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Improved Utilization of a Plant By-Products Mixture by Supplementing Dietary Bamboo Charcoal for Juvenile Amberjack *Seriola dumerili*

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Abstract

A 3×2 factorial experiment was carried out to investigate the effects of a plant by-products mixture (PBM; shochu distillery by-products: okara, 4:1) and bamboo charcoal (BC) on growth performance, feed utilization, carcass composition and hematological parameters of juvenile amberjack (*Seriola dumerili*) by feeding low fishmeal based diets. Six isonitrogenous and isolipidic diets were prepared by adding three levels of PBM at 0, 5 and 10% and two levels of BC at 0 and 4%. Triplicate groups of juvenile amberjack (3.00 g, 20 fish per tank) were fed the respective test diets twice daily to apparent satiation for 45 days. Results indicated that PBM had no significant ($P>0.05$) effect on growth performance and feed utilization of fish. Inclusion of BC significantly ($P<0.05$) improved the performance of fish. However, no interaction was found between PBM and BC. Survival was not affected by PBM, BC or their interaction. Whole body proximate composition was also significantly influenced by BC supplementation. Blood chemistry showed some differences but the values were comparable among the dietary groups. The present study demonstrated that BC supplementation is effective for improving the utilization of a plant by-product mixture in low fishmeal and comparatively higher level of plant protein based diet for juvenile amberjack. It is assuming that BC could absorb the noxious substances present in PBM which might improve the utilization of diets. Based on the present experiment, it is concluded that 5-10% PBM along with 4% BC in 25% fishmeal based diet is effective supplementation for better performances of juvenile amberjack.

Key words: amberjack *Seriola dumerili,* bamboo charcoal, okara, shochu distillery by-products

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Introduction

Charcoal is the carbonaceous residue of wood, bamboo, cellulose, coconut shells or various industrial wastes left after heating organic matter in the absence of oxygen. This very fine and tasteless black powder is an adsorbent for many toxins, gases and drugs without any specific action (Moe Thu *et al*. 2010). Charcoal has an extraordinarily large surface area and pore volume that gives it a unique adsorption capacity (Baker *et al*. 1992). Commercial food grade charcoal products range between 300 and 2000 m^2/g (Burdock 1997). Nowadays, charcoal has been used in animal feed formulation as an additive because it absorbs ammonia and nitrogen, and activates the intestinal function through eliminating the poisons and impurities from the gastrointestinal tract of different animals (Mekbungwan *et al*. 2004, Van *et al*. 2006, Moe Thu *et al*. 2010). Supplementation of charcoal significantly increased feed intake, body weight and feed utilization in chicken (Kutlu *et al*. 2001). Addition of 0.5-4.0% bamboo charcoal (BC) significantly improved growth, feed utilization and ammonia nitrogen excretion of Japanese flounder, *Paralichthys olivaceus* and tiger puffer, *Takifugu rubripes* (Moe Thu *et al*. 2009, 2010). BC is considered more economical than activated charcoal and it possesses a higher surface area and adsorption capacity than the standard wood charcoal (Van *et al*. 2006, Moe Thu *et al*. 2009). However, very limited information is currently available on the effectiveness of the BC for use as a feed additive for the commercial aquaculture industry.

Shochu is traditional Japanese liquor made from rice, sweet potato, barley, fruits and other ingredients (Yamamoto *et al*. 2004, Sasaki *et al*. 2005). Sweet potato shochu is popular in the southern region of Japan and shochu industries generate a huge amount of shochu distillery by-products (SDBP). In 2005, about 480,000 tons of SDBP were produced in Japan. Only a small part of these by-products is utilized for animal feed production. Most of them are treated as waste products and then incinerated, dumped into the sea or deposited on land, causing serious environmental problems (Teramoto *et al*. 1994). However, SDBP contains valuable nutrients such as protein, carbohydrate, vitamin and some other bioactive compounds. Mahfudz *et al*. (1996) have found that SDBP contains unidentified growth promoting factors for broiler chicken. SDBP have been tested as dietary feedstuff for chicken (Mahfudz *et al*. 1996), pig and cattle (Yanagita *et al*. 1999) and fish (Mokolensang *et al*. 2003a, 2003b). As SDBP is a wet by-product, their utilization in formulated feed and long time storage is difficult. Therefore, in the present study, SDBP was mixed with a by-product from the tofu industry called “okara” at a ratio of 4:1 and co-dried to produce a plant by-products mixture (PBM). As PBM is the mixture of two plant sources, there is high possibility that it contains some anti-nutritional factors, toxins etc. Moreover, SDBP is a distillery product and it might contain some organic acids, antinutritional factors and an alcoholic smell.

