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RESEARCH PAPER

The Effects of Dietary Supplement of Rounded Brittle Fern Weed (*Laurencia obtusa*) (Hudson) (J.V.Lamouroux, 1813) as a Natural Carotenoid on Pigmentation in Rainbow Trout (*Oncorhynchus mykiss*)

Birol Baki[1,*], Murat Kerim[1], Dilara Kaya Ozturk[1], Bora Eyuboglu[2], Ali Karacuha[3]

[1] Sinop University, Faculty of Fisheries, Aquaculture Department, 57200 Sinop, Turkey.
[2] Sinop University, Vocational High School, 57200 Sinop, Turkey.
[3] Sinop University, Faculty of Fisheries, 57200 Sinop, Turkey.

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Corresponding Author
Tel.: +90.368 2876265-3104
E-mail: bbaki@sinop.edu.tr

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Abstract

The aim of this study was to determine the effects of *Laurencia obtusa* (Hudson) as a natural carotenoid on the growth performance, biochemical composition and skin and fillet pigmentation in rainbow trout (*Oncorhynchus mykiss*).

Laurencia obtusa was collected manually from the shores of Sinop (The Black Sea-Turkey) and included in the diet at two different ratios as 30 (L30) and 60 (L60) mg/kg diet. The fish with an average weight of 248.9±6.1 g were fed by the experimental diets for 6 weeks.

The best growth performance was determined in L0. The differences between protein, fat and ash values of fish were not significant (P>0.05). At the end of the study, the L* value was highest in the L0 group (59.94±1.28) and lowest in the L60 group (53.43±1.98) and, in fillet, it decreased in all experimental groups compared to the initial values (P<0.05). The a* values of skin and fillet were determined between 4.48±0.31 and 4.68±0.41, 1.45±0.42 and 1.73±0.39, respectively (P>0.05). The b* value increased in all groups in skin (P<0.05), whereas it was decreased in fillet (P>0.05).

Introduction

Sea product origin raw materials, especially fishmeal is very extensively used in feeds for fish as well as other animals. A recent global survey estimated the aquaculture consumption of fishmeal at 4539 thousand tonnes in 2016 (IFFO, 2017). Nowadays, it is becoming increasingly evident that such rapid and unconscious consumption of this natural resource will ultimately become both environmentally and economically unsustainable. Alternative feed ingredients instead of fishmeal must be able to supply comparable nutritional value at competitive costs. Vegetable raw materials, due to their low costs, have proved successful in some applications when they were used as substitutes for a portion of the fishmeal (Valente *et al*. 2006; Diler, Tekinay, Güroy, Güroy & Soyutürk, 2007; Ergün, Soyutürk, Güroy, Güroy & Merrifiled, 2009; Güroy, Cirik, Güroy, Sanver & Tekinay, 2009; Dantagnan, Hernandez, Borguez & Mansilla, 2009; Soler-Vila, Coughlan, Guriy & Kraan, 2009; Wassef, El-Sayed & Sakr, 2013). However, even when these vegetable raw materials substitutes can support growth, they can cause significant changes in the nutritional quality of the fish produced.

In 1970, global fisheries production (capture and aquaculture) was 67.4 million tonnes and reached 199.7 million tonnes in 2015. In those years, the aquaculture production which constitutes 5% (3.5 million tonnes) of the world production, reached 53% (106 million tonnes) in 2015. Total production of rainbow trout (*Oncorhynchus mykiss*), one of the most cultured species in the world, was 812,939 tonnes in 2014 (FAO, 2016). The recent increase in the aquaculture production has also made quality fish production and marketing important in the world. Besides the physical appearance and the freshness of the fish, consumers also consider meat color, especially in species such as rainbow trout and

salmon. It has been reported that the pink-red color of the wild trout is originated from the plankton and benthic crustaceans which are the natural food of the fish (Choubert and Blanc, 1985), therefore it has been stated that a pigmentation source should be added to the diet of the cultured fish (Yuangsoi, Jintasataporn, Areechon & Tabthipwon, 2010; Shields & Lupatsch, 2012). However, synthetic pigment materials (astaxanthin and cantaxantine) are usually used for the red-pink color fillet in intensive trout culture (Ergün, Bircan, Türker & Baki, 2000; Lee, Pham, & Lee, 2010; Brown, Barnes, Parker, & Fletcher, 2016). These synthetic materials are receiving negative feedback by consumers (Pham, Byun, Kim, & Lee, 2014) as they increase the feed cost (Doolan, Booth, & Allan, 2009).

