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Effect of Ration Level and Feeding Frequency on Growth, Nutrient Utilization and Body Composition of Juvenile Black Fin Sea Bream, *Acanthopagrus berda* (Forsskal 1775)

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Authors' Contributions
GA conceived and designed the study and wrote the article. AR executed all the experimental work. MHR analyzed feed components. AM analyzed fish meat samples. AG statistically analyzed the data. SR and LG helped in preparation of manuscript.

Key words
Sea bream (*Acanthopagrus berda*), Feeding frequency, Ration level, Nutrient utilization, Growth.

ABSTRACT

In this study, effect of different feeding level and frequency on growth performance, nutrient utilization and body composition of juvenile black fin sea bream, *Acanthopagrus berda* (weight 23.5g−125.8 g) were investigated. Fish were randomly distributed in rectangular glass tanks (150 liter) and were fed at six ration levels of 2.0, 2.5, 3.0,3.5, 4.0 and 4.5% body weight per day (BW d^{-1}) and four feeding frequency *i.e.*, once, twice, thrice and four times per day. Fish in all replicates were fed with diet comprising 40% protein and 20% lipid for 75 days. Higher percent weight gain (% WG), best feed conversion ratio (FCR) and specific growth rate (SGR) were recorded at ration level from 2.5 to 4.5% BW d^{-1} and feeding frequency of three to four times daily. The moisture, protein and ash contents of whole body of the fish were not significantly (P>0.05) affected by feeding frequency. The highest lipid contents were observed in fish at feeding frequency of three to four times daily. The condition factor (CF), viscerosomatic index (VSI) and hepatosomatic index (HSI) were significantly (P< 0.05) higher in fish at ration levels from 2.5 to 4.5% BW d^{-1} and feeding frequency of three to four times daily. These results concluded that the optimum ration level and feeding frequency of the juvenile sea bream *A. berda* (weight 23.5g to 125.8 g) are 2.5% BW d^{-1} and three times daily, respectively, under similar culture conditions.

INTRODUCTION

Sea breams are the most important commercial fishes constituting a significant demersal fish resource of Asia, Africa and Europe (Mourente and Bell, 2006; Abbas *et al*., 2015; Rahim *et al*., 2015). Black fin sea bream, *Acanthopagrus berda* has high market value in Pakistan due to its best taste and public demand (Abbas *et al*., 2011; Anonymous, 2012). But, captive stocks of sea bream have been drastically decreased than other groups of fishes like snappers and grunts in the last decade in our waters due to over exploitation and mismanagement of fisheries resources (Anonymous, 2012). Feasible remedy for government and farmers in this regard is the culture of this species on commercial scale in order to fulfill the protein needs of the country (Abbas *et al*., 2015; Rahim *et al*., 2015). Fish feed is considered as an important component for such type of aquaculture development on commercial scale (Abbas *et al*., 2011). Feed contributes considerably to the running cost of any culture system. In addition, determination of daily ration is important for sustainable growth and suitable feed conversion of fish (Wang *et al*., 2007; Aderolu *et al*., 2010; Abbas *et al*., 2015). Ration size and frequency varies from species to species and also depend on many physical factors like temperature (Russel *et al*., 1996). Moreover, feeding frequency and ration

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level greater than optimum level may decrease digestive efficiency and also have adverse effect on the water quality (Andrews and Page, 1975; Ng *et al*., 2000). Therefore, an optimum ration level must be investigated for sustainable aquaculture which will not only reduce the running cost but will prevent water quality from deterioration (Abbas and Siddiqui, 2009). Although, some studies have been conducted on the nutrient requirements of demersal fish (Catacutan *et al*., 2001; Abbas and Siddiqui, 2003; Catacutan and Pagador, 2004; Mourente and Bell, 2006). However, no information about ration level and feeding frequency of black fin sea bream, *A. berda* is available. Therefore, present study was planned to determine the optimum ration size and feeding frequency of *A. berda* juvenile growing from 23.5 g to 125.8 g for best growth.

MATERIALS AND METHODS

Experimental feed preparation

The experimental feed (42% protein and 20% lipid) was prepared from the available ingredients containing fish meal as source of protein, and tapioca was used as source of carbohydrate. Cod liver oil was used as source of lipid for energy. Minerals and vitamins were also added to the experimental diets. All components were weighed, grounded and mixed mechanically to realize homogeneity of ingredients. Water (150 ml/kg) was added to the mixture and was remixed. Thus, soft dough was pelleted by 2mm die. These pellets were then dried under shade for 10 h and stored for further use.