The amberjack, *Seriola dumerili*, is one of the commercially important species in Japan because of its delicacy and comparatively higher market value. It is distributed throughout the tropical and subtropical seas except the Pacific Ocean (Laroche *et al.* 1984). Nowadays, aquaculture of this species is intensified in the Mediterranean region and Japan (Takakuwa *et al*. 2006). However, nutritional studies of this species are scarce. The present study was conducted to evaluate the interactive effect of BC and PBM on growth performance, feed utilization, whole body composition and hematological parameters of juvenile amberjack, *Seriola dumerili*.

Materials and Methods

Experimental design and test diets

A 3×2 factorial design was used to study the effect of PBM and BC levels in a low fishmeal based diets on growth performance, feed utilization, whole body carcass composition and hematological parameters of juvenile amberjack. The formulation of the experimental diets is shown in Table 1. Six isonitrogenous and isolipidic diets were prepared

Table 1. Ingredients and chemical composition of the test diets.

Ingredients (g kg^{-1} diet)	Test diets					
	D1	D2	D3	D4	D5	D6
Fishmeal[1]	250	250	250	250	250	250
Soybean protein isolate[2]	150	150	150	150	150	150
Squid meal[1]	130	130	130	130	130	130
Krill meal[1]	60	50	30	60	50	30
PBM[3]	-	50	100	-	50	100
Pollack liver oil[4]	80	80	80	80	80	80
Soybean lecithin[4]	50	50	50	50	50	50
HUFA[5]	10	10	10	10	10	10
α-Starch	60	40	20	40	20	-
Vitamin mixture[6]	43	43	43	43	43	43
Mineral mixture[7]	40	40	40	40	40	40
Lactoferrin[8]	1	1	1	1	1	1
Seaweed powder[9]	16	16	16	16	16	16
BC[10]	-	-	-	40	40	40
Attractants[11]	10	10	10	10	10	10
Activated gluten	50	50	50	50	50	50
α-Cellulose	50	30	20	30	10	-
Proximate composition (g kg^{-1} diet)						
Dry matter	888	909	894	905	899	904
Crude protein	504	498	509	496	514	518
Total lipid	194	190	196	197	201	203
Ash	120	124	125	123	124	125

[1] Nippon Suisan Co. Ltd., Tokyo, Japan.
[2] Fuji Pro Company, Tokyo, Japan.
[3] Plant by-products mixture.
[4] Riken Vitamin, Tokyo, Japan.
[5] Poweash A, Oriental Yeast Co, Ltd., Tokyo, Japan.
[6] Vitamin mixture (g kg^{-1} diet): ß-carotene 0.13; Vitamin D_3 0.01; Menadione $NaHSO_3.3H_2O$ (K_3) 0.06; DL-α-Tochopherol Acetate (E) 0.51; Thiamine-Nitrate (B_1) 0.08; Riboflavin (B_2) 0.26; Pyridoxine-HCl (B_6) 0.06; Cyanocobalamine (B_{12}) 0.0001; d-Biotin 0.01; Inositol 5.13; Niacine (Nicotic acid) 1.03; Ca Panthothenate 0.36; Folic acid 0.02; Choline choloride 10.49; ρ-Aminobenzoic acid 0.51; L-Ascorbyl-2-phosphate-Mg 3.0; Cellulose 2.57.
[7] Mineral mixture (g kg^{-1} diet): $MgSO_4$ 5.07; Na_2HPO_4 3.23; K_2HPO_4 8.87; Fe Citrate 1.10; Ca Lactate12.09; Al $(OH)_3$ 0.01; $ZnSO_4$ 0.13; $CuSO_4$ 0.004; $MnSO_4$ 0.03; Ca $(IO_3)_2$ 0.01; $CoSO_4$ 0.04.
[8] Morinaga Milk Industry Co. Ltd., Japan.
[9] Marui Products Co. Ltd., Japan.
[10] Ishiyama Co. Ltd., Japan.
[11] Attractants: alanin: betaine (1:1).

