pigmentation of rainbow trout fillet.

## DISCUSSION

The diets supplemented with 6% red beet had negative effect on growth performances of the fish. Several studies showed that the use of high level of plant materials in the diet of fish, retards their growth and survival (Baron, 2007). As reported by Xiangjun *et al.*, (2012), the increase in various pigments from some plant sources were not improved the growth and feed utilization of Japanese ornamental Carp (Koi) considerably. The main reason for this could be the high levels of carbohydrate and cellulose in plant sources. In addition, it might lead to negative effect on taste of food, physical quality of the pellets and nutritional imbalance in diet (Furr and Clark, 1997).

The degree of this effect depends on the feeding regime of fish, since rainbow trout is carnivorous fish. Therefore, adding plant materials to their diet will be naturally restricted (Christianen, 1996). There is no relevant reference regarding to the use of red beet in diet of fish or other aquatic organism.

In this study, 4% level of red beet was found to be adequate to get desired coloration in rainbow trout. Further more, this level of red beet did not retarded the growth performances of studied fish. Similar results were obtained with Buyukcaparm (2007) when he used natural carotenoid sources like red pepper in the diet of rainbow trout. They found that 6% level was the best ratio to obtain desired coloration and weight gain.

In present study, the optimum duration for perfect antocyanin pigmentation in rainbow trout was 60 days. Similar results were reported with Schiedt (1993) for optimum duration for absorption, retention and metabolic transformation of pigments such as carotenoids in salmonids and crustaceans. There was no trace of yellow color in rainbow trout in this study using red beet for pigmentation. However, in a study conducted by Buyukapar *et al.* (2007), they found yellow trace pigmentation in the fillet of rainbow trout when they used marigold flower of carotenoid sources in the diet of fish. This might be due to the deposition of Lutein or other xanthophylls from characterized by yellow color, however, the yellow pigmentation formed in the fish fed with marigold flower may not be desirable by consumers. Therefore, consumer preferences should be taken into consideration. Recently, Asadi Sharif *et al.* (2014) reported that the red beet juice powder up to 6% represented good effect on pigmentation and growth performances of Oscar fish. In another study, Noverian and Shabanipour (2012) reported that the beet-root juice powder had a positive effect on coloration of skin and fillet of rainbow trout.

## CONCLUSION

It was found that the most appropriate dose of red beet in the diet for best growth performances and better pigmentation of rainbow trout was 4%. Furthermore, this experiment is a base line research in using red beet for other organisms such as Lobster, shrimp, crab etc. in which their coloration are commercially important.

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## REFERENCES

- Adams, J. P., Elbe, J. H., & Amundson, C. H. (1976). Production of a betacyanine concentrate by fermentation of red beet juice with *Candida utilis*. *Journal of Food Science*, 41(1), 78-81. ISO 690.
- Asadi Sharif, E, Noverian, R, Noverian, H, Razaghi, M. and Shabanipour, N. (2014). The effect of different level of red beet (*Beta vulgaris* L.) on pigmentation and growth performances of Oscar fish (*Astronorusocellatus*). *18th National and 6th International Congress of Biology in Iran*. p 290.
- Baron, M. Davies, S. Alexander, L. Snellgrove, D. Sloman, K.A. (2007). The effect of dietary pigments on the coloration and behavior of flame-red dwarf gourami, *Colisalalia*. *Animal Behaviour*, 75, 1041-1051 doi:10.1016/j.anbehav.08.014.
- Boeuf, G., and Le Bail, P.Y. (1999). Does light have an influence on fish growth?. *Aquaculture*, 177(1), 129-152.
- Buyukapar. H.M. Yanar. M. Yanar. (2007). Pigmentation of Rainbow Trout (*Oncorhynchusmykiss*) with Carotenoids from Marigold Flower (*Tagetes erecta*) and Red Pepper (*Capsicum annum*). *Turk. J. Vet. Anim. Sci* 31(1): 7-12.
- Cai, Y.Z., Sun, M. and Corke, H. (2005). Characterisation and application of betalain pigments from plants of the Amaranthaceae. *Trends in Food Science & Technology*, 16, 370-376.
- Sinclair, C.G. (Ed.). (1998). International dictionary of food and cooking. Taylor & Francis.?
- Choubert, G. and Storebakken, T., (1989). Doseresponse to astaxanthin and canthaxanthin pigmentation of rainbow trout fed various dietary carotenoids concentrations. *Aquaculture*, 81: 69-77.
- Elbandy, M.A., Abdelfadeil, M.G., Stability of Betalain Pigments from A Red Beetroot (*Beta vulgaris*). *The First International Conference of Food Industries and Biotechnology & Associated Fair*.
- Elena. C. Yahia, M. (2008). Identification and Quantification of Betalains from the Fruits of 10 Mexican Prickly Pear Cultivars by High-Performance Liquid Chromatography and Electrospray Ionization Mass Spectrometry. *J. Agric. Food Chem.* 56: 5758-5764.
- Furr, H.C. & Clark, R.M. (1997). Intestinal absorption and tissue distribution of carotenoids. *Journal of Nutritional Biochemistry*, 8, 364-377.
- Goldman, I.L. & Navazio, J.P. (2003). History and breeding of table beet in the United States. *Plant Breeding Reviews*, 22, 357-388.
- Gomelsky, B., Cherfas, N.B., Ben-Dom, N., Hulata, G., (1996). Color inheritance in ornamental (Koi) carp (*Cyprinus carpio* L.) inferred from color variability in normal and gynogenetic progenies. *The Israeli Journal of Aquaculture – Bamidgeh*, 48(4), 219-230.
- Gouveia, L., E. Gomes and J. Empis, (1997). Use of *Chlorella vulgaris* in diets for rainbow trout to pigmentation of muscle. *J. Appl. Aqua. Cult.*, 7: 61-70.