Fish juvenile collection and management

Sea bream juveniles were collected from Sonari coast located towards west from Karachi, and they were acclimatized for 15 days prior to start the experiment. After acclimatization, they were randomly distributed into the glass tanks (150 liter) 10 fish in each. All these tanks were supplied with sand-filtered sea water which was aerated continuously. All the fish were placed in similar photoperiod of 12L:12D. Feed was supplied by hand and uneaten feed was collected after 2 h. The water was cleaned daily by siphoning. Fish length and weight were noted fortnightly.

Experimental procedure and feeding trial

In order to determine the effect of ration level and feeding frequency on growth, two separate trials were conducted in the laboratory of Aquaculture, CEMB, University of Karachi. In Experiment I, 10 fish (mean weight 23.5±0.6 g) were stocked in rectangular tanks in three replication. Fish were randomly distributed in rectangular tanks, and were individually weighed. Six levels (2.0, 2.5, 3.0, 3.5, 4.0 and 4.5% of their body weight per day, BW d^{-1}) of the experimental diet were fed to the fish three times a day in each replication for 75 days.

In Experiment II, juvenile sea bream were treated in the same experimental facility as mentioned in Experiment I. Sea bream juvenile (mean weight 23.5±0.6 g) were randomly distributed in rectangular tanks, 10 fish in each tank with three replication. Feed were supplied with 2.5 BW d^{-1} with four feeding frequency (once daily at 0800 h, twice daily at 0800 and 1800h, three times daily at 0800, 1300 and 1800 h, and four times daily 0800, 1120, 1440 and 1800 h) for 75 days. The length and weight of fish were noted fortnightly. Uneaten diet was removed by siphoning and thus feed intake was recorded.

Chemical analysis and measurement

At the end of the experiment, three fishes from each tank were killed and then dissected to calculate the weight of liver and viscera for determining the hepatosomatic index (HSI) and viscerosomatic index (VSI). Three fishes were also collected and killed for carcass analysis. Crude lipid (CL), moisture and crude protein (CP) were determined by using the procedure of Association of Official Analytical chemist (AOAC, 2000). The moisture was estimated at 105°C for 24 h with the help of an oven (Labostar-LG122 Tabia Espec, Osaka, Japan). Crude lipid was estimated by chloroform/ methanol (2:1v/v) extraction procedure (Folch *et al*., 1957). Crude protein was determined by using Kjeldahl method (N×6.25) using automatic Kjeldahl system (Buchi 430/323). Ash was calculated by burning in a muffle furnace. Energy in each treatment was determined with the help of automatic bomb-calorimeter (Parr Instruments, model1265, Moline IL, USA). The data of these parameters were taken as mean of three replicates.

Statistical analysis

The experimental data was analyzed by one way analysis of variance (ANOVA) to determine biological and chemical indices of fish. Difference among means was calculated by 5% probability levels addressing Duncan's multiple range tests (Zar, 1996). Optimum ration level and feeding frequency for maximum growth of black fin sea bream were estimated by the maximum percent weight gain of initial weight. The weight gain (WG), protein efficiency ratio (PER), specific growth rate (SGR), feed conversion ratio (FCR), feed intake (FI) and condition factor (CF) hepatosomatic index (HSI) and viscerosomatic index (VSI) were determined by the following formulae:

$$CF = 100 \times \text{weight} / \text{length}^3.$$

$$SGR = 100 \times (\ln \text{final weight} - \text{in initial weight/period}).$$

FCR = diet given / weight gain).
HSI = weight of liver (g)/empty fish weight (g) ×100.
PER = wet weight gain / N×6.25 intake.
VSI = 100 × [wet weight of visceral organs and associated fat tissue (g) / wet body weight (g)].

RESULTS

Water quality

During the study period, water temperature ranged from 27+0.5°C to 28+0.4°C. Salinity was maintained at 20.2±0.7%. Dissolved oxygen (DO) remained 6.8 ml $l^{-1}$to 7 ml $l^{-1}$and pH was found to be 6.9 to 7.7. Ammonia (NH_4-N) and nitrites (NO_2-N) were not more than 0.1±0.008 ml l^{-1} and nitrates were less than 0.02 ml l^{-1}.

Chemical composition of the experimental diet

The experimental feed was analyzed for achieving desired amounts of protein and lipid. The chemical composition showed that feed contained approximately 42.1% protein, 20.0% lipid, 13.6 % ash, 20.9% carbohydrate, 7.6% moisture and 25.2kJ/g energy (Table I).