by adding three levels of PBM (0, 50 and 100 g kg^{-1}) and two levels of dietary BC (0 and 40 g kg^{-1}). The diets were designated as D1 (PBM 0, BC 0); D2 (PBM 5, BC 0); D3 (PBM 10, BC 0); D4 (PBM 0, BC 4); D5 (PBM 5, BC 4) and D6 (PBM 10, BC 4). PBM was received from a local shochu production company of Kagoshima Prefecture, Japan. It was prepared by mixing a sweet potato distillery by-product or SDBP and okara supplied by a local industry in Kagoshima Prefecture, Japan at 4:1 ratio. The resulting PBM contains 267, 48, 61 g kg^{-1} of crude protein, total lipid and ash respectively on dry matter basis. All other ingredients used in the present study were received commercially. Brown fishmeal, soy protein isolate, squid meal and krill meal were used as protein sources. Krill meal was used to keep the diets isonitrogenous. The lipid sources were Pollack liver oil, soybean lecithin and HUFA.

All the dietary ingredients were first ground to a small particle size in a hammer mill and passed through a 100 μm mesh sieve. The diets were prepared by thoroughly mixing all the dry ingredients in a food mixer for 15 min. Pollack liver oil, soybean lecithin and HUFA were premixed with a sonicator (CA-4488Z, Kaijo Corporation, Tokyo, Japan), added to the dry ingredients and mixed for another 15 min. The required amount of water (35-40% of the dry ingredients) was then added to the premixed ingredients and mixed for another 15 min. The pH of the diets was adjusted to 7.0 – 7.5 with 4N sodium hydroxide. The mixture was then passed through a meat grinder with an appropriate diameter (1.2 to 2.2 mm) to prepare pellets which were then dried in a dry-air mechanical convection oven (DK 400, Yamato Scientific, Tokyo, Japan) at 60 ^{o}C for 120 min. The moisture content of each diet was checked with a moisture detector (MM30 Yamato Co., Japan). The test diets were stored at –28 ^{o}C in a refrigerator until use.

Experimental fish and feeding protocol

Juvenile amberjack were obtained from Kinki University, Wakayama Prefecture, Japan and transferred to the Kamoike Marine Production Laboratory, Faculty of Fisheries, Kagoshima University, Japan. The fish were maintained on a commercial formulated diet (Higashimaru Foods, Kagoshima, Japan) for two weeks prior to the feeding trial. One hundred liter polycarbonate circular tanks were used for the experiment. Six groups of fish with an initial average weight of 3.04 ± 0.05 g (mean ± S.D.) were stocked in triplicate in 18 tanks at a density of 20 fish $tank^{-1}$. The tanks were equipped with continuous aeration and flow through sea water system (1.5 l min^{-1}). Natural illumination conditions were followed during the feeding period. The fish were supplied the respective test diets to apparent satiation**.** Daily ration size was divided into two equal feedings at 9.00 and 17.00 h. Uneaten feed was collected and fecal matter was removed from the tank by siphoning after each feeding. The duration of the feeding trial was 45 days. Every 10 days, fish were counted and weighed in bulk to adjust the ration size. During each sampling, all the experimental tanks were cleaned and the water was totally renewed. The water quality parameters (mean ± SD) monitored during the experimental period were temperature 24.9 ± 1.9 ^{o}C, pH 8.0 ± 0.3, salinity 33.5 ± 0.6 ppt and dissolved oxygen 6.1 ± 0.5 mg l^{-1} . These ranges are considered within optimal values for juvenile amberjack.

Sample collection

During stocking a sample group of 15 fish was stored at −20 °C for initial whole body analysis. At the end of the feeding trial, all fish were left fasted for 24 h prior to final sampling. The total number, and individual body weight of fish in each tank was measured. Five fish from each replicate tank were randomly collected and stored at −20 °C for final whole body analysis. Blood was drawn by puncturing the caudal vein of individual fish. Plasma samples were collected after spinning down the heparinized blood at 3000 × *g* for 15 min at 4 °C and kept at −80 °C until analysis.

Analytical procedure

The ingredients, diets and fish whole body were analyzed for moisture, crude protein, total lipid and ash, in triplicates, using standard AOAC methods (AOAC 1990). Moisture was determined by drying the sample at 105 °C to a constant weight. The Kjeldahl method was used to determine nitrogen levels and crude protein was calculated multiplying by 6.25. Total lipid was analyzed using the BLIGH and DYER (1959) method and ash by combustion at 550°C in a muffle furnace. Plasma chemical parameters were measured spectrophotometrically with an automated analyzer (SPOTCHEM™ EZ model SP-4430, Arkray, Inc. Kyoto, Japan).

