



Effects of Varying Dietary Protein Level on Growth, Nutrient Utilization and Body Composition of Juvenile Blackfin Sea Bream, *Acanthopagrus berda* (Forsskal, 1775)

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ABSTRACT

The optimum dietary protein requirement of juvenile blackfin sea bream, *Acanthopagrus berda* (live body weight 8.1-89.2 g) was investigated. Fish juvenile were collected from Sonari Channel Hawks bay and were maintained in recirculating water system (rearing glass aquaria of 120 lit, water temperature 25°C). Four isoenergetic (20.1 kJ/g digestible energy) diets were formulated to contain protein levels of 20%, 30%, 40% and 50%, and fed one of the experimental diets at a daily ration of 2% body weight for 90 days. Higher weight gain and growth rate were observed in the fish fed with 40% and 50% protein diets. Broken line regression analysis generated an optimum protein levels of 42.0% for blackfin sea bream. Fish whole body composition showed that lipid content of the fish fed with 40% and 50% was lower and moisture content was higher. The hepatosomatic index (HSI) of the fish fed with 40% and 50% was greater than that of the fish fed with 20%-30% protein level. No significant difference was observed in protein and ash contents of whole fish for the diets of 20% to 50% protein. Fish fed 40% and 50% protein diets showed higher nitrogen gain and nitrogen retention efficiency than those fed on other diets. These results suggest that under similar culture conditions, the optimum protein level of the juvenile sea bream (from initial weight of 8.1 g to 89.2 g) is 42.0%.

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Authors' Contribution

AR designed the study and executed the experimental work. AG performed chemical analysis. AM formulated feed. BW managed fish juveniles. MHR helped in data analysis. AR wrote the article and SF and LG helped in writing. GA supervised the research work.

Key words

Protein level, sea bream *Acanthopagrus berda*, meat composition, growth, feed conversion.

INTRODUCTION

Blackfin sea bream, *Acanthopagrus berda* (Forsskal, 1775), is widely distributed in the neritic zone of the temperate, subtropical, and tropical oceans of the world (FAO, 2012). In Pakistan, it is highly prized as a food fish and constitutes a major demersal fish resource of the northern Arabian Sea. Although, there is a considerable commercial fishery (Anon, 2012), its demand has increased to such a level that development and management of cost-effective sea bream culture is important for the expansion of its industry (Sing *et al.*, 2014; Zhang *et al.*, 2014). A preliminary trial (Sarwat, 2014) indicated that sea bream has potential for aquaculture in Pakistan because of its large size, fast growth and resistance to extreme environmental conditions (Sa *et al.*, 2006; Rigos *et al.*, 2011; Rahim *et al.*, 2015). The sustainable aquaculture of this fish depends on nutritionally balanced fish feed. Since protein is the most expensive component of fish feed, optimizing

its dietary concentration is essential to minimize feed cost and to formulate feed, which allows good growth and protein utilization (Alvarez-Gonzalez *et al.*, 2001; Hecht *et al.*, 2003; Ai *et al.*, 2004; Ngandzali *et al.*, 2011; El-Dahhar *et al.*, 2013; Serrana *et al.*, 2013). Mostly, the increase in dietary protein concentration is related with improved fish production and broodstock, particularly for carnivorous fish (El-Sayed and Kawanna, 2008; Zhang *et al.*, 2010). Nevertheless, surplus amount of protein incorporated in diet will be metabolized as an energy source and will upsurge nitrogenous waste products. These products are excreted in water, which may be harmful to fish growth and culture environment (Tibbetts *et al.*, 2000; Lupatsch *et al.*, 2001, 2003; Zhang *et al.*, 2010; Kousar and Javed, 2014; Mahboob *et al.*, 2014). Furthermore, optimal dietary protein requirement of an animal is influenced by some factors like water temperature, feeding habits, stage of development, optimal dietary protein-to-energy balance, the essential amino acid composition and digestibility of the test protein, and the amount of non-protein energy sources in the test diet (Wilson, 2002; Das *et al.*, 2014; Daudpota *et al.*, 2016). Keeping in view the economical as well as environmental perceptions, it is important that diets must comprise protein at levels that not exceed requirements

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for aquaculture animals (Ozorio *et al.*, 2006; Sa *et al.*, 2006; Serrana *et al.*, 2013; Mongile *et al.*, 2014).