Effects of ration size

No disease was noted during the entire experimental duration and thus no mortality was observed. Weight gain (WG) increased considerably (P<0.05) with increasing feeding level up to 2.5% BW d^{-1} (Table II). Best specific growth rate (SGR) was also noted in fish fed with 2.5% BW d^{-1}. But no further increase was observed when feeding level was increased away from 2.5% BW $d^{-1}$to 4.5BW d^{-1}.

Feed conversion ratio (FCR) shows considerable best value for fish fed with 2.5% BW d^{-1} and no enhancement were found when it was increased up to 4.5% BW d^{-1}. Protein efficiency ratio (PER) increased slightly with increase in ration level from 2 to 3.5 BW d^{-1}, but further increase in ration level showed no effect on the PER ratio.

Table I.- Feed formulation and chemical analysis of test diet.

Ingredients[1]	**g 100 g-[1] diet (dry)**
Fish meal	37.5
Tapioca flour	13.6
Lupine seed meal	6.8
Corn gluten meal	7.5
Wheat flour	12
Vitamin-mineral premix	2.6
Cod liver oil	20
Proximate composition[2]	
Moisture	7.6±0.5
Crude protein[3]	42.1±2.3
Crude lipid	20.0±1.0
Crude fiber	3.4±0.7
Ash	13.6±0.9
Carbohydrates[4]	20.9±1.6
Energy (kJ/g)	25.2±1.9

[1]Rahim *et al*., 2015. [2]Dry matter (%): number of samples = 5. [3]Measured as N × 6.25. [4]Carbohydrates = 100 – (%protein + %fat + %ash + %fiber).

Table II.- The weight gain, specific growth rate, feed conversion ratio, protein efficiency ratio, feed intake of juvenile *A. berda* fed at different feeding levels in rectangular tanks (Experiment I).

Parameter	**Ration level (% BW d⁻¹)**					
	2.0	**2.5**	**3.0**	**3.5**	**4.0**	**4.5**
Final weight	85.2±0.7[a]	125.5±0.4[a]	125.8±0.9[b]	125.6±2.4[b]	125.8±1.4[b]	125.9 ±3.8[b]
WG% [1]	262.55±0.5[a]	434.04±0.8[a]	435.31±0.4[b]	434.4±0.6[b]	435.31±0.5[b]	435.7±0.4[b]
SGR[2]	1.71±0.4[a]	2.23±0.3[b]	2.23±0.5[b]	2.23±0.3[b]	2.23±0.4[b]	2.23±0.3[b]
FCR[3]	0.4±0.01[c]	0.20±0.02[a]	0.27±0.01[b]	0.27±0.02[b]	0.27±0.03[b]	0.27±0.02[b]
PER[4]	1.2±0.2[a]	1.3±0.3[b]	1.3±0.3[b]	1.5±0.2[c]	1.4±0.3[bc]	1.5±0.3[c]
FI[5]	35.1±1.3[a]	35.3±1.3[a]	35.2±1.4[a]	35.2±1.3[a]	35.1±1.3[a]	35.1±1.2[a]
CF[6]	2.6±0.1[a]	3.1±0.2[a]	3.1±0.1[a]	3.2±0.2[a]	3.1±0.1[a]	3.1±0.2[a]
HSI[7]	1.2±0.2[a]	1.4±0.1[b]	1.4±0.1[b]	1.4±0.2[b]	1.4±0.2[b]	1.4±0.2[b]
VSI[8]	6.1±0.1[a]	6.9±0.1[b]	7.1±.02[b]	7.5±0.1[bc]	7.7±0.2[bc]	7.7±0.1[bc]
Survival	100	100	100	100	100	100

Similar superscripts show no significant (*P*>0.05) difference among treatments. [1]WG, % of initial weight =100 × [final weight – initial weight / initial weight. [2]CF =100 × weight / length³. [3]FI = diet given as % body weight – remaining diet pellets. [4]SGR =100 × ln final weight – ln initial weight / period. [5]FCR = diet given / WG). [6]HSI = wet of liver (g) / empty fish weight (g) ×100: total of initial was 1.24%. [7]PER = wet weight gain / N×6.25 intake. [8]VSI = 100 × [wet weight of visceral organs and associated fat tissue (g) / wet body weight (g)].

The proportion of moisture, protein and ash contents of fish whole body were not significantly ($P>0.05$) affected by feeding levels (Table III). Hepatosomatic index (HSI) viscerosomatic index (VSI), and condition factor (CF) of fish fed 2.0 BW $d^{-1}$was considerably less than the 2.5, 3.0, 4.0 and4.5% BW d^{-1} (Table III). Feed intake was not influenced by dietary treatments.