Statistical analysis

Data were tested using two-way analysis of variance (Package Super ANOVA, Abacus Concepts, Berkeley, CA). Percentage survival data were arcsin-square-root transformed before statistical analysis. Comparison between two treatments ($P < 0.05$) was evaluated by Duncan’s new multiple range test (Package Super ANOVA, Abacus Concepts, Berkeley, CA).

Results

Growth performance

The effect of test diets on growth performance and survival of juvenile amberjack are summarized in Table 2. Mean survival of fish in all the treatments ranged between 72.5% and 82.5% and was not significantly ($P > 0.05$) affected by the dietary levels of PBM, BC or the interaction between PBM and BC. At the end of the feeding trial, fish attained a weight gain of 360% to 465%. Only BC was a significant factor on weight gain of fish. Significantly higher weight gain was found in fish fed with diets supplementing 4% BC regardless of dietary PBM levels. The highest weight gain was obtained from the fish fed with diet containing 5% PBM and 4% BC levels. Specific growth rate (SGR, % day) of fish followed the similar trend as with weight gain.

Feed utilization

Feed utilization parameters are given in Table 3. Feed intake (FI) was significantly influenced by the inclusion of dietary BC. Although PBM was not a significant factor,

Table 2. Growth parameters in juvenile amberjack fed test diets for 45 days[1].

Diets			Growth parameters				
	PBM level[2]	BC level[3]	Initial BW[4] (g)	Final BW (g)	Weight gain (%)[5]	SGR (% day^{-1})[6]	Survival (%)[7]
D1	0	0	3.0 ± 0.01	15.0 ± 0.0	391 ± 8.7	3.5 ± 0.04	82.5 ± 7.5
D2	5	0	3.0 ± 0.02	13.9 ± 0.5	360 ± 19.4	3.4 ± 0.09	72.5 ± 2.5
D3	10	0	3.0 ± 0.02	14.7 ± 0.2	385 ± 5.0	3.5 ± 0.02	82.5 ± 7.5
D4	0	4	3.0 ± 0.01	16.7 ± 0.4	451 ± 11.2	3.8 ± 0.05	72.5 ± 2.5
D5	5	4	3.0 ± 0.02	17.1 ± 1.0	465 ± 29.6	3.9 ± 0.12	72.5 ± 7.5
D6	10	4	3.1 ± 0.07	17.3 ± 0.6	458 ± 7.7	3.8 ± 0.03	75.0 ± 0.0
Statistical analysis (*P* value)							
PBM			NS[8]	NS	NS	NS	NS
BC			NS	0.0015	0.0009	0.0007	NS
PBM × BC			NS	NS	NS	NS	NS

[1] Values are means of triplicate groups ± S.E.M. (standard error of the mean) .

[2] Plant by-products mixture.

[3] Bamboo charcoal.

[4] Body weight.

[5] Weight gain (%) = (final body weight – initial body weight) / initial body weight × 100.

[6] Specific growth rate (%, day^{-1}) = (ln final weight – ln initial weight) × duration / ln initial weight.

[7] Survival (%) = (final no. of fish / initial no. of fish) × 100.

[8] No significant difference.

Table 3. Feed utilization in juvenile amberjack fed test diets for 45 days[1].

Diets			Feed utilization		
	PBM level[2]	BC level[3]	FI (g dry fish^{-1})[4]	FE (%)[5]	PER[6]
D1	0	0	20.4 ± 1.1	57.5 ± 1.7	1.2 ± 0.04
D2	5	0	19.1 ± 0.3	56.9 ± 2.0	1.1 ± 0.04
D3	10	0	18.6 ± 0.5	62.9 ± 0.6	1.2 ± 0.02
D4	0	4	21.8 ± 0.6	62.8 ± 3.2	1.3 ± 0.07
D5	5	4	21.0 ± 0.4	67.1 ± 3.4	1.3 ± 0.07
D6	10	4	20.0 ± 0.8	70.9 ± 0.1	1.4 ± 0.0
Statistical analysis (*P* value)					
PBM			NS[7]	NS	NS
BC			0.0297	0.0048	0.0078
PBM × BC			NS	NS	NS

[1] Values are means of triplicate groups ± S.E.M.

[2] Plant by-products mixture.

[3] Bamboo charcoal.

[4] Feed intake (g dry fish^{-1}) = (dry diet given – dry remaining diet recovered) / no. of fish.

