Potential of poultry by-product meal as a main protein source in diets formulated for juvenile sobaity (*Sparidentex hasta*)

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Abstract

A 60-day feeding trial was conducted to evaluate the potential of using poultry by-product meal (PBM) as a partial replacement for fish meal (FM) in the diets for juvenile sobaity. Six iso-nitrogenous and iso-lipidic diets were formulated to contain graded levels of PBM, at 0 (as control diet), 15, 25, 35, 45, and 55% of FM replacement. Each diet was fed to groups of 20 juvenile sobaity in triplicate 300-L tanks, three times a day to apparent satiation. Survival, feed conversion ratio and efficiency ratio showed that there were no significant differences between fish fed PBM based diets compared to fish fed the reference diet. Growth performance and protein efficiency ratio of fish fed the PBM15 and 25 were higher than in other treatments. The somatic indices, biochemical content of whole body and fillet, hematological factors were not significantly affected by the replacement level of FM with PBM, with the exception of serum cholesterol and triglyceride content. The results of the present study indicated that PBM is a suitable replacement for fish meal in juvenile sobaity diet up to 55% substitution.

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Introduction

Sobaity, *Sparidentex hasta*, (Valenciennes, 1830) is a carnivorous marine fish distributed in tropical waters of the Western Indian Ocean and the Persian Gulf. It usually inhabits coastal shallow waters and gulf waters, and feeds on shrimp, cephalopod, fingerlings and crabs (Pavlidis and Mylonas, 2011). Because of its rapid growth, effective feed utilization, high market value, spawning in captivity, ability to withstand a wide range of culture conditions, it has been regarded as the greatest potential among all candidate species for culture in the Persian Gulf and Oman Sea regions, where it currently supports a small but well established sector of aquaculture (Pavlidis and Mylonas, 2011). In recent years, this species has been regularly propagated in the Mariculture Research Station of the South Iranian Aquaculture Research Center (SIARC, Sarbandar, Iran). Juvenile fish have subsequently been released into the Persian Gulf to increase stock enhancement or transferred to sea cages for marine fish aquaculture extension projects (Mozanzadeh *et al.*, 2015). In 2011, an estimated 550 metric tons of sobaity was produced from farming activities in the region (FAO, 2014). Some nutrient requirements including dietary optimal level of protein, and energy have been investigated (Mozanzadeh *et al.*, 2016). However, currently the limited supply of trash fish as the main source of food for grow-out, difficulties in storage, variable nutritional quality and cost and low feed conversion rate could be the crucial constraint for sobaity culture in Iran. Thus, nutritionally complete artificial diets at lower cost (especially reducing fish meal usage) could be a substitute for the trash fish feed (Zhou *et al.*, 2005).

Fish meal (FM) is well recognized as the best dietary protein source in formulated diets of most marine carnivorous fish (NRC, 1993), for its balanced amino acid profile, high digestibility and palatability, the presence of potential growth factors (Craig and McLean, 2005). However, FM production has been nearly constant annually and demand for FM is growing, putting pressure on fishery stocks and causing the price of FM to increase. The search for FM substitutes and alternative dietary protein sources has become an international research priority (Lee, 2002). Therefore, alternative proteins, containing dietary plant and animal protein, have been studied by many fish nutritionists and the feed industries (Tacon and Jackson, 1985; Enyidi *et al.*, 2014; Rodríguez-Miranda *et al.*, 2015). However, plant protein inclusion has normally been limited due to deficiencies in essential amino acids, anti-nutrient factors and poor palatability (Gomes *et al.*, 1995). Rendered animal protein ingredients are good sources of amino acids, with high protein content (Bureau *et al.*, 1999; Zhou *et al.*, 2004). Poultry by-product meal (PBM) is one potentially rendered animal protein which has been tested in diets for some fish species (Fowler, 1991; El-Sayed, 1994; Quartararo *et al.*, 1998; Nengas *et al.*, 1999; Kureshy *et al.*, 2000; Goto *et al.*, 2001; Turker *et*

al., 2005; Wang et al., 2006; Yigit et al., 2006; Shapawi et al., 2007; Usman et al., 2007; Zhou et al., 2011; Amirkolaie et al., 2014; Gunben et al., 2014; Hernandez et al., 2014; Wang et al., 2015) and it was concluded that this ingredient is cost-effective and a valuable protein source for these species. In this sense, some studies suggest that FM may be partially replaced by PBM, consequently providing a substantial savings in feed costs without a decrease in growth performance.

As far as we know, currently there is no published information on the use of PBM in the diets of sobaity. The primary objective of the present research was to evaluate the effects of iso-nitrogenous replacement of FM with PBM in practical diets on weight gain, feed efficiency, body composition, hematological and serum biochemical parameters in juveniles of this species.

