

Effects of graded activated charcoal in rice husk diets for mud catfish, *Clarias gariepinus* juveniles (Teleostei: Clariidae)

Ademola Zaid ADEROLU, Muyideen Owonire LAWAL*, Toheeb Tomiwa ADESOLA

Department of Marine Sciences, University of Lagos, Akoka, Lagos, Nigeria.

*Email: mlawal@unilag.edu.ng

Abstract: This study investigated the growth performance, nutrients utilization and haematology of *Clarias gariepinus* juveniles fed with graded activated charcoal (AC) in rice husk (RH) diet. Eight diets were formulated; Control diet 1 (without RH and AC), Control diet 2 (+AC), Diet 3 (2.5% RH), Diet 4 (2.5% RH+AC), Diet 5 (5.0% RH), Diet 6 (5.0% RH+AC), Diet 7 (7.5% RH), Diet 8 (7.5% RH+AC). Diets were fed to triplicate groups of ten fish per tank thrice daily to satiation for eight weeks. Fish fed Diet 4 recorded highest values for mean weight gain (MWG) (75.71 ± 1.76 g), SGR (2.40 ± 0.03 %/day) and PER (3.22 ± 0.15) while fish fed Diet 7 had the least values for MWG (59.50 ± 1.21 g), SGR (2.09 ± 0.03 g) and PER (2.39 ± 0.10). The AFI did not record significant difference ($P > 0.05$) while FCR had significant difference ($P < 0.05$) across diets, with Diet 4 having the best value for FCR (0.86 ± 0.04). Diets of RH lower cholesterol, PCV and Hb levels in the experimental fish though, the inclusion of AC to the test diets resulted in a non-significant increase in the levels of these parameters. The values of WBC and RBC did not show significant differences ($P > 0.05$) amongst diets however, Diets 1 and 2 recorded the highest values for WBC (14500 ± 707.11) and RBC (6.15 ± 0.91) with the least values (9000 ± 2828.43 and 5.15 ± 0.21 , respectively) for these parameters recorded with Diets 3 and 7. The improved growth recorded at 2.5% inclusion level of AC suggests that activated charcoal could favourably be added to the feed of *C. gariepinus* juveniles.

Keywords: Growth performance, Haematology, Nutrients utilization Catfish, Fisheries.

Citation: Aderolu, A.Z.; Lawal, M.U.; Adesola, T.T. 2016. Effects of graded activated charcoal in rice husk diets for mud catfish, *Clarias gariepinus* juveniles (Teleostei: Clariidae). Iranian Journal of Ichthyology 3(3): 203-209.

Introduction

Rice husk, an agricultural by-product of rice milling, it is available in Nigeria and presently constitute a nuisance to the environment (Belewu 1998). In spite of rice husk abundance, nutritionists have neglected its use in monogastric animals feed production because of its fibre content, poor nutritive value and bulkiness (Dafwang & Shwaremen 1996; Aderolu & Oyedokun 2009). Rice husk contains 2.9-3.6% crude protein, 0.8-1.2% ether extract, 39-42% crude fibre and 15-22% ash (Oyenuga 1968). Attempts at increasing the utilization of fibrous feed ingredients

like rice husk include, physical and chemical pre treatments, the use of microbial enzymes and antibiotics (Wing-Keong et al. 2002; Aderolu & Oyedokun 2009) and adequate fortification with additives.

Feed additives usage includes the preservation of the nutritional characteristics of a diet or feed ingredient prior to feeding (antioxidants and mould inhibitors), the enhancement of ingredient dispersion or feed pelletizing (emulsifiers, stabilizers and binders), facilitating feed ingestion and consumer acceptance of the product (feed stimulants and food

Table 1. Feed composition of experimental diets (kg).