Table III.- Whole body composition of *A. berda* fed with diets of different feeding frequency for 75 days. (Experiment I).

Parameter	Feeding frequency (number of meals d-1)			
	1.0	2.0	3.0	4.0
Moisture	70.5±0.07[a]	70.6±0.21[a]	70.8±0.08[a]	70.7±0.21[a]
Protein[1]	17.8±0.14[a]	18.5±0.27[a]	18.9±0.31[a]	18.8±0.14[a]
Lipid	10.1±0.14[a]	12.1±0.71[a]	12.5±0.14[a]	12.9±0.28[a]
Ash	4.4±0.05[a]	4.3±0.06[a]	4.4±0.06[a]	4.1±0.07[a]

Similar superscripts show no significant ($P>0.05$) difference among treatments. Initial body proximate composition was: moisture 71.1%, Protein 17.3%, lipid 9.2% and ash 4.3%. [1]Measured as nitrogen × 6.25.

Table IV.- The weight gain, specific growth rate, feed conversion ratio, feed intake, protein efficiency ratio and condition factor of juvenile *A. berda* at different feeding frequencies for 75 days (Experiment II).

Parameter	Feeding frequency			
	1.0	2.0	3.0	4.0
Final weight	85.5±0.92[a]	95.5±1.41[b]	125.8±0.72[c]	123.6±0.21[c]
WG%[1]	263.8±1.28[a]	306.3±1.21[b]	435.3±1.41[c]	425.9±1.21[c]
SGR[2]	1.72±0.21[a]	1.86±0.28[a]	2.2±0.35[b]	2.2±0.25[b]
FCR[3]	0.13±0.02[b]	0.11±0.02[b]	0.08±0.04[a]	0.082±0.02[a]
FI[4]	34.1±0.21[a]	35.2±0.74[b]	35.2±0.35[b]	35.1±0.61[b]
PER[5]	1.3±0.04[a]	1.3±0.51[a]	1.5±0.08[b]	1.4±0.04[a]
CF[6]	3.1±0.35[a]	3.2±0.21[a]	3.1±0.35[a]	3.1±0.21[a]
HSI[7]	1.3±0.14[a]	1.2±0.35[a]	1.4±0.07[b]	1.4±0.21[b]
VSI[8]	6.5±0.31[a]	7.5±0.21[b]	7.7±0.31[b]	7.5±0.31[b]
Survival	100	100	100	100

Similar superscripts show no significant ($P>0.05$) difference among treatments. [1]WG, % of initial weight =100 × [final weight – initial weight / initial weight. [2]CF =100 × weight / length[3]. [3]FI = diet given as % body weight – remaining diet pellets. [4]SGR = 100 × (ln final weight – ln initial weight / period). [5]FCR = diet given / WG). [6]HSI = wet of liver (g) / empty fish weight (g) ×100: total of initial was 1.24%. [7]PER = wet weight gain / N×6.25 intake. [8]VSI = 100 × [wet weight of visceral organs and associated fat tissue (g) / wet body weight (g)].

Table V.- Whole body composition of *A. berda* fed with diets of different feeding frequency for 75 days (Experiment II).

Parameter	Feeding frequency (number of meals d-1)			
	1.0	2.0	3.0	4.0
Moisture	70.2±1.03[a]	71.3±1.11[a]	70.5±1.04[a]	70.6±1.22[a]
Protein[1]	17.5±0.14[a]	18.8±0.25[a]	18.7±0.21[a]	18.5±0.34[a]
Lipid	10.3±0.12[a]	12.2±0.51[a]	12.6±0.12[a]	12.4±0.17[a]
Ash	4.3±0.03[a]	4.2±0.05[a]	4.3±0.03[a]	4.2±0.03[a]

Similar superscripts show no significant ($P>0.05$) difference among treatments. Initial body proximate composition was: moisture 71.2%, protein 17.1%, lipid 9.3% and ash 4.1%. [1]Measured as nitrogen × 6.25.

Effects of feeding frequency

No pathological symptom and mortality among treatments was noted during this study. Percent WG, SGR, and best FCR were noted in fish fed with three and four times daily. While low percent weight gain and specific growth rate were recorded for the fish fed with once and twice daily (Table IV). PER of fish fed for all treatments was not significantly different. No significant different in feed intake was observed among all treatment. VSI of the fish fed with three and four time a day was found greater than that of one and two times daily. HIS of the fish fed with one time daily was less than that of two, three, and four times. The whole body protein, lipid, moisture, and ash contents of fish were not significantly ($P>0.05$) affected by feeding frequency (Table V). However, lipid content of fish fed two, three and four times daily was considerably ($P<0.05$) greater than fish fed one time daily.