Materials and methods

Ingredients and experimental diets

The reference diet, which utilized kilka fish meal (FM) and soy-bean meal as protein sources and kilka oil as the lipid source, was formulated to contain 50 % crude protein and 18 % lipid (dry matter basis). This diet satisfied all known nutrient requirements of sobaity (Mozanzadeh, et al., 2015). Six is-onitrogenous (ca 50%) and iso-lipidic (ca 18%) experimental diets were formulated with WUFFDA software and the proximate analysis of the diets is given in Tables 1 and 2. The experimental diets were formulated to produce diets in which 0 (PBM0), 15 (PBM15), 25 (PBM25), 35 (PBM35), 45 (PBM45) and 55% (PBM55) of protein from FM was replaced by that from poultry by-product (PBM). Poultry by-product and kilka FM were provided by the Beiza-Iranian company, and the other feed ingredients were obtained from local markets. All the dry ingredients were thoroughly mixed for 30 minutes till homogenous in a Hobart type mixer, then lipid and water were added and thoroughly mixed, pellets (3.0 mm in diameter) were produced and air-dried to about 10% moisture, sealed in packed bags , and stored frozen (-20 $^\circ$C) prior to use in trial.

Fish management

This study was carried out in the Mariculture Research Station of the South Iranian Aquaculture Research Center (SIARC), Sarbandar, Iran. Two hundred and forty juveniles of sobaity produced at the same hatchery of Sarbandar station were used. Fish were randomly distributed into 18 cylindrical polyethylene tanks of 300 L in volume, and each tank was stocked with 20 fish (mean body weight 29.27±0.1, mean±standard error). Fish were acclimated for 2 weeks before the onset of the nutritional trial.

Tanks were supplied with filtered running sea water 1(l min^{-1}). Salinity was 48.0±0.5‰ and temperature was 23.48±1.2 $^\circ$C during the experimental period. Average values for dissolved oxygen and pH were 6.8±0.4 mg L^{-1} and 7.37±0.2, respectively. The photoperiod was natural during the trial

(30°32′ N, 49° 20 ′ E). Triplicate groups of fish were fed one of the above mentioned diets by hand to visual satiation three times daily (08:00 h, 13:00 h and 17:00 h) for 60 days.

Table 1: Formulation of the experimental diets were fed by juvenile sobaity for 60-day.

Ingredients	Diets					
	PBM0	PBM15	PBM25	PBM35	PBM45	PBM55
FM[1]	61	52	46	40	34	28
PBM[2]	0	11	18	25	32	39
SM[3]	11	11	11	11	11	11
Wheat middling	5	5	5	5	5	5
Fish oil	8.5	7.5	6.5	5.5	4.5	3.5
Soybean lecithin	1.5	1.5	1.5	1.5	1.5	1.5
Corn starch	5.5	4.5	4.5	4.5	4.5	4.5
Gelatin by-prod	4	4	4	4	4	4
Vitamin Premix[a]	1.5	1.5	1.5	1.5	1.5	1.5
Mineral Premix[b]	1.5	1.5	1.5	1.5	1.5	1.5
Stay-C	0.5	0.5	0.5	0.5	0.5	0.5
Betain	0.5	0.5	0.5	0.5	0.5	0.5

1 FM: Fish meal, 2. PBM: Poultry by- product, 3: SM: soy bean meal
a)Vitamin premix: Vit. A, 2000 IU Kg^{-1}; Vit. D3, 800 IU Kg^{-1}; Vit E, 88 IU Kg^{-1}; Vit K, 3 mg Kg^{-1}; Vit C, 200 mg Kg^{-1}; Vit B1, 12 mg Kg^{-1}; Vit B2, 14 mg Kg^{-1}; Vit B5, 70 mg Kg^{-1}; Vit B3, 50 mg Kg^{-1}; Vit B6, 12 mg Kg^{-1}; Vit B9, 3 mg Kg^{-1}; Vit B12, 0.016 mg Kg^{-1}; Vit H2, 0.14 mg Kg^{-1}. DamloranPharmaceutical Company, Broujerd, Iran.
b) Mineral Premix: Selenium, 0.168mg Kg^{-1}; Iron sulfate, 20mg Kg^{-1}; Copper sulfate, 2 mg Kg^{-1}; Calcium iodate, 2 mg Kg^{-1}; Zinc oxide, 33.2 mg Kg^{-1}; Cobalt, 0.336 mg Kg^{-1}; Manganese oxide, 16.8mg Kg^{-1}.