Although some work has been done on the nutrition and feeding strategies of different species of sea bream (Johansen *et al.*, 2001; Hernandez *et al.* 2001; Ozorio *et al.*, 2006; Aydin *et al.*, 2011; El-Dahhar *et al.*, 2013), data regarding nutritional requirements and utilization of dietary protein for maximum growth of juvenile black fin sea bream is scarce. In the present study, test diets containing different protein concentration (20%, 30%, 40% and 50%) were supplied to the juvenile sea bream to determine optimal protein level and its effect on growth, feed conversion and body composition to reduce feed cost and enhance aquaculture on commercial basis in the coastal region of Pakistan.

MATERIALS AND METHODS

Experimental diets

Four isoenergetic diets (23.5 kJ/g digestible energy) were formulated to contain different protein level (20%, 30%, 40% and 50%) with fishmeal as the major source of protein (Table I). Wheat flour was used as rich source of carbohydrates. Fish oil was included as energy source. Minerals and vitamins were also added to the experimental diets. All the components were weighed and 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. The pellet of diet was then dried at 27°C for 10 h. The experimental diets were then placed in plastics bags and stored at -4°C till used for the trial.

Fish collection and husbandry

Black fin sea bream juveniles of the same size were collected from Sonari Channel Karachi Coast, Pakistan. Fish juveniles were acclimated to the experimental conditions for 15 days. After completing acclimatization period, a group of 6 fish were randomly distributed into the experimental glass aquaria (120 liters each) supplied with sand-filter and aerated seawater continuously. All aquaria were provided with a photoperiod of 12L:12D (light: 08:00-20:00 h). Dissolved oxygen was maintained up to 6.0 ml/l throughout the experimental period. Temperature was kept constant at 25°C and ammonia was not more than 0.1 ml/l. Fish were fed on daily ration of 2% wet body weight per day (BW day⁻¹) for 45 days. The daily ration was divided into three equal meals and given to the fish at 09:00, 13:0 and 17:00 h. The daily feed was supplied by hand and uneaten feed was collected 2 h after the start of feeding to determine the feed intake. Half amount of the seawater in each

aquarium was replenished daily. Fishes were individually weighed and measured their length weekly.

Chemical analysis

At the end of the experiment, three fish were randomly sampled from each aquarium, anesthetized, dissected and their liver were weighed to estimate hepatosomatic index (HSI). After weighing, the liver samples of the three fish from each aquarium were pooled and stored frozen at -20°C for further proximate composition analysis. Three fish were collected from each aquarium, anesthetized, killed and pooled for whole body composition analysis. Fish whole body samples were taken out of the -20°C cold store and thawed at room temperature. All these samples were homogenized, dried and ground into a powder form for the chemical composition analysis. Prior to start the experiment, two replicate samples with two fish per replicate were taken and kept frozen at -20°C for subsequent analysis of the liver and whole body composition. The moisture, protein, lipid and ash contents of test diets and fish meat samples were analyzed according to the standard methods (AOAC, 2000). The Moisture was determined by drying in an oven (Labostar-LG 122, Tabai Espec, Osaka, Japan) at 105°C for 24h; crude lipid by chloroform/methanol (2:1v/v) extraction procedure (Folch *et al.*, 1957); crude protein by Kjeldahl method (N×6.25) using automatic Kjeldahl system (Buchi 430/323, Flawil, Switzerland); ash by burning in a muffle furnace (Isuzu Seisakusho, Tokyo, Japan) at 550°C for 18 h; gross energy in each treatment was estimated with the help of automatic bomb-calorimeter (Parr Instruments, model 1265, Moline, IL, USA). All chemical analyses were done in triplicate and averaged.

Calculation and statistical analysis

At the end of the experiment, all fish from each tank were individually weighed and their total length was measured for calculation of the condition factor [CF = (100 × body weight in g)/(TL in cm)³]. Growth and feed efficiency were monitored in terms of the final weight, weight gain (expressed as the percent of initial body weight at the end of the experiment), specific growth rate (SGR) (ln final body weight – ln initial body weight/time, expressed as % per day), feed conversion ratio (FCR) (feed fed / wet weight gain), protein efficiency ratio (PER) (wet weight gain/protein intake), protein retention efficiency [(final whole body protein – initial body weight) – (initial whole body protein – initial body weight) / total protein intake] and energy retention efficiency [(final whole body energy – initial body weight) – (initial whole body energy – initial body weight)/total energy intake].