[5] Feed efficiency (%) = {weight gain (g) / feed intake, dry (g)} × 100.

[6] Protein efficiency ratio = weight gain (g) / protein intake, dry (g).

[7] No significant difference.

there was a decreasing trend in FI of fish with the increasing level of PBM in diets. Supplementation of BC with PBM significantly improved FI in all the respective groups. Similar effects were also found in the cases of feed efficiency (FE) and protein efficiency ratio (PER). However, no significant interaction was determined between PBM and BC level on feed utilization parameters.

Whole body proximate composition

Table 4 represents the whole body proximate analysis of fish. There were no significant effects of PBM or BC on whole body ash content. However, dietary BC was a significant

Table 4. Whole body proximate composition (% wet basis) in juvenile amberjack fed the test diets for 45 days[1].

Diets			Parameters			
	PBM level[2]	BC level[3]	Moisture	Crude protein	Total lipid	Ash
Initial[4]			80.9	13.1	1.3	3.5
D1	0	0	79.6 ± 0.7	13.7 ± 0	1.5 ± 0.3	4.2 ± 0.2
D2	5	0	79.5 ± 0.8	14.0 ± 0.2	1.5 ± 0.2	4.1 ± 0.1
D3	10	0	79.2 ± 0.4	14.6 ± 0.3	2.1 ± 0.2	3.9 ± 0.1
D4	0	4	78.8 ± 0.2	14.9 ± 0.2	2.0 ± 0.1	3.8 ± 0.1
D5	5	4	77.7 ± 0.4	15.1 ± 0.3	2.4 ± 0.2	4.1 ± 0.1
D6	10	4	78.3 ± 0.1	15.5 ± 0.3	2.2 ± 0.1	3.8 ± 0.0
Statistical analysis (*P* value)						
PBM			NS[5]	NS	NS	NS
BC			0.0279	0.0018	0.0212	NS
PBM × BC			NS	NS	NS	NS

[1] Values are means of triplicate groups ± S.E.M.
[2] Plant by-products mixture.
[3] Bamboo charcoal.
[4] Data were not included in statistical analysis.
[5] No significant difference.

Table 5. Blood parameters in juvenile amberjack fed the test diets for 45 days[1].

Diets			Blood parameters			
	PBM level[2]	BC level[3]	Hematocrit (%)	T-pro[4]	T-cho[5]	Glucose[6]
D1	0	0	33.5 ± 2.5	3.7 ± 0.1	245 ± 10	103 ± 2
D2	5	0	32.5 ± 0.5	3.1 ± 0.0	235 ± 1	85 ± 7
D3	10	0	29.5 ± 2.5	3.5 ± 0.3	229 ± 16	105 ± 4
D4	0	4	40.0 ± 1.0	4.4 ± 0.1	297 ± 6	100 ± 1
D5	5	4	32.0 ± 1.0	3.8 ± 0.3	264 ± 2	92 ± 6
D6	10	4	32.5 ± 1.5	3.9 ± 0.2	267 ± 2	77 ± 3
Statistical analysis (*P* value)						
PBM			0.0319	NS[7]	NS	0.0456
BC			NS	0.0110	0.0010	0.0478
PBM × BC			NS	NS	NS	0.0297

[1] Values are means of triplicate groups ± S.E.M.
[2] Plant by-products mixture.
[3] Bamboo charcoal.
[4] Total protein ($g\ dl^{-1}$).
[5] Total cholesterol ($IU\ l^{-1}$).
[6] Glucose ($IU\ l^{-1}$).
[7] No significant difference.

factor on moisture, crude protein and total lipid contents. Comparatively low moisture and high lipid and protein contents were found in all the groups received 4% BC level supplemented diets.

Blood parameters

Blood parameters are presented in Table 5. PBM had significant effect on hematocrit and plasma glucose content. Total protein (T-Pro), total cholesterol (T-Cho) and glucose were significantly affected by BC. Moreover, significant interaction was only found in plasma glucose level of fish.

Discussion

The present study demonstrated that supplementation of BC with PBM is effective to improve growth performance, feed utilization, whole body composition and blood parameters of juvenile amberjack. The results are in harmony with previous reports by Moe Thu *et al*. (2009, 2010) showing that supplementation of BC significantly improved the performance of Japanese flounder and tiger puffer fish.