Table 2: proximate compositions of protein ingredients and diets were fed by sobaity for 60-day feeding trail (% in dry matter).

Ingredients	Dry matter	Crude protein	Crude lipid	Crude fiber	Ash	Calcium	Phosphorus
FM	92.6	70.17	9.42	0.89	13.09	2.63	2.35
PBM	93.12	60.52	23.38	1.2	4.11	3.9	6.62
SM	95.42	42.11	0.16	2.1	4.04	nd[*]	nd[*]
Diets							
PBM0	93.65	50.24	17.18	2.38	10.32	3.50	3.68
PBM15	93.18	50.39	17.85	2.40	10.52	2.90	3.62
PBM25	92.90	50.05	17.52	2.42	9.95	3.08	3.21
PBM35	92.62	50.29	17.41	2.44	10.01	3.00	3.19
PBM45	92.34	50.47	17.30	2.46	8.49	2.57	4.27
PBM55	92.06	50.45	17.19	2.35	9.25	3.80	3.42

*None detected

Samples collection techniques

At the termination of the 60-day feeding trial, 24 hours after the last feeding fish in each tank were also anesthetized with (2-phenoxyethanol at 0.5 ml L^{-1}; Merck, Schuchardt, Germany) and individually weighed. Blood was collected from the caudal vein of 4 fish (n=12 fish per diet treatment) by 2 mL heparinized syringes, and it was liquated into three parts (each 1 ml). An aliquot of blood was evaluated for hematological factors and another aliquot for hemoglobin, and the rest was centrifuged (3000 g, 10 min, 4 °C), to separate serum. Vials containing serum samples were transferred into liquid nitrogen and

stored at -80 $^{\circ}$C until their analysis, then these fish were sacrificed with an overdose of this anesthetic to evaluate somatic indexes and fillet composition. Ten fish from the initial population and three fish from each tank at the end of experiment were sampled randomly and sacrificed with an overdose then stored in (-80 $^{\circ}$C) to determine the whole body composition.

Standard formulae were used to assess growth performance: percentage weight gain (%WG), specific growth rate (SGR); survival rate (SR); somatic indexes: viscera somatic index (VSI), intraperitoneal fat ratio (IPF), hepatosomatic index (HSI), condition factor (CF); feed utilization: feed conversion ratio (FCR), protein efficiency ratio (PER), net protein utilization (NPU) and economic performance of the diets: reduced cost (RC) and profit index were calculated as follows:

$WG(\%)=100 \times (W_t - W_0)/W_0$;

$SGR\ (\%\ \text{fish-1})=100 \times (LnW_t - LnW_0)/t$;

$SR(\%)=100 \times N_t/N_0$;

$VSI(\%)=100 \times W_v/W_t$;

$IPF(\%)=100 \times W_{pf}/W_t$;

$HSI(\%)=100 \times W_l/W_t$;

$CF(\text{g cm}^{-3})=100 \times W_t/Ls^3$;

$FCR=I/(W_t - W_0)$;

$PER=(W_t - W_0)/(I \times C_{Nf})$;

$NPU=[(C_{Nt} - C_{N0})/C_{Nf}] \times 100$;

$RC=100-(100 \times (ECR\ diet/ECR\ Control\ diet))$;

$PI=C_f(kg)/C_N$

Where I (g) is the total amount of the test diets fed on a dry matter basis; W_0(g) is the total initial body weight and W_t(g) is the total final body weight; L_s(cm), W_v(g), W_{pf} and W_l(g) are the final body length, viscera weight interperitoneal fat and liver weight; t (d) is the duration of the feeding trial; N_t is the number of fish at the end of the feeding trial and N_0 at the start; C_{Nt} is the crude protein of the whole body at the end of the feeding trial and C_{N0} is the crude protein sobaity at the start of trail, C_{Nf}, is the crude protein, contents of the test diets, ECR is economic coefficient ratio=FCR×Cost of feed, C_f is the value of fish; C_N is the cost of feed.

Biochemical analyses

Proximate analyses of ingredients, diets, whole fish bodies and fillet were determined using standard methods (AOAC, 2005). Moisture was determined using a moisture analyzer (AM B5 0, AD AM, UK). Protein was determined by measuring nitrogen using the Kjeldahl method (BÜCHI, Auto-KjeldahlK-370, Switzerland). To convert total nitrogen to total protein content, as a percentage of dry weight, the factor 6.25 (100/16) was used. Total body lipid was extracted by petroleum benzene using the Soxhlet method (Barnstead/Electrothermal, UK). Fiber content was analyzed with a fiber analyzer (VELP® Scientifica, Italy) while the ash content was determined for each dried sample in a porcelain crucible using a muffle furnace (Finetech, Shin Saeng Scientific, South Korea) a t 600 °C for 8 h.

