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Comparative study of carcass composition and total carotenoids of two important cold water fishes *viz. Barilius bendelisis* and *Schizothorax rechardsonii* fed on *Spirulina platensis* fortified diets

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Abstract
This work is a portion of our previous PhD thesis work and represents a comparative data of separately published works. The results related to change in carcass composition and total carotenoids of two important coldwater fishes *viz*. *Barilius bendelisis* and *Schizothorax rechardsonii* of almost similar length and weight as collected from local streams and reared in laboratory condition for sixty days in triplicate to study the effect of spirulina (*Spirulina platensis*) fortified diets (0, 3, 5, 7 & 10%) are compared and represented here. Supplementation of 3-10% dietary spirulina (T_1 to T_4) meal significantly increased crude protein (CP)% in both the experimental fishes, when compared with control group (T_0). There was a significant change in crude fat also in case of *B. bendelisis*, while no significant effect for the same on *S. rechardsonii*. Whereas, supplementation of 5-10% dietary spirulina (T_2 to T_4) significantly increased body carotenoids (µg/g) compared to control group (T_0). Spirulina meal Supplementation has potential to enhance the coloration and body composition of *Schizothorax richardsonii* and *Barilius bendelisis*. Spirulina meal at 5-10% supplementation of the diet provided highest carotenoid accumulation in the flesh among the groups which may help farmers as well as other stakeholders in realizing more profits in the culture of these species and considering as ornamental fish.

Keywords: Coldwater fishes, spirulina, carcass composition, total carotenoids

1. Introduction

The Indian hill trout (*Barilius bendelisis)* and Snow trout (*Schizothorax richardsonii*) are small indigenous fish endemic to Himalaya, thrives well in coldwater streams, lakes and rivers [1]. Both are having commercial importance due to their ornamental and food value hence widely cultured in hilly regions of Himalaya [2].

The food value of fish is determined with the quality and quantity of protein and other nutrients present in the muscle while the ornamental value is associated to the coloration due to carotenoids or pigment bearing substance in the tissue [3]. Inadequate pigmentation, retarded growth with degraded muscle composition is always noticed in cultured fishes due to lack of carotenoid and other nutrient content in their artificial diets [4]. Carotenoids are most conspicuous in petals, pollen, fruit, tomatoes, citrus fruits and some roots [5]. Higher animals, including fish, are unable to synthesize carotenoids *de novo* [6], and are contingent upon a dietary source [7]. Therefore, a direct relationship between dietary carotenoids and pigmentation exists in fish [3]. In wild, fishes are getting quality food required for their proper growth, pigmentation and nutrient profile. But, in captive condition, lack of nutrients and pigment bearing substance, results in retarded growth, faded coloration and degraded nutrient profile of the fish.

Fish skin colour is primarily dependent on the presence of chromatophores containing pigments, pteridines and purines [8]. Fish can modify alimentary carotenoids and store them in the integument and other tissues [3]. The effectiveness of carotenoid source in terms of deposition and pigmentation is species specific [9]. The loss of pigments, due to inadequate carotenoid reached diets, can be overcome by their addition to the artificial diet. Several studies addressed this issue and have evaluated different potential pigment sources like red pepper (*Capsicum annum*), marigold flower- *Tagetes erecta* and Spirulina-*Spirulina platensis* [10, 11]. These additives, besides pigments, influence muscle quality, survival, resistance to diseases and growth [12, 13, 14] while the effects are species specific. Therefore, the purpose of this comparative study was to investigate the effect of spirulina (*Spirulina platensis*) fortified diets on carcass composition and total carotenoids content of above mentioned two important fishes in order to increase their production with enhanced pigmentation and muscle composition, and to provide species specific recommendations.

2. Materials and Methods

2.1 Experimental animal

Fingerlings of *B. bendelisis* and *S. richardsonii* of the average weight of about 5.6g were collected from a local stream of Nainital (Uttarakhand), transported in a circular container (500 L) with sufficient aeration, to the experimental site at hatchery complex of Directorate of Coldwater Fisheries Research (ICAR), Bhimtal and were acclimatized to the experimental rearing conditions for one week. During acclimatization, fish were fed with control diet. After acclimatization, fishes were transferred to 15 uniform size experimental fiberglass tanks of 100L capacity and reared for 60 days.