Table I.- Formulation and chemical analysis of the experimental diets.

Ingredients ¹ (%)	Dietary protein (% dry matter DM)			
	A (20%)	B (30%)	C (40%)	D (50%)
Fish meal	19.5	29.5	39.5	49.5
Soybean meal	8.0	16.0	24.0	32.0
Rice bran	14.6	10.8	7.0	3.2
Wheat bran	15.6	11.8	8.0	4.2
Tapioca	14.0	10.0	6.0	2.0
Mustard oil cake	17.6	12.4	7.2	2.0
Cod liver oil	6.5	5.3	4.1	2.9
Vitamin/mineral premix ²	3.0	3.0	3.0	3.0
Fish protein hydrolysate	1.2	1.2	1.2	1.2
Vitamin/mineral premix²				
Moisture	8.9±0.2	9.2±0.3	9.3±0.4	10.2±0.5
Crude protein ⁴	19.8±0.2	24.6±0.3	29.9±0.5	34.7±0.5
Crude lipid	7.4±0.1	7.5±0.3	7.4±0.5	7.4±0.3
Crude fiber	3.5±0.6	5.3±0.5	7.6±0.6	10.5±0.5
Ash	6.5±0.5	7.5±0.4	8.3±0.6	9.8±0.6
NFE ⁵	62.8±0.5	55.1±0.8	46.8±0.3	37.6±0.3
Energy (kJg ⁻¹)	23.2±0.6	23.3±0.4	23.5±0.5	23.6±0.6
P/E (mg crude protein kJ ⁻¹)	8.5±0.5	10.6±0.3	12.7±0.5	14.7±0.4

¹Fish meal (CP=62.1%); soybean meal, *Glycine max* (CP=45.5%); rice bran, *Oryza sativa* (CP=6.2%); wheat bran, *Triticum aestivum* (CP=7.4%); tapioca flour, *Metroxylon sago* (CP=3.1%); soluble fish protein hydrolysate (CP=75.3%) purchased from the local market of Karachi. CP represents crude protein.

²Vitamin and mineral mixture contained the following ingredients (g 100 g⁻¹ diet): Ascorbic acid (vit C), 15.3; thiamin HCl (vit B₆), 1.0; inositol, 39.5; calcium, 1.25; zinc, 1.0; retinol (vit A), 1.0; phosphorus, 3.5; choline chloride, 3.5; magnesium, 2.5; copper, 1.0; pyridoxine (vit B₆), 1.3; phospholipids, 3.5; α -tocopherol acetate (vit E), 5.5; folic acid, 0.4; cholecalciferol (vit D₃), 7.5; cyanocobalamine (vit B₁₂), 0.006; riboflavin (vit B₂), 1.5; menadione sodium bisulphite (vit K₃), 0.03; manganese, 2.0; iodine, 2.0; sodium, 1.0; iron, 1.0; nicotinic acid, 4.3; biotin, 0.35.

³Dry matter basis (%): mean \pm SE, number of determination = 3.

⁴Measured as nitrogen \times 6.25.

⁵Nitrogen-free extract = 100 - (% protein + % lipid + % ash + % fiber).

The data regarding fish growth rate, feed utilization efficiency and body constituents were subjected to one-way analyses of variance (ANOVAs) to determine whether there was a significant difference ($P < 0.05$) among fish fed at different protein levels. Differences between means were assessed at the 5% probability level using Duncan's multiple range test, as described by Steel and Torrie (1980). The data are presented as mean \pm SE of the replicate groups. The optimal dietary protein requirements were estimated from percent weight gain of initial weight using the broken line regression analysis (Robbins *et al.*, 1979; Cowey, 1992).