Apart from these synthetic materials, the algae are the base of the aquatic food chains that produce the food resources that fish are adapted to consume. A few algae are used as pigment sources in fish feeds. Microalgae such as *Haematococcus sp*. is used to produce astaxanthin, which is responsible for the pink colour of the flesh of salmon (Anonymous, 2018). Spirulina is used as a source of other carotenoids that fishes such as ornamental koi can convert to astaxanthin and other brightly colored pigments. Various species of macroalgae has been included in cultured fish feed formulations to enhance their nutritional value, and many have been shown to be beneficial: *Ascophyllum*, *Porphyra* or *Ulva* fed to Sea Bream (*Sparus aurata)* (Mustafa, Wakamatsu, Takeda, Umino, & Nakagawa, 1995); *Gracilaria* or *Ulva* fed to European Sea Bass (*Dicentrarchus labrax*) (Valente *et al*. 2006); Ulva or Pterocladia fed to Gilthead Sea Bream (WassefEl-Sayed, Kandeel, &Sakr, 2005).

Laurencia obtusa, widely found at the Turkish coasts, is from the family Rhodomelaceae and is a red sea alga (İslimyeli, Üstün, & Yılmaz, 1990). *Laurencia obtusa*, in addition to its carotene content, is also rich in fucosantine (0.14 mg/g) and β-carotene (0.09 mg/g) which are also found in the structure of natural salmonids (Wahbeh, 1997; Hegazi, 2002). Therefore, the aim of this study was to determine the effects of rounded brittle fern weed (*Laurencia obtusa*) as a natural carotenoid on the growth performance, biochemical composition and skin and fillet pigmentation in rainbow trout.

Materials and Methods

Experimental Setup

The study was carried out at Sinop University, Faculty of Fisheries, Research and Application Center for 6 weeks. In this study, 9 circular tank of 300L volume were used. In the test unit, a direct flow water system and aeration were used for each tank. Groundwater was used in the system. During the study period, the ammonia value was 0.003mg/L and the average salinity were 1-4‰ in the system. UV filter was used for the sterilization of water. The tanks were cleaned daily from faeces and feed waste by siphon method. In this study, natural daylight was used. A total of 90 rainbow trout with an average weight of 248.9±6.1 g were stocked in three replicates (stocking density:12 kg/m^3). Fish were fed to satiation with commercial feed (color additive-free) in the adaptation period. Mean water temperature was 15.45±0.71°C, mean dissolved oxygen was 6.42±0.16 and mean pH was 8.03±0.05 during the study.

Experimental Diets

Three different diets were prepared for the study (L0, L30 and L60) (Table 1). Trial feeds were produced in the feed technology laboratory at room temperature (18-21°C). Feeds were prepared as isonitrogenic (49%) and isolipidic (16.5%). The red marine algae, *Laurencia obtusa* used in the feed as the natural carotenoid raw material were collected manually from the shores of Sinop at The Black Sea (42°02´ N and 35°05´ E).

The raw materials used in the trial feed production were obtained from Black Sea Feed company. The feed components were first passed through a 500 µm mesh sized sieve and weighed and composed according to the ratio. *Laurencia obtusa* (crude protein 7.78±0.16%, crude fat 0.39±0.01%, crude ash 16.67±1.14%, moisture 87.44±1.16%) was washed with pure water and freed from sand and other materials. Then, it was dried in the oven (60ºC 24 h), shredded using a mixer andhomogenized. It has been reported that *Laurencia obtusa* contains inhibitory compounds (Anonymous, 2018). Therefore, in order not to have a negative effect on the fish, *Laurencia obtusa* was added to feeds as 30 and 60mg/kg, respectively.

The feed mixture was stirred for 20 minutes until a homogenous mash was obtained. The mash was supplemented with lipid source addition and mixed for an additional 10 minutes. Subsequently, hot water was added to the mixture at 35% ratio and kneaded for 15 minutes and a dough structure was obtained. The mixture dough was pelletized by passing through a 4 mm diameter pellet machine. The prepared feed was dried for 6 hours in a drying cabinet set to 60ºC and then for 5 hours at room temperature. Breaking down to the size of the mouth opening of the fish, dried feed was stored at -20°C. The fish were given twice a day manually until satiation.