2.2 Experimental design and feeding

Five iso-nitrogenous (35.25±0.9% crude protein) diets were prepared with graded level of (0, 3, 5, 7 and 10% of diet) spirulina (SP) meal. Both the fishes (150 nos. of each) were randomly distributed in 5 experimental groups, separately, in triplicate following a completely randomized design (CRD). There were 5 treatment groups, *viz*. T_0 (control), T_1 (3% SP), T_2 (5% SP), T_3 (7% SP) and T_4 (10% SP). The control (T_0) was common for both the fishes, while treatments were divided into eight group *viz*. for *Barilius bendelisis* it was BS-1 (fed on T_1), BS-2 (fed on T_2), BS-3 (fed on T_3) and BS-4 (fed on T_4) and for *Schizothorax richardsonii* it was SS-1 (fed on T_1), SS-2 (fed on T_2), SS-3 (fed on T_3) and SS-4 (fed on T_4). The physicochemical parameters of water were analyzed by APHA [15] methodology, and were within the optimum range (dissolved oxygen: 6.0–8.5 mg/l, pH: 7.3–8.2 and temperature: 18–20 °C) throughout the experimental period. All the groups were fed their respective diets. Feeding was done at 5% of the body weight. Daily ration was divided into 2 split doses: about 2/3rd of total ration was given at 09:00 h and the rest at 18:00 h. The fecal matters were removed by siphoning and a constant water flow (2-3 L/M) was maintained by providing inlet at one and outlet at the other end to ensure optimum dissolved oxygen throughout the experimental period. A very few accidental mortality (10%) was observed during the 60 day experimental feeding trial. The composition of the diets are given in the table-1.

Table 1: Diet composition (%)

Ingredients	Diets				
	T_0	T_1	T_2	T_3	T_4
Fish meal	20.27	20.27	20.27	20.27	20.27
Soybean meal	20.27	20.27	20.27	20.27	20.27
Rice bran	29.73	26.73	24.73	22.73	19.73
Wheat bran	21.73	21.73	21.73	21.73	21.73
Vitamin-Mineral Mix[1]	2.00	2.00	2.00	2.00	2.00
Vegetable oil[2]	2.00	2.00	2.00	2.00	2.00
Fish oil[3]	2.00	2.00	2.00	2.00	2.00
Sodium alginate[4]	2.00	2.00	2.00	2.00	2.00
Spirulina meal	0	3.00	5.00	7.00	10.00
Total	100	100	100	100	100

[1]Agrimin Forte (Virbac Animal Health India Pvt. Ltd., Mumbai-59, India). Each kg contains- Vitamin A-7, 00,000 I.U., Vitamin D_3-70, 000 I.U., Vitamin E-250 mg, Nicotinamide-1000mg, Cobalt-150 mg, Copper-1200 mg, Iodine-325 mg, Iron-1500 mg, Magnesium-6000 mg, Manganese-1500 mg, Potassium-100 mg, Selenium-10 mg, Sodium-5.9 mg, Sulphur-0.72%, Zinc-9600 mg, Calcium-25.5%, Phosphorus-12.75%
[2]Ruchi Soya Industries Ltd., Raigad, India. [3]Procured from local market; [4]Himedia Ltd. India

2.3 Proximate composition analysis

The proximate composition of the experimental diets and fish muscle was determined following the standard methods of AOAC [16]. The moisture content was determined by drying at 105 °C to a constant weight. Nitrogen content was estimated by Kjeldahl method and crude protein was estimated by multiplying nitrogen% by 6.25. Crude fat (ether extract) was measured by solvent extraction method using diethyl ether (boiling point, 40–60 °C) as a solvent and ash content was determined by incinerating the samples in a muffle furnace at 600 °C for 6h.

2.4 Total carotenoids analysis

Total carotenoids were determined following the methodology given by Olson [17]. The samples (skin and muscles) were gently meshed with a glass rod against the side of the vial and then 5 ml of chloroform was added and left overnight at 0°C. When the chloroform formed a clear 1–2 cm layer above the caked residue, the optical density was read at 380, 450, 470 and 500 nm, in a spectrophotometer taking 0.3 ml aliquots of chloroform diluted to 3 ml with absolute ethanol. A blank prepared in a similar manner was used for comparison. The wavelength, at which maximum absorption observed, was used for the calculation.

2.5 Statistical analysis

Table 2: Proximate (% dry matter basis) and total carotenoids (µg/g diet) composition of different diets

Particulars	Diets				
	T_0	T_1	T_2	T_3	T_4
Moisture	13.28±0.02	13.40±0.03	13.15±0.01	13.34±0.05	13.30±0.02
Crude protein	35.21±0.08	35.32±0.05	35.10±0.02	35.20±0.01	35.09±0.02
Ether extract	7.65±0.02	7.80±0.01	7.25±0.02	7.97±0.03	7.45±0.02
Ash	10.85±0.05	11.01±0.02	10.90±0.04	11.00±0.03	11.08±0.05
TC	29.1±0.94	100.23±3.41	170.24±4.63	250.14±3.74	350.25±5.51

Mean values of all the parameters were subjected to one way ANOVA to study the treatment effect, and Tukey's test (HSD) were used to determine the significant differences between two means. Comparisons were made at 5% probability level. All the data were analyzed using statistical package SPSS (Version 12.01).