Diet	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	Diet 7	Diet 8
Ingredients	Control (C)	C+AC	2.5% RH	2.5%RH+AC	5.0%RH	5.0%RH +AC	7.5%RH	7.5%RH+AC
Fish meal	25	25	25	25	25	25	25	25
SBM	36	36	36	36	36	36	36	36
Maize	35	35	32.5	32.5	30	30	27.5	27.5
Rice husk	-	-	2.5	2.5	5.0	5.0	7.5	7.5
Methionine	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
DCP	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Lysine	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Oil	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Salt	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Premix	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
CP %	34.87	34.87	34.73	34.73	34.61	34.61	34.48	34.48
CF	3.07	3.07	4.05	4.05	5.03	5.03	6.02	6.02
Energy kcal	2980	2980	2975	2972	2955	2960	2905	2952

AC = Activated charcoal (0.25g), CP = Crude protein, SBM = Soybean meal, CF = Crude fibre, DCP = Dicalcium phosphate and RH = Rice Husk.

colourants), growth promoting effects (growth promoters, including antibiotics and hormones) and the supply of essential nutrients in purified forms (vitamins, minerals and amino-acids) (Ajiboye et al. 2012), or to mop-up the effect of toxin present in the feed ingredients (activated charcoal).

Activated charcoal (AC), could be made by dry distillation of a thick-stemmed bamboo and powder of which is known as a universal adsorbent, because it can bind with variety molecules since it contains a complex network of pores of various shapes and sizes (Zhao et al. 2008). Now-a-days, AC has been used in animal feed formulation as an additive because it absorbs ammonia and nitrogen, control or mop-up the effect of toxin secreted by different microbes such as *Aspergillus* spp. and activates the intestinal function through eliminating the poisons and impurities from the gastrointestinal tract of land animals (Mekbungwan 2004; Quaiyum et al. 2014).

Although activated charcoal has been used as feed additives, however; its supplemental effects in high fiber fish diet have not been evaluated. Hence, the current study investigated the inclusion effects of activated charcoal in high fiber rice husk diets on growth, nutrients utilization and haematology of African mud catfish, *Clarias gariepinus* juveniles.

Materials and Methods

An eight weeks experiment was carried out on

African mud catfish juveniles. The fish were purchased from a farm at Cele-Egbe area of Ikotun, Lagos and transported to the nutritional unit, Department of Marine Sciences, University of Lagos. The fish were allowed to acclimatize for two weeks before the commencement of the experiment, during which they were fed with commercial feed. At the expiration of the acclimatization, they were weighed (OHAUS model CS2000 series) and sorted into plastic experimental tanks (52.5×33.5×21cm³) at the rate of ten fish per tank, with average weight 26.63±0.31g. The plastic tanks were covered with netting to prevent the fish from jumping out.

All the feed ingredients used were bought from Sabina Pad Enterprises Ltd., at Abattoir, Oko-Oba, Agege, Lagos, except the experimental ingredient (rice husk, RH) which was bought from a rice milling factory at Imota, Ikorodu, Lagos. All ingredients were grind into fine powder and stored in an air-tight container to preserve ingredient integrity. Eight experimental diets (Table 1) were formulated; Control diet 1 (without RH and AC), Control diet 2 (+AC), Diet 3 (2.5% RH), Diet 4 (2.5% RH+AC), Diet 5 (5.0% RH), Diet 6 (5.0% RH+AC), Diet 7 (7.5% RH), Diet 8 (7.5% RH+AC). The experiment was run in triplicate, making a total of 24 tanks. The fish were fed to satiation by hand three times daily (9:00am, 1:00pm and 5:00pm). The water was changed every other day to ensure the fish were kept

Table 2. Growth performance and nutrients utilization of *Clarias gariepinus* juveniles fed different experimental diets.

	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	Diet 7	Diet 8
INWT	26.63±0.38	26.92±0.31	26.58±0.38	26.79±0.19	26.63±0.38	26.92±0.31	26.75±0.25	26.80±0.38
FNWT	97.46±0.69 ^{bc}	99.10±3.46 ^b	97.17±0.80 ^{bc}	102.50±1.88 ^a	94.92±0.50 ^c	95.61±5.81 ^{ab}	86.25±1.28 ^d	87.43±1.71 ^d
MWG	70.83±0