RESULTS

Body weight gain and SGR of black fin sea bream juvenile fed the 40% and 50% protein diets were significantly ($P < 0.05$) higher than of those fed the 20% and 30% protein diets (Table II). Weight gain and SGR tended to plateau at around 959.6 g and 2.6% day⁻¹ respectively. Based on weight gain, the appropriate

supplementation of dietary protein for the fish was estimated to be 42.0% of diet using broken line regression analysis (Fig. 1). Feed intake, expressed on a dry matter basis, decreased slightly with an increase in dietary protein level. Fish fed the 40% and 45% protein diets showed significantly lower ($P < 0.05$) feed intake than the other groups. The same trend was observed in FCR and PER values. The hepatosomatic index (HSI), of fish fed diets containing 40% and 50% protein were significantly ($P < 0.05$) higher than for those fed diets of 20% to 30% protein (Table II). The survival remained 100% among all groups.

The chemical composition of whole body showed that the moisture content of fish fed diets of 40% and 50% protein was significantly ($P < 0.05$) higher than that of fish fed diets containing protein levels of 20% to 40% in 10% increments, although the lipid contents were lower (Table III). No significant differences were observed in the protein and ash contents of fish fed the diets in all treatments.

Nitrogen intake increased with an increase in dietary

Table II.- Growth and feed utilization of juvenile blackfin sea bream fed practical diets for 90 days.

Ingredients ¹ (%)	Diets (% protein level)			
	A (20%)	B (30%)	C (40%)	D (50%)
Initial weight (g)	8.3±1.2 ^a	8.4±1.6 ^a	8.4±1.3 ^a	8.1±1.5 ^a
Final weight (g)	74.6±1.8 ^a	74.7±2.0 ^a	89.2±1.7 ^b	88.1±1.9 ^b
Weight gain ¹ (%)	798.4±43.1 ^a	789.5±88.3 ^a	959.6±73.5 ^b	984.1±73.5 ^b
SGR ²	2.4±0.02 ^a	2.42±0.05 ^a	2.6±0.04 ^b	2.6±0.05 ^b
Feed intake ³ (g fish ⁻¹)	31.2±1.5 ^b	30.1±2.0 ^b	28.4±1.5 ^a	28.2±1.2 ^a
FCR ⁴	0.47±0.6 ^b	0.46±0.2 ^b	0.35±0.3 ^a	0.35±0.4 ^a
PER ⁵	1.4±0.06 ^b	1.3±0.5 ^b	1.0±0.7 ^a	0.9±0.3 ^a
CF ⁶	2.6±0.05 ^a	2.5±0.06 ^a	2.6±0.04 ^a	2.5±0.3 ^a
Survival (%)	100	100	100	100

Values (means±SE, n = 3 and each n consists of 6 fish per replicate) in the same row with different superscripts are significantly different ($P>0.05$). Initial body weight of the fish was 8.0 ± 0.3 g.

¹Weight gain (%) = $100 \times [\text{final body weight} - \text{initial body weight} / \text{initial body weight}]$.

²Specific growth rate = $100 \times [\ln \text{ final body weight} - \ln \text{ initial body weight} / \text{time in days}]$.

³Feed intake = total feed fed as % body weight - total uneaten feed.

⁴Feed conversion ratio = total feed fed (g) / total wet weight gain (g).

⁵Protein efficiency ratio = wet weight gain / protein ($N \times 6.25$) intake.

⁶Condition factor (CF) = $100 \times (\text{weight} / \text{length}^3)$.

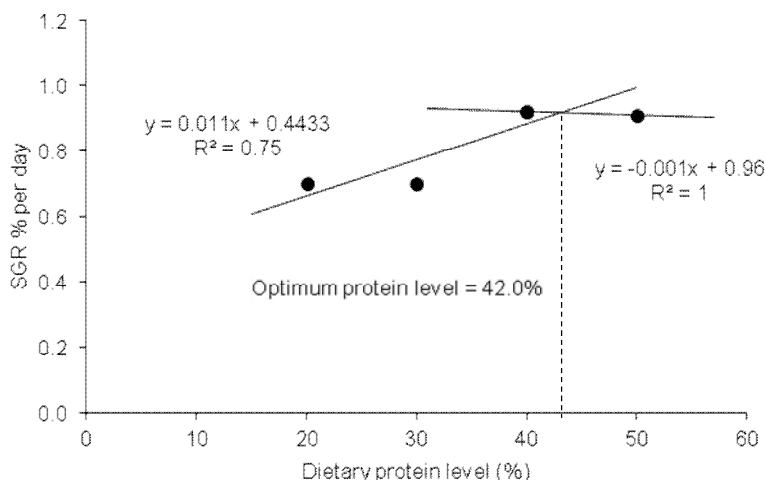


Fig. 1. Optimal protein level of juvenile blackfin sea bream, *A. berda* based on percent SGR as determined by the broken line model.