Hematological and serum biochemical analyses

Hematocrit (Hct%) was measured by micro-centrifugation and the

determination of the percentage of packed cell volume after blood centrifugation in standard heparinized micro hematocrit capillary tubes; 3500 g, 10 min at room temperature (Barros *et al.*, 2002). Hemoglobin (Hb; g dl^{-1}) concentration was spectrophotometrically assayed by the cyanmethemoglobin method (Blaxhall and Daisley, 1973). Blood indices including (MCHC) were calculated according to the formula (Dacie and Lewis, 2001):

Mean cell hemoglobin concentration MCHC (g dl^{-1})=Hb(g dl^{-1})/Hct(%)

Serum biochemical parameters were analyzed by means of an auto-analyzer (Mindray BS-2 00, China) using commercial clinical investigation kits (Pars Azmoon Kit, Tehran, Iran). Biochemical measurements were conducted for glucose, total protein, albumin, total cholesterol, triglyceride, calcium, magnesium and inorganic phosphorous. Moreover, the content of total globulin was estimated by subtracting albumin from total protein (Kumar *et al.*, 2005).

Statistical analyses

The data are presented as means±standard error of the mean calculated from three replicates. The data for each parameter were tested for normality and homoscedasticity. A one-way analysis of variance was performed with diet as the independent variable. A Tukey's HSD test was used for post hoc identification of significant differences among the dietary treatment groups at a significance level of 95%. All of the statistical procedures were performed using SPSS ver.19.0 software (Chicago, Illinois, USA).

Results

Growth performance, feed utilization and economic indexes

All fish were acclimated quickly to the experimental diets from the beginning of the trail period. Initial weight of fish stocked in the growth trial did not differ among dietary treatments and averaged 29.27±0.178 g. The fish growth performance and feed utilization are shown in Table 3. No significant differences were observed for final body weight (FBW), weight gain (%WG), specific growth rate (SGR), protein efficiency ratio (PER) of juvenile sobaity between reference group and other experimental groups (p>0.05). Fish fed diets PBM15, PBM25 had significantly higher protein efficiency ratio (PER) than PBM55 (p<0.05). Net protein utilization (NPU) did not differ between experimental diets, except PBM15, that had a significantly higher value than PBM0. FCR and survival (%S) were not significantly different between PBM0 and other experimental groups (p>0.05). A quadratic regression analysis indicated that %WG (%WG=0.037 (%FM replacement)^2+1.76 (%FM replacement)+103.0; R^2=0.87) and PER (Fig. 1) reached the maximum value, where the replacement level of fish meal with PBM occurred at 23.75%. Reduced cost (RC) of diets increased with increasing level of FM substitution, RC of PBM55 was about 41% in comparison with the control

diet. Profit index (PI) showed the same trend with level of inclusion of PBM in

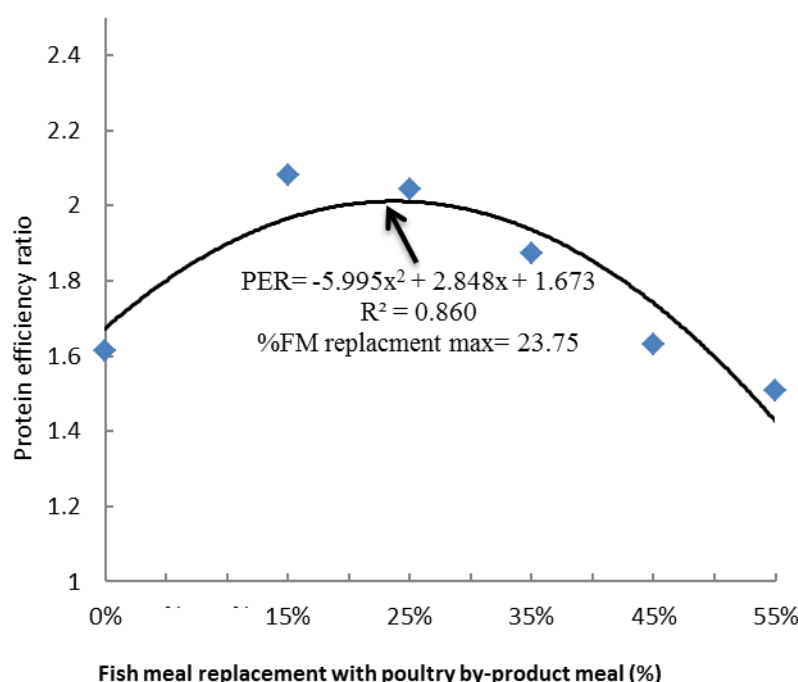diets and ranged from 4.32 to 7.28 in PBM0 to 55.