The original study was conducted as per the details as provided above, however for the present comparative study, the results derived from original works are compared and explained.

3. Results and Discussion

The proximate composition of the diets used in this study is given in the table-2. The proximate composition of the diets did not varied significantly.

Data expressed as Mean±SE, n=3

The carcass composition and total carotenoids content of *Barilius bendelisis* and *Schizothorax richardsonii* at the end of feeding trial are shown in Table-3.

Table 3: Carcass composition and total carotenoids of *Barilius bendelisis* and *Schizothorax richardsonii* fingerlings fed diets with graded levels of spirulina meal (mean±SD, n = 3 (10 fish per replicate).

Treatments	CP Mean±SD	CF Mean±SD	Caro. Mean±SD
Control	$14.9{\pm}0.04^{a}$	$3.5{\pm}0.11^{a}$	$2.02{\pm}0.04^{a}$
BS-1	$16.6{\pm}0.13^{e}$	$4.3{\pm}0.47^{b}$	$2.54{\pm}0.1^{a}$
BS-2	$16.9{\pm}0.1^{f}$	$4.1{\pm}0.31^{b}$	$3.97{\pm}0.18^{c}$
BS-3	$16.9{\pm}0.21^{f}$	$4.3{\pm}0.3^{b}$	$3.97{\pm}0.18^{c}$
BS-4	$16.9{\pm}0.24^{f}$	$4.1{\pm}0.13^{b}$	$3.94{\pm}0.27^{c}$
SS-1	$15.7{\pm}0.03^{c}$	$3.5{\pm}0.18^{a}$	$2.54{\pm}0.21^{a}$
SS-2	$16.5{\pm}0.07^{e}$	$3.3{\pm}0.14^{a}$	$3.73{\pm}0.17^{c}$
SS-3	$16.5{\pm}0.07^{e}$	$3.2{\pm}0.05^{a}$	$3.73{\pm}0.17^{c}$
SS-4	$16.5{\pm}0.07^{e}$	$3.3{\pm}0.11^{a}$	$3.71{\pm}0.14^{c}$

Values in a column with different superscripts differ significantly ($p<0.05$).

Supplementation of 3-10% dietary spirulina (T_1 to T_4) meal significantly ($P<0.05$) increased crude protein (CP)% in both the experimental fishes, when compared with control group (T_0). There was a significant change in crude fat also in case of *B. bendelisis*, while no significant ($P>0.05$) effect on *S. rechardsonii*.

The comparative results of the body composition analysis under present study are in conformity with those of Boonyaratpalin *et al.* [18], Gurung *et al.* [19] and Jha *et al.* [20, 21] who reported that different sources of carotenoids might have different impact on body composition of fish and prawn.

Supplementation of 5-10% dietary spirulina (T_2 to T_4) significantly ($P<0.05$) increased body carotenoids (µg/g) compared to control group (T_0) in both the experimented fishes. Chromatophores containing pigments such as melanins, carotenoids, pteridines, and purines [8] causes brilliant coloration of fish skin. Fishes like other animals, can modify alimentary carotenoids and store them in the integument and other tissues which is species specific [9] and they are unable to biosynthesize carotenoids *de novo* [6]. Farmed fish have no access to natural carotenoid-rich feeds and, therefore, the necessary carotenoids must be added to their diet. Fishes under present investigation utilized experimental diets better than the control one, which may be a ground for better pigmentation.

The outcome of the present comparative analysis are in agreement with those of Jha *et al.* [20] who used beet root and marigold as a carotenoid source on pigmentation and growth of *Schizothorax richardsonii*. Total carotenoid levels in the skin of rainbow trout, *Oncorhynchus mykiss* increased significantly with an increase in green algae, *Haematococcus pluvialis* in their diet [22], while, Choubert [23] reported that the quantity of total pigments in the skin was greater in trout receiving the feed supplemented with increased amount of spirulina.

4. Conclusion

This comparative study showed that supplementation of 3-10% dietary spirulina meal significantly increased crude protein (CP)% in both the experimental fishes. There was a significant change in crude fat also in case of *B. bendelisis*, while no significant effect for the same on *S. rechardsonii*. Whereas, supplementation of 5-10% dietary spirulina significantly increased body carotenoids (µg/g). Spirulina meal Supplementation has potential to enhance the coloration and body composition of *Schizothorax richardsonii* and *Barilius bendelisis*. Spirulina meal at 5-10% supplementation of the diet provided highest carotenoid accumulation in the flesh among the groups which may help farmers as well as other stakeholders in realizing more profits in the culture of these species and considering as ornamental fish.

5. References

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