protein (Table IV). The amount of protein taken in by the fish fed 30% and 50% protein diets was significantly different ($P<0.05$) from that of fish fed diets containing 20% protein and 30% protein diet being intermediate. A similar trend was observed in nitrogen gain of the fish whole body. Fish fed 40% and 50% protein diets showed higher nitrogen gain than those fed on all other diets ($P<0.05$). Fish fed diets containing 40% and 50% protein had a significant better NRE than those of fish given 20% and 30% protein (Table IV).

Gross energy intake (GEI) of fish showed a linear decrease as protein level increased over the whole range of dietary protein levels. Although GEI in the fish fed 50% protein was lower (569.2 kJ) than that of 40% protein diet (573.2 kJ), the differences were not statistically significant ($P>0.05$); GEI ranging from 702.7 kJ to 713.2 kJ at remaining two diets (20% to 30% protein) did not appear to differ significantly ($P>0.05$, Table IV). The highest energy retention efficiency (ERE) of 91.7% was found in fish fed diet of 40% protein.

Table III.- Whole body composition (% wet weight) of juvenile blackfin sea bream fed practical diets for 90 days.

Ingredients ¹ (%)	Diets (% protein level)			
	A (20%)	B (30%)	C (40%)	D (50%)
Moisture	72.3±1.1 ^a	73.1±2.1 ^a	72.3±1.2 ^a	73.6±3.5 ^a
Crude protein	18.3±2.4 ^a	18.2±2.2 ^a	18.6±1.5 ^a	18.5±3.3 ^a
Crude fat	8.84±1.2 ^b	7.28±1.3 ^b	6.95±1.20 ^a	6.12±1.10 ^a
Ash	1.4±0.1 ^a	1.4±0.2 ^a	1.7±0.2 ^a	1.6±0.4 ^a
APR	32.1±2.6 ^b	31.52±1.5 ^b	29.27±3.13 ^a	28.2±3.3 ^a
HSI ²	0.32±0.01 ^a	0.41±0.01 ^a	2.0±0.1 ^b	1.9±0.1 ^b

Values (mean±SE, n=3 and each n consists of 6 fish per replicate) in the same row with different superscripts are significantly different ($P>0.05$). Chemical composition of initial body was: moisture 72.8%, protein 18.7%, lipid 6.3% and ash 1.5%, and total lipid contents of muscle, liver and viscera were 0.6%, 7.4% and 12.9%, respectively.

²Hepatosomatic index (HSI) = wet liver weight (g) / empty fish weight (g) × 100; that of the initial fish was 1.24%.

Table IV.- Nutrient utilization of juvenile blackfin sea bream fed practical diets for 90 days

Ingredients ¹ (%)	Experimental diets (% protein level)			
	A (20%)	B (30%)	C (40%)	D (50%)
Nitrogen intake ¹	1.4±0.3 ^a	1.5±0.2 ^a	1.8±0.1 ^b	2.2±0.4 ^b
Nitrogen gain ²	0.3±0.01 ^a	0.4±0.03 ^a	0.8±0.05 ^b	0.9±0.02 ^b
Nitrogen retention ³	20±0.01 ^a	20±0.02 ^a	40±0.01 ^b	40±0.02 ^b
Energy intake	713.2±4.7 ^b	702.7±3.5 ^b	573.2±6.7 ^a	569.2±4.9 ^a
Energy gain ⁴	572.4±22.2 ^b	583.4±14.2 ^b	523.5±25.4 ^a	513.1±10.5 ^a
Energy retention ⁵	80.26±0.13 ^a	83.02±0.11 ^a	91.7±0.01 ^b	90.2±0.01 ^b

Values (means±SE, n = 3 and each n consists of 6 fish per replicate) in the same row with different superscripts are significantly different ($P>0.05$). Initial body weight of the fish was 8.0±0.3 g.