Figure 1: The relationship between protein efficiency ratio and dietary FM replacement with PBM in juvenile sobaity diets.

Table 3: Growth performance and feed utilization of juvenile sobaity and economic indexes of experimental diets.

Diets	PBM0	PBM15	PBM25	PBM35	PBM45	PBM55
IBW[d]	29.17±0.104	29.29±0.131	29.44±0.122	29.00±0.115	29.28±0.167	29.41±0.162
FBW[e]	58.24±2.369[ab]	66.74±1.656[a]	66.22±2.013[a]	62.71±1.248[ab]	58.65±0.95[ab]	56.58±2.473[b]
WG(%)[f]	99.60±7.528[ab]	127.78±4.692[a]	124.92±6.853[a]	116.23±3.652[ab]	100.32±2.840[ab]	92.45±9.284[b]
SGR [g]	1.15±0.062[ab]	1.37±0.034[a]	1.35±0.05[a]	1.28±0.028[ab]	1.16±0.24[ab]	1.09±0.082[b]
FCR[h]	1.25±0.092	0.96±0.039	0.98±0.052	1.07±0.038	1.23±0.037	1.24±0.131
PER[i]	1.62±0.128[ab]	2.08±0.085[a]	2.04±0.112[a]	1.87±0.064[ab]	1.63±0.049[ab]	1.51±0.145[b]
NPU[j]	20.96±1.596[b]	31.22±2.114[a]	30.20±2.685[ab]	29.03±2.829[ab]	24.73±0.396[ab]	24.95±2.276[ab]
S(%)[k]	95.00±5.00	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00	96.67±3.33
RC[l]	0	31.55	35.88	36.26	33.95	40.69
PI[m]	4.32	6.31	6.74	6.78	6.54	7.28

IBW[d]: initial body weight (gram), FBW[e]: final body weight (gram), WG(%)[f]: Percent weight gain, SGR[g]: specific growth rate, FCR[h]: feed conversion ratio, PER[i]: Protein efficiency ratio, NPU[j]: net protein utilization, S(%)[k] :Survival, RC[l]: Reduced cost, PI[m]: profit index.

Somatic indexes

No significant differences were observed for hepatosomatic index (HSI) and viscerosomatic index (VSI) of juvenile sobaity fed the reference diet and PBM-based diets (p>0.05; Table 4). Fish fed the PBM15 diet had a

significantly higher intra-peritoneal fat index (IPF) than fish fed the other experimental diets (p<0.05). Condition factor (CF) was not significantly different between the PBM (0, 25, 35 and 45).

Table 4: Somatic indexes, whole body and fillet composition (% in dry matter) of juvenile sobaity fed the experimental diets for 60 days.

Somatic indexes	PBM0	PBM15	PBM25	PBM35	PBM45	PBM55
CF[d]	3.06 ± 0.043^a	2.90 ± 0.028^b	2.96 ± 0.034^a	2.96 ± 0.031^a	3.06 ± 0.044^a	2.85 ± 0.037^b
HSI[e]	1.43 ± 0.097^{ab}	1.74 ± 0.071^a	1.27 ± 0.046^b	1.74 ± 0.066^a	1.44 ± 0.061^{ab}	1.67 ± 0.094^a
VSI[f]	6.96 ± 0.255^{ab}	7.82 ± 0.238^b	6.27 ± 0.250^b	7.21 ± 0.280^{ab}	6.48 ± 0.229^b	7.26 ± 0.371^{ab}
IPF[g]	2.01 ± 0.240^{bc}	3.53 ± 0.240^a	1.80 ± 0.224^c	2.92 ± 0.304^{ab}	1.63 ± 0.198^c	2.54 ± 0.253^{abc}
Whole body composition						
Moisture	70.18 ± 0.584	68.82 ± 0.219	$69.13\pm.808$	68.95 ± 0.078	68.92 ± 0.549	68.91 ± 0.596
Crude protein	16.10 ± 0.168	16.92 ± 0.279	16.56 ± 0.225	17.16 ± 0.373	16.68 ± 0.257	17.12 ± 0.193
Crude lipid	6.87 ± 0.417	7.66 ± 0.206	7.78 ± 0.802	7.53 ± 0.118	7.35 ± 0.136	7.26 ± 0.334
Ash	5.07 ± 0.021	4.95 ± 0.152	4.94 ± 0.089	4.90 ± 0.066	5.29 ± 0.116	5.04 ± 0.094
Calcium	5.6 ± 0.172	5.43 ± 0.090	5.21 ± 0.353	5.16 ± 0.086	5.71 ± 0.130	5.61 ± 0.034
Phosphorus	2.73 ± 0.269	2.41 ± 0.156	2.96 ± 0.157	2.62 ± 0.297	2.27 ± 0.015	2.78 ± 0.234
Fillet composition						
Moisture	69.11 ± 0.155^{abc}	68.89 ± 0.086^{bc}	68.94 ± 0.119^{bc}	69.73 ± 0.313^a	68.36 ± 0.182^c	69.38 ± 0.108^{ab}
Crude protein	19.60 ± 0.242^{ab}	19.59 ± 0.129^{ab}	19.24 ± 0.193^b	19.80 ± 0.197^{ab}	19.66 ± 0.247^{ab}	20.25 ± 0.207^b
Crude lipid	5.83 ± 0.131^{ab}	6.36 ± 0.310^a	5.82 ± 0.141^{ab}	5.88 ± 0.096^{ab}	5.56 ± 0.106^b	5.48 ± 0.100^b
Ash	3.50 ± 0.064^{ab}	3.27 ± 0.146^b	3.96 ± 0.071^a	3.29 ± 0.113^b	3.62 ± 0.161^{ab}	3.59 ± 0.049^{ab}
Moisture	69.11 ± 0.155^{abc}	68.89 ± 0.086^{bc}	68.94 ± 0.119^{bc}	69.73 ± 0.313^a	68.36 ± 0.182^c	69.38 ± 0.108^{ab}