DISCUSSION

In the present study, best growth performance was noted in juveniles of black fin sea bream (*A. berda*) fed at 2.5% BW d^{-1} with feedings of three and four times daily for 75 days. Similar findings were reported by Haruna *et al*. (2014) and Aderolu *et al*. (2010). Fish fed at feeding level of 2.5% BW d^{-1} and feeding frequency of three time daily showed significantly higher WG, SGR and FCR. These results were in agreement with the observations noted for other carnivorous species (Russel *et al*., 1996; Kayano *et al*., 1993; Cho *et al*., 2003; Aderolu *et al*., 2010; Nekoubin and Sudagar, 2012; Al-Zahrani *et al*., 2013). However, it is fact that higher feeding level and feeding frequency have adverse effects on water quality and may increase operation cost (Jobling, 2012). Therefore, feeding to fish should be reduced to a minimal level. This minimal level corresponds to 2.5% BW d^{-1} with three times a day in the present study. Hence optimization of feeding level and feeding frequency is necessary (Abbas *et al*., 2015). Moreover, feeding level less than 2.5% BWd^{-1} decreased weight gain, this indicates that sea bream must require 2.5% BWd^{-1} feeding level for best growth similar to the findings for other fishes with similar feeding behavior (Al-Zahrani *et al*., 2013; Abbas *et al*., 2015). Moreover, when feeding was at 2.0% BW d$^{–1}$ with one and two times a day, fish showed the lowest growth which might have been due to the nutrient requirement for maintenance. It appears that a large proportion of nutrient in the diet was used to maintain life, and only a small proportion was available for growth. Low feeding level and high feeding frequency proved that with excessive ration level, the supplied feed are partially consumed by fish and large amount of feed become useless by dissolved in water deteriorating water quality (Cho *et al*., 2003; Ribeiro *et al*., 2012; Abbas *et al*., 2015). While in moderate ration level and feeding frequency the required amount of feed was available for fish to meet the current feed requirement which enhanced the growth and inhibit water from deterioration parallel with study of many scientist for other demersal fish like sea bass *Dicentrarchus labrax* (Tsevis *et al*., 1992). They found best results about growth performance on low feeding level and high feeding frequency.

Feed intake increased with the increase in feeding frequency from one to three times a day, but further increase in feeding frequency from three to four times do not affect feed intake values. This indicates that further increase in feeding frequency dose not enhance the growth but causes wastage of food. Studies conducted on many fishes revealed that feed consumption as well as growth performance generally improved with feeding frequency up to a cretin limit as described by Abbas *et al*. (2015) which is in line with the present study. It was also noted that excessive feeding of fish caused extra stress on the gut and create gastro problem in digestion (Jobling, 2012). In the present study, PER was not affected by the feeding frequency but ration level slightly affected the PER, increasing slightly with increase in ration level from 1.0% BW d^{-1} to 3.5% BW d^{-1} but further increases up to 4.5% BW d^{-1} have no effects on PER value.

Indices of condition like CF, HIS and VSI play important role for the assessment of nutritional status of fish (Ng *et al*., 2000; Abbas *et al*., 2015). In this study, HSI and VSI are not significantly affected by the feeding frequency. These results tally with the findings of Zakes *et al*. (2006) and Iqbal *et al*. (2015). In addition, similar results were found by Gines *et al*. (2004) for gilthead sea bream *Sparus aurata*. CF was similar among all treatments agreeing with the study of Al- Zahrani *et al*. (2013). They found that feeding frequency did not affect the condition indices.

Keeping in view the effects of ration size and feeding frequency on whole body composition, crude protein was not significantly affected by the feeding level and feeding frequency. This indicates that all diets can provide the dietary protein for maintenance of the fish body but less feeding level and feeding frequency did not enhance the growth as described by Cho *et al*. (2003). Positive correlation was found between feeding frequency and whole body lipid in the present study which is in agreement with the study of Jegede and Olorunfemi (2013). He found direct relationship between lipid and feeding frequency. It is fact that optimization of feeding frequency and feeding level not only improve the growth, FCR, and SGR but also decreases the running cost and water deterioration of any culture operation. On the basis of these results, it is suggested that black fin sea bream *A. berda* (weight 23.5g to 125.5 g) perform best growth on feeding level of 2.5% BW d^{-1} and feeding frequency of three times a day under the present culture conditions.

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Statement of conflict of interest

Authors have declared no conflict of interest.

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