¹Nitrogen intake (g fish⁻¹) = feed intake per fish × nitrogen content of feed.

²Nitrogen gain (g fish⁻¹) = nitrogen in whole body of final fish – nitrogen in whole body of initial fish.

³Nitrogen retention (%) = nitrogen gain / nitrogen intake × 100.

⁴Energy gain (kJ fish⁻¹) = energy in whole body of final fish – energy in whole body of initial fish.

⁵Energy retention (%) = energy gain / energy intake × 100.

DISCUSSION

In the present study, the dietary protein levels of 40% and 50% with 23.6 kJ g⁻¹ digestible energy were adequate to optimize both the weight gain, and the SGR in juvenile blackfin sea bream growing from 8.1 g to 89.2 g. On the basis of maximum SGR, the estimated protein requirement of the fish was 42.0%. Similar results have been reported in other fish such as sharp snout sea bream, *Diplodus puntazzo* (Coutinho *et al.*, 2012), black spot sea bream, *Pagellus bogaraveo* (Silva *et al.*, 2006), Japanese sea bass, *Lateolabrax japonicus* (Ai *et al.*, 2004), blake sea bream, *Sparus macrocephalus* (Zhang, 2010), golden snapper, *Lutjanus johni* (Hussain and Abbas, 1995), red snapper, *Lutjanus campechanus* (Miller *et al.*, 2005), mangrove red snapper, *Lutjanus argentimaculatus* (Abbas and Siddiqui, 2013), blackspot seabream, *Pagellus bogaraveo* (Silva *et al.*, 2006), gilthead seabream, *Sparus aurata* (Santinha *et al.*, 1996), spotted sand bass, *Paralabrax maculatofasciatus* (Alvarez-

Gonzalez *et al.*, 2001); haddock *Melanogrammus aeglefinus* (Kim and Lall, 2001) and singhi, *Heteropneustes fossilis* (Siddiqui and Khan, 2009) which have shown that growth and FCRs improve with high protein diets. In this study, the dietary protein requirements for the growth of blackfin sea bream seem to be in the same range as other marine carnivorous fish species (NRC, 1993). Some studies in gilthead seabream (Santinha *et al.*, 1996), European seabass, *Dicentrarchus labrax* (Peres and Oliva-Teles, 1999), spotted sand bass (Alvarez-Gonzalez *et al.*, 2001) and Japanese seabass, *Lateolabrax japonicus*, Cuvier (Ai *et al.*, 2004) have estimated 40% to 55% as the optimal dietary protein level in terms of growth performance. In the present study, when dietary protein concentration was above 42.0%, mean percent weight gain decreased significantly ($P<0.05$). This indicates that weight gain maxima may be identified in a range of dietary protein concentration from 40% to 50% as suggested by Abbas and Siddiqui (2013). According to him, broken line model or an asymptotic

model is preferable in attempting SGR gain maxima similar in the present study.

Although, an increase in dietary protein causes a decrease in PER and NRE (Lupatsch *et al.*, 2003; Guerreiro *et al.*, 2012; Abbas and Siddiqui, 2013; El-Dahhar *et al.*, 2013), a linear increase in nitrogen gain is generally observed until the requirement level is met. This indicates that excess protein is catabolized to provide energy for growth (Sa *et al.*, 2006; Rigos *et al.*, 2011; El-Dahhar *et al.*, 2013). Similar trend was observed in Arctic char (Gurure *et al.*, 1995), haddock (Kim and Lall, 2001), mangrove red snapper (Abbas and Siddiqui, 2013) and blackfin sea bream in the present study. As the dietary protein level increased, feed intake decreased resulting in a decrease in FCR. This shows that an increase in dietary protein energy could be more beneficial to feed utilization than an increase in lipid energy in the diet (Ai *et al.*, 2004, Ngandzali *et al.*, 2011; El-Dahhar *et al.*, 2013; Abbas and Siddiqui, 2013). High protein utilization of low protein diets has been observed in many species (El-Sayed and Kawanna, 2008; Zhang *et al.*, 2010; Serrana *et al.*, 2013; Mongile *et al.*, 2014). In this study, although the diets of protein levels 20% and 30%, had significantly high PER, the SGR values were low. This indicates that blackfin sea bream could have efficiently utilized the low protein diet for protein synthesis, thus increasing PER value and suggesting a compensatory mechanism (Catacutan *et al.*, 2001; Ozorio *et al.*, 2009; Abbas *et al.*, 2011, 2015; Aydin *et al.*, 2011; El-Dahhar *et al.*, 2013; Abbas and Siddiqui, 2013).