Sampling and Measurement

Biometric measurements, meat yield, biochemical composition and color measurement

Table 1. Raw material content and biochemical composition of the feed used in the experiment

Feed Raw materials (g/kg dry matter)	Feed Rations		
	L0	L30	L60
Fish meal[1]	350	350	350
Soybean meal[2]	250	250	250
Corn gluten[3]	150	150	150
Semolina meal[4]	110	109.97	109.94
Fish oil[5]	130	130	130
Vitamin mixture[6]	5	5	5
Mineral mixture[7]	5	5	5
Laurencia obtusa[8]	0	0.03	0.06
Total	100	100	100
Biochemical Composition of Feed (%)			
Crude Protein	48.62±0.14	48.96±0.03	49.06±0.30
Crude Fat	17.07±0.24	16.84±0.81	16.09±0.17
Crude Ash	5.92±0.09	6.02±0.08	6.03±0.12
Moisture	7.29±0.55^{b}	6.04±0.36^{a}	6.03±0.39^{a}

Values in the same row with different superscripts are significantly different (P<0.05).

[1] Fishmeal: Agros, Mocaco-France

[2] Soybean meal: Obtained from genetically modified soybean. [ACS-GM005-3]:[MON-89788 (MON89788)]:[MON-04032-6], USA

[3] Corn gluten: Cargill, Bursa-Turkey,

[4] Semolina meal: Farin chemistry-China

[5] Fish oil: Mocaco-France

[6] Vitamin mixture: Vitamin A15.625IU/kg, Vitamin D_3 3126 IU/kg, Vitamin E 275 mg/kg

[7] Mineral mixture: Zinc oxide (Zn) 150mg/kg, bicarbonate of cobalt (Co) 4.5 mg/kg

[8] *Laurencia obtusa*: The red marine algae was collected manually at the Black Sea Sinop cost

were conducted at the beginning and the end of the study for each group. Growth performance in fish was determined by daily weight gain (g/day), thermal-unit growth coefficient (TGC), feed conversion ratio (FCR), hepatosomatic index (HSI), visceromatic index (VSI), carcass yield (CR) and condition factor (CF) values (Skalli & Robin, 2004; Cui, Wang, & Qin, 2006).

Daily weight gain = (Final weight,g – Initial weight,g) / Days experiments

Thermal-unit growth coefficient= (Final weight,g 1/3 – Initial weight,g 1/3)/(Temp. x Days)x100

Feed conversion rate=Total Consumed amount of feed,g/Total weight Gain,g

HSI (%)= (Liver Weight,g/Total Body Weight,g)x100

VSI* (%)=(Vicera Weight,g/Total body weight,g)x100

*VSI: (Heart, esophagus, stomach, liver, intestine, splenic, gall bladder, kidney)

Carcass yield (%)=(Edible Fillet Weight.g/Total Body Weight,g)x100

Condition factor =(W/L^3)x100

Biochemical Analyzes and Color Measurement

Fillet process was performed manually. The fillet was dried at 105^{o}C for 20 h to obtain their dry weight and moisture content. Ash weight was determined by combusting a known dry weight of tissue at 500^{o}C for 15 h in a muffle furnace and reweighing the tissue (AOAC, 2000). Triplicate dry meat samples were analyzed for lipid and protein according to the AOAC (2000) methods. Crude lipid content was conducted according to the Soxhelet method. Crude protein content was determined as total nitrogen content by Kjeldahl method and using coefficient 6.25 for calculation.

The L*, a* and b* values of skin and fillets were measured using a Minolta Chroma Meter (Model CR-400, Konica Minolta, Tokyo, Japan) by standardizing using white tiles (Commission Internationale de l´Eclairage, CIE, 1976). L* defines brightness (lightness-darkness), a* redness-greenness, b* yellowness-blueness (Nickell and Bromage, 1998) values. The L*, a* and b* values of the skin and fillet samples were determined by measuring the samples in three different regions in three replications and are given as mean values (Figure 1).

Compliance with Ethical Standards

Ethical Approval

This study was conducted in compliance with the rules for animal experiments for scientific purposes and permission was given by the Sinop University, Animal Experiments Local Ethics Committee with the permission No. 2014/19 on July 24th 2014.

Statistical Analysis

The results are presented as mean±standard error. Statistical analysis of data was carried out using the IBM SPSS 21 statistical package program. The difference between the results was tested by one-way analysis of variance (ANOVA, P<0.05). The differences were determined by the Tukey multiple comparison test.