CF[d]: condition factor (%), HSI[e]: hepatosomatic index (%), VSI[f]: viscerosomatic index (%), IPF[g]: intra-peritoneal fat index (%); Different superscript in the same row denotes statistically significant differences ($p<0.05$).

Whole body and fillet composition
Biochemical content of the whole body and fillet are presented in Table 4. There were no significant differences in moisture, crude lipid, crude protein, ash, calcium and phosphorus content among fish fed the experimental diets ($p>0.05$). In fillet proximate composition did not show significantly differences between fish fed reference and PBM-based diets, although crude lipid was significantly higher in PBM 15 than PBM45, 55 and ash in PBM 25 was significantly the highest value.

Hematological factors
The hematological factors and serum biochemical parameters are presented in Table 5. Serum glucose concentration, triglyceride, total protein, urea, calcium, magnesium, albumin, globulin, hemoglobin, hematocrit, MCHC were not significantly affected by the replacement level of fish meal with poultry by-product meal ($p>0.05$). The lowest and highest cholesterol values were observed in PBM0 and PBM55, respectively. Significantly higher triglyceride values were recorded in fish fed the FM based diet ($p<0.05$; Table 5) and the lowest values were observed in PBM25 and 35 ($p<0.05$).

Table 5: Hematological and serum biochemical parameters of juvenile sobaity fed the experimental diets for 60 days.

parameters	PBM0	PBM15	PBM25	PBM35	PBM45	PBM55
			Diets			
Hemoglobin (g dl^{-1})	8.88±1.19	7.47±0.14	7.67±0.071	7.62±0.84	9.34±1.33	8.21±0.63
Hematocrit (%)	43.00±4.16	42.67±3.38	39.67±0.67	40.33±3.18	43.00±0.58	43.33±0.33
MCHC (g dl^{-1})	21.77±2.23	17.87±1.45	19.51±0.17	18.98±1.13	22.01±2.49	22.71±2.76
Total protein (g dl^{-1})	5.67±0.38	5.37±0.08	5.25±0.21	5.41±0.25	4.41±0.66	5.29±0.29
Albumin (g dl^{-1})	0.53±0.05	0.36±0.08	0.48±0.15	0.37±0.23	0.27±0.16	0.47±0.12
Globulin (g dl^{-1})	4.86±0.19	5.01±0.11	4.77±0.13	5.04±0.19	4.14±0.49	4.82±0.19
Glucose (mmol L^{-1})	94.67±1.86.	88.67±0.88	85.00±1.53	94.67±2.91	92.67±2.96	92.00±1.73
cholesterol (mg dl^{-1})	412.00±6.00[c]	519.33±9.43[b]	464.00±3.75[b]	607.33±3.46[a]	575.00±10.39[a]	630.67±8.65[a]
Triglyceride (mg dl^{-1})	352.67±8.450[a]	263.67±9.33[b]	224.33±6.47[c]	228.00±9.54[c]	265.00±7.74[b]	266.33±4.17[b]
urea	8.63±0.09	7.00±0.29	8.00±0.15	8.03±0.95	9.50±1.07	10.17±0.22
Calcium (mg dl^{-1})	15.60±1.78	20.50±0.90	19.13±1.34	20.3±1.20	19.57±0.48	21.83±1.20
Phosphorous (mg dl^{-1})	12.90±0.66[a]	10.77±0.38[ab]	11.27±0.55[ab]	9.33±0.76[b]	9.67±1.139[b]	10.30±0.635[ab]
Magnesium	4.80±0.51	5.77±0.54	4.90±0.61	4.50±0.42	4.00±1.20	5.50±1.19

Different superscript in the same row denotes statistically significant differences ($p<0.05$).