Dietary protein contents are generally known as the criterion constituents for determining the quality of fish flesh (Serrana *et al.*, 2013; Mongile *et al.*, 2014). In the present study, as dietary protein concentration increased, fat content decreased as in another fish (Ozorio *et al.*, 2006; Sa *et al.*, 2008). These results are substantiated by the findings of Hecht *et al.* (2003), Ai *et al.* (2004), Ngandzali *et al.*, (2011), Zhang *et al.* (2010), Aydin *et al.* (2011), Mongile *et al.* (2014). They observed that the fat contents of fish appeared to be influenced by feeding rhythm with age; correlation among them was positively significant. Similar strong correlation was also observed in the results of the present study. This relationship suggests that as the fish grows its weight increases and proportionately most of this increase is present in the form of fat in fish (Zhang *et al.*, 2010). The fish first consumes this fat from the liver and starts mobilizing muscle protein only when fat-derived energy has been nearly used up (El-Dahhar *et al.*, 2013). After that as protein is utilized, water moves in to take its place. Such a shift results an increase in moisture content of the body. Abbas and Siddiqui (2013) found that moisture content was not significantly correlated with either dietary

protein or metabolizable energy for mangrove red snapper. In the present study, no statistically significant differences in moisture and crude protein contents were found among fish fed with diets 20% to 50%, though moisture content showed a clear inverse relationship with crude fat contents (El-Sayed and Kawanna, 2008; Zhang *et al.*, 2010). Evidence to support this is available in other studies of the relationship between protein and water contents in different fish species. Tibbetts *et al.* (2000), for example, found that in non-fatty fish, as protein is removed from the muscle, the moisture content rises steadily. Mongile *et al.* (2014) and Zhang *et al.* (2010) concluded that with increasing fat content, the water content fell (*i.e.*, the dry matter content increased and vice versa). A clear inverse relationship between fat and water content was found and there appeared to be a mechanism for some homeostasis of tissue volume. Additional energy stored as fat replaced body water and did not adversely affect the deposition of protein. The protein content was approximately constant since fat has a protein sparing action in fish. Sa *et al.* (2006) reported that the dry matter and fat in muscle of rainbow trout were positively correlated. A variation in dry content was caused mainly by a variation in fat content. Fat and water to a certain degree substitute each other. In the present study, body fat contents reflected the same of the diets. From this fact, it could be said that 6.5% lipid and 42% protein should be included in practical diets if appropriate protein and energy levels are provided (Aydin *et al.*, 2011). The apparent protein retention (APR) varied inversely with dietary protein. The APR was significantly different in fish fed with diets of 40% and 50% protein than fish fed with diets of 20%–30%.

In the present study, except for lipid content, which was low in fish fed with diets containing 40% and 50% protein, whole body composition was not affected by the dietary treatments. Since protein constitutes the expensive component of the diet and its high concentrations in the diet are counterproductive (Fotedar, 2004; Ozorio *et al.*, 2009; Maldonado-Garcia *et al.*, 2012; Bonaldo *et al.*, 2014). Therefore, protein concentration when developing nutritionally balanced diet should be reduced to a minimum level as suggested by Mongile *et al.* (2014). The dietary protein to energy ratio (P/E) that provided the maximal SGR (2.6%) was found to be 12.7–14.7 mg protein kJ^{-1} digestible energy for juvenile blackfin sea bream fed diets of 40% and 50% protein. This ratio was very close to the reported optimum value (22.0 mg protein kJ^{-1}) for the other fish fed 42.6% dietary protein (Catacutan *et al.*, 2001; Abbas and Siddiqui, 2013; Abbas *et al.*, 2015). In conclusion, the diet containing 40 to 42% dietary protein with P/E ratio of 12.7 mg protein kJ^{-1} could be considered as optimum for

the growth of blackfin sea bream juveniles under the experimental conditions of the present study.

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Statement of conflict of interest

Authors have declared no conflict of interest.

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