Results

At the end of the study, fish weights of L0, L30 and L60 groups were 368.6±23.4g, 328.8±31.5g and 293.6±32.7g, respectively (P<0.05). The FCR values of the L30 and L60 groups (1.24±0.05-1.26±0.02) were higher than those in L0 group (1.16±0.01) (P<0.05). The thermal growth coefficient (TGR) was the highest in the L0 group (0.114±0.014), followed by L30 (0.078±0.009) and L60 (0.045±0.011) groups, respectively (P<0.05) (Table 2). The analyses revealed that the difference between protein, fat and ash values were not significant (P>0.05), however dry matter value in L0 group was statistically higher (P<0.05) (Table 3).

At the beginning of the study, L, a* and b* values were 58.16±1.34, 4.39±0.42 and 8.39±1.46, respectively. At the end of the study, L* value in the

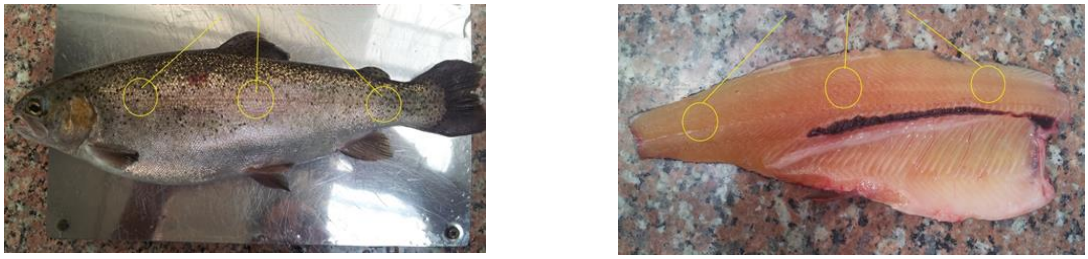

Figure 1. Color measurement areas.

Table 2. Growth performance of rainbow trout

Growth parameters	L0	L30	L60
Final weight (g)	368.6±23.4[b]	328.8±31.5[a]	293.6±32.7[a]
Daily weight (g/day)	2.85±0.71[b]	1.91±0.85[a]	1.06±0.18[a]
TGC (%)[1]	0.114±0.014[b]	0.078±0.009[a]	0.045±0.011[a]
FCR[2]	1.16±0.01[a]	1.24±0.05[b]	1.26±0.02[b]
HSI (%)[3]	1.32±0.17[b]	0.95±0.13[a]	1.08±0.06[a]
VSI (%)[4]	12.87±0.79[b]	10.50±1.05[a]	10.86±0.82[a]
CY (%)[5]	49.23±0.77	49.20±0.58	47.59±1.44
CF[6]	1.41±0.02[b]	1.25±0.04[a]	1.25±0.05[a]
Survival Rate (%)	93.33±6.67	93.33±3.33	86.67±6.67

Values in the same row with different superscripts are significantly different (P<0.05).
[1]TGC: Thermal-unit growth coefficient
[2]FCR: Feed conversion rate
[3]HSI: Hepatosomatic index
[4]VSI: Viscerosomatic index
[5]CY: Carcass yield
[6]CF: Condition factor

Table 3. Biochemical composition of fillet

%	Initial	L0	L30	L60
Protein	18.37±0.24	19.06±0.35	19.61±0.19	18.58±0.38
Lipid	6.55±0.16	6.18±0.07	5.43±0.16	5.95±0.79
Moisture	70.79±0.12	71.38±0.27[a]	73.10±0.24[b]	73.32±0.13[b]
Ash	1.47± 0.07	1.54±0.03	1.49±0.04	1.31±0.14

Values in the same row with different superscripts are significantly different (P<0.001).

skin increased in L0 and L30 groups whereas decreased in L60 group (53.43±1.98) (P>0.05). In terms of a* value, the differences between the groups were not significant (P<0.05) and the highest value was in L60 group (4.68±0.41). The b* value decreased in L30 group, the difference between L30 and L60 group was significant in the skin (P<0.05), and the highest value was in L30 group (6.49±0.82) (Table 4).

As a result of the initial analysis in the fillet, the L*, a* and b* values were 46.92±0.59, 1.43±0.38 and 11.78±0.92, respectively. At the end of the study, L* values of fillet increased in L0 and L30 compared to those of the initial. The lowest L* value of fillets was determined in L60 group (45.63±0.58). In terms of a* values, the differences between the groups were not significant (P>0.05), and the highest value was in L60 group in fillet (1.73±0.39).

In terms of b* values of fillet, the differences between the groups were not significant (P>0.05), and the highest value was in L0 group (13.86±0.71).