Discussion

The results of the present study indicated that PBM is a suitable replacement for fish meal in well-balanced practical diets formulated for juvenile sobaity. This species had better tolerance for dietary PBM without significant reduction in growth performance (FBW, %WG, SGR), and feed utilization (FCR, PER, NPU) when the replacement level of FM with PBM was up to 55% (39% of total diet, 23.6% crude protein or 47.2 % of the total dietary protein). Juvenile sobaity showing a capacity similar to that of other species such as: juvenile red drum up to 13% crude protein or 30% of the total dietary protein (Kureshy *et al*., 2000), gilthead sea bream up to 18% crude protein or 40% of dietary protein (Nengas *et al*., 1999), Australian snapper up to 27% of the protein provided by fish meal or 12% crude protein (Quartararo *et al*., 1998) and

cobia up to 45% of total dietary protein (Zhou *et al*., 2011).

A significantly lower growth performance of fish fed the control diet compared to fish fed low level of PBM-based diets suggests that PBM can be efficiently utilized by sobaity juveniles. On the other hand the use of local FM as a sole source of protein in PBM0 did not give superior performance compared to PBM diets. Also dietary PBM could positively improve the feed utilization of fish. It is noteworthy that FCR of the control diet was higher than of PBM diets. The highest PER and %WG were observed where *S. hasta* were fed diets including two animal protein sources: FM + PBM (at 23.75% level of FM replacement). Dietary supplementation of PBM at low inclusion levels has been reported to result in positive impact on growth performance and feed utilization of *Epinephelus fuscoguttatus, Lateolabrax japonicas; Anabas testudineus,*

Carassius auratus (Ghosh and Das, 2005; Yang *et al*., 2006; Gunben *et al*., 2014; Wang *et al*., 2015). However, some studies revealed that replacement of fish meal protein with PBM resulted in decreased feed utilization in *Clarias gariepinus* (Abdel-Warith *et al*., 2001), *Psetta maeotica* (Yigit *et al*., 2006), and *Lutjanus guttatus* (Hernandez *et al*., 2014). The differences could be attributed to the different quality of the PBM (Yang *et al*., 2006), tolerance level of the fish species, developmental stage of the fish, type and degree of processing used for alternative protein sources (Dong *et al*., 1993), protein level of the diets, quality of fish meal (Hardy, 1996), or other protein sources in the control diet (Krogdahl and Bakke-McKellep, 2005).

In the present study, condition factor was significantly lower in PBM15 and 55, and IPF significantly higher in PBM15 than in the control group, although no relation and trend between increasing level of FM replacement and somatic indexes was observed. These findings were in agreement with those reported by Shapawi *et al*. (2007) for humpback grouper and Hu *et al*. (2008) for gibel carp. However, increasing levels of PBM in the diet, significantly increased HSI in rainbow trout (Steffens, 1994; Zoccarato *et al*., 1996), red sea bream (Takagi *et al*., 2000), hybrid striped bass (Rawles *et al*., 2006) and gibel carp (Yang *et al*., 2006). Whole-body and fillet proximate composition did not show significant differences between control and PBM-based diets. This is in agreement with Takagi *et al*. (2000) and Gunben *et al*.

(2014) who reported no significant difference in whole-body composition of red sea bream and grouper fed different levels of dietary PBM. In the present study there was no relation between HSI, IPF and lipid of whole body with level of PBM in the diet, but Rawles *et al*. (2009) observed the positive correlation of major depots of dietary energy (HSI and IPF) with dietary PBM. Increase in body lipid and HSI (Steffens, 1994) and VSI (Zoccarato *et al*., 1996) have been observed in rainbow trout as the level of PBM in the diet increased. Other than species specific differences, other compounding dietary and environmental factors might be the main cause of these differences found in the whole body composition and somatic indexes of various fish fed PBM-based diets (Shapawi *et al*., 2007; Hernandez *et al*., 2014). Nevertheless, the tendencies for whole body protein to decrease and energy depots (HSI and IPF) to increase as PBM replaced fish meal indicate a nutrient imbalance in the replacement diets (Rawles *et al*., 2006). Thereby, it appears that the low level of FM ($280g\ kg^{-1}$; in PBM55) was able to provide sufficient nutrient and energy to meet the requirements of fish for normal growth and body composition in the presence of PBM.