At the end of the feeding trial, survival of the juvenile amberjack was not significantly affected ($P > 0.05$) by PBM, BC or their interaction. Inclusion of PBM had no significant effect on growth performance, feed utilization, whole body carcass composition and most of the hematological parameters of fish. Moe Thu *et al*. (2009) also found that inclusion of 10% SDBP had no negative effect on the normal growth rate of fish. However, higher inclusion levels might have detrimental effect on the performance of fish as SDBP might contain phenolic compounds since sweet potato storage root have high content of phenolic compounds (Yoshimoto *et al*. 2004). Moreover, Mokolensang *et al*. (2003a, 2003b) reported that common carp, *Cyprinus carpio* L. shows better growth performance than the control when fed 4.2% SDBP as dry basis. On the other hand, present experiment showed slightly lower ($P > 0.05$) growth performance in fish fed with diets supplementing 5 and 10% PBM (SDBP contains at 3.3 and 6.6% respectively on dry matter basis) compared to the diet without PBM. Supplementation of BC with PBM significantly improved growth performance and feed utilization of fish to the extent of being approximately equal to the fish fed high fishmeal based diet (Takeuchi *et al*. 1992). Several studies reported that growth was significantly improved by the inclusion of dietary BC in goat (Van *et al*. 2006), tiger puffer (Moe Thu *et al*. 2009) and Japanese flounder (Moe Thu 2010); wood charcoal in chickens (Kutlu *et al*. 2001) and dietary charcoal with wood vinegar in piglets (Mekbungwan *et al*. 2004) and Japanese flounder (Yoo *et al*. 2005).

Improved growth of fish in BC supplemented groups might be attributed by the increased feed intake in all the respective groups. Charcoal has been used as an adsorbent and detoxifier since long. Previous studies showed activated carbon is effective in removing various mycotoxins such as aflatoxin, ocharatoxin A, etc. (Dalvi and Ademoyero 1984, Rotter *et al*. 1989). Natural toxins from plants are also removed or attenuated by activated charcoal treatment or supplementation (Banner *et al*. 2000, Bisson *et al*. 2001). Charcoal also acts as sequestering agent for some toxins and anti-nutritional metabolites which have detrimental effects on animals (Moe Thu 2010). Dietary wood charcoal and vinegar compound activated the intestinal functions and increased feed efficiency of piglets and chicken (Samanya and Yamauchi 2001, Mekbungwan *et al*. 2004). Activated charcoal not only binds toxins or organic acids but it also controls pathogenic bacteria (Almagambetov *et al*. 1992) and complexes with phenolics in the gastrointestinal track in order to prevent hydrosable tannins interfering with enzyme function and protein digestion (Murdiati *et al*. 1991). In the present study, PBM were used which contains two by-products from plant based industries: SDBP and okara. As a distillery product, SDBP might contain some organic acids, polyphenolic compounds, toxins and strong alcoholic smell. It was assumed that supplementation of BC might adsorbed these noxious substances of PBM which facilitated to increase feed intake, growth performance and feed utilization of fish.

Inclusion of PBM had no significant effects on whole body proximate composition of amberjack. Whole body moisture was decreased; and crude protein and lipid were significantly increased by the supplementation of BC with PBM in all the respective groups. There was a strong negative correlation between the percentages of body lipid and moisture in fish (Jobling 2001). Increased whole body protein and lipid content could be a reflection of higher feed intake which would also increase the amount of protein and lipid intake and subsequent accumulation in the carcass. Moreover, increased nitrogen retention by BC supplementation might be one of the reasons for increasing carcass protein content of fish (Moe Thu *et al*. 2010). In the present experiment, there was no significant effect on whole body ash content. Yoo *et al*. (2005) also found similar results by feeding dietary charcoal and wood vinegar mixture to Japanese flounder. In contrast, Kutlu *et al*. (2001) reported that charcoal increased the mineral retention which ultimately increased the whole body ash content in broiler chicken.

Blood parameters are important tools for the indication of the physiological stress response as well as the general health condition of fish. Blood parameters obtained in the present study are considered to be within the normal range for juvenile amberjack although some variations were found among treatments (Ikeda and Minami 1982, Shimeno *et al*. 1992a, 1992b, Watanabe *et al*. 1998).

Conclusion

The observations in the present study on growth performances, feed utilization, whole body carcass composition and hematological parameters therefore indicated that BC supplementation was necessary for the effective utilization of PBM in low fish meal based diet for juvenile amberjack. The research concluded that 5-10% PBM along with 4% dietary BC in 25% fishmeal based diet could be effectively utilized by juvenile amberjack.

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