Discussions and Conclusions

Different studies have stated that the plant-derived raw materials participating in the diet have an effect on the growth (Olvera-Novoa, Campos, Sabido, & Martinez Palacios, 1990; Büyükçapar,Yanar and Yanar 2007). For instance, Dantagnan *et al*. (2009) have reported that the inclusion of <3% *Macrocystis pyrifera* meal in diets for rainbow trout did not have a negative impact on growth and feed parameters and improved the quality of meat. In studies with green algae *Ulva rigida;* Ergün *et al*. (2009) have reported that 5% inclusion of Ulva meal at both dietary lipid levels improved the growth performance, feed efficiency, and body composition of Nile tilapia (*Oreochromis niloticus*). In another study, Güroy, Ergün, Merrifield, and Güroy, (2013) have stated that the dietary inclusion of Ulva meal at levels of up to 10% can be used without significant negative effects on the growth performance of rainbow trout. At the end of the study, it was determined that the feed containing *Laurencia obtusa,* had an effect on feed intake and, therefore, on the growth of fish.

In this study, the addition of *Laurencia obtusa* in the feed increased the moisture content in the fillet. As a result of the biochemical analysis, the differences between the crude protein and crude fat values of the experimental groups were not significant and the addition of *Laurencia obtusa* in the feed did not significantly affect the flesh quality. In other studies, it has been reported that the effect of macroalgae added to feeds on fish meat, particularly, lipid values (Ergün *et al*., 2009; Güroy *et al*., 2013).

In different studies, it has been reported that skin pigmentation values increased in fish that were fed with feeds containing natural carotenoid sources (Gomes *et al*., 2002; Hancz, *et al*. 2003; Diler, Hoşsu, Dilek, Emre, & Sevgili, 2005; Tejera, *et al*. 2007; Kalinowski, Izquierdo, Schuchardt and Robaina, 2007; Lee *et al*., 2010). Sole-Vila *et al*., (2009) have observed a pigmentation in the flesh of rainbow trout, which were fed with feed containing red algae, from pinkish-white in the control fish to pinkish orange to dark orange in the group that were fed with the feed containing the highest ratio fo red algae in the study. Red algae contain yellow xanthophylls, especially lutein and zeaxanthin which may possibly cause the coloration as these pigments are soluble in fat. It was found that *Laurencia obtusa* had no positive effect on lightness (L*) value, however redness (a*) and yellowness (b*) values increased in the skin. In addition, *Laurencia obtusa* had an effect on redness (a*) value and lightness (L*) values in fillet. I twas determined that, yellowness (b*) values decreased as the amount of *Laurencia obtusa* increased in the feed. In different studies, it was determined that a* and b* values increased and L* values of carotenoid concentration decreased in salmon fillet, and these results were similar to those obtained in the present study.

In culture rainbow trout, synthetic carotenoids (astaxanthin and cantaxantine) are added to the feed to obtain a color close to its natural state and this significantly increases the feed costs. Therefore, coloration of fillet in rainbow trout using macroalgae such as *Laurencia obtusa* as a natural pigment source may enhance the potential of algae inclusion in fish feed and may perhaps replace or reduce artificial colorants currently used by the industry.

Table 4. L*, a* and b* values of skin and fillet of rainbow trout

		İnitial	L0	L30	L60
L*	Skin	58.16±1.34	59.94±1.28^{b}	59.75±1.06^{ab}	53.43±1.98^{a}
	Fillet	46.92±0.59	45.78±0.76	45.94±0.58	45.63±0.57
a*	Skin	4.39±0.42	4.48±0.31	4.57±0.33	4.68±0.41
	Fillet	1.43±0.58	1.45±0.42	1.52±0.36	1.73±0.39
b*	Skin	8.39±1.46	3.96±0.56^{a}	6.49±0.82^{b}	6.07±0.57^{b}
	Fillet	11.78±0.92	13.86±0.71	13.81±0.52	12.36±1.19

Values in the same row with different superscripts are significantly different (P<0.05).

Consequently, natural and alternative carotenoid raw materials, such as *Laurencia obtusa,,*require further research to determine the growth performance, biochemical composition, and their effects on skin and meat color of the fish. This study suggests that *Laurencia obtusa* can be included as an ingredient for rainbow trout feed, used in harvested period, up to 30mg/kg without significant negative effects on growth performance. However, a more thorough study would be necessary to consolidate these findings.

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