Mozanzadeh *et al*. (2015) and Peres *et al*. (2013) showed that feeding conditions strongly affect most serum biochemistry parameters of sobaity and gilthead sea bream. In the present study, hemoglobin value was not affected by experimental diets. It varied between 7.47-9.34 ($g\ dl^{-1}$), close to the range

reported for gilthead sea bream (8.3-10.6, Fazio *et al*., 2013) and yellow fin sea bream (8.0-9.8, Karimi *et al*., 2013). Hematocrit (Hct) is a reliable index for checking the anemic condition as well as fish health relative to nutrition, disease and stress status (Brill *et al*., 2008), which in our study was not affected by the replacement of fish meal. The range of Hct in sobaity (39.74–43%) was comparable to normal values reported for gilthead sea bream (45.19%; Fazio *et al*., 2013), four studied marine species (31-44%; Satheeshkumar *et al*., 2010) and yellow fin sea bream (Karimi *et al*., 2013). Hrubec and Smith (2000) reported that the normal MCHC values for many marine species ranged between 18 to 30%. The MCHC value of sobaity in experimental groups was comparable with values reported for other fish species (Fazio *et al*., 2013; Peres *et al*., 2014). The normal ranges of hematological parameters recorded in the present study suggested that substitution of FM up to 55% by PBM did not lead to any sign of anemia in sobaity.

In sobaity, variation of total protein (TP; 4.4- 5.7 g dl^{-1}) was close to the normal range reported for the marine species (Coeurdacier *et al*., 2011; Peres *et al*., 2014). As TP did not vary among the treatments it could be stated that replacement of FM with PBM had no adverse effect on control of protein synthesis and food intake (Xu *et al*., 2012). Replacement up to 55% FM did not show any adverse influence on albumin, globulin or on innate immune system of sobaity. The mean glucose

values were close to the normal range reported for sparids (Yildiz, 2009; Peres *et al*., 2013). Plasma glucose values of sobaity were not affected by dietary treatments suggesting that PBM had no significant effect on energy metabolism in *S. hasta*.

Cholesterol was significantly modified with inclusion of PBM in diets which could be attributed to the characteristic of the substituted protein source, although variation of it up to PBM55 was close to the normal range reported for the same size sobaity fed FM based-diet (Mozanzadeh *et al*., 2016). Increased cholesterol was reported in fish fed animal protein sources (Kjaer *et al*., 2008; Mozanzadeh *et al*., 2015) as compared to fish fed diets containing plant protein sources (Soltanzadeh *et al*., 2016; Yaghoubi *et al*., 2016). Gaylord *et al*. (2007) suggested that the protein source plays the main role in serum cholesterol content by increasing or decreasing the rate of its metabolite by inducing the synthesis of salt bile in the liver. In the current study diets containing PBM showed significantly lower triglyceride level than the control diet, whereas Yaghoubi *et al*. (2016) reported increased serum triglyceride when FM was replaced with plant protein source of sobaity diet. Mozanzadeh *et al*. (2016) suggested that a minimum of 5% fish oil in diets could result in optimum performance of sobaity. In our study inclusion of fish oil (FO) in dietary treatments of up to PBM55 was more than 5% (3.5% FO + ca 2% inclusion of oil in FM) suggesting that replacement of FM with PBM could meet the

requirements of sobaity for optimum lipid metabolism. The lowest triglyceride level was observed in PBM25 and 35, which was associated with the higher growth and nutrient performance. The decrease in serum triglyceride could be attributed to the higher rate of lipid hydrolysis and consequently higher metabolism in fish fed these diets in comparison with fish fed control diet (Mozanzadeh *et al.*, 2016). Economic evaluation of the feeding trials after 60 days showed that replacing FM with PBM lowered the cost of diets, therefore the profit indices of the fishes fed PBM diets increased similar to reported economic indexes by Hernandez *et al.* (2014). Based on the economic performance of the sobaity fed with the experimental diets, the replacement of FM with PBM is recommended.

In conclusion, the results of the present study showed that in the *Sparidentex hast*, up to 55% of the fish meal protein in formulated diets can be replaced with PBM food grade without lysine and methionine supplementation, without any negative effects on health and growth performance. The quadratic regression analysis suggested that the best performance of sobaity was achieved in 23.75% level of replacement of fish meal with poultry by-product meal, not in fish meal based diet. The present study represents the first research conducted on the nutritional capacity of the sobaity and may serve as a basis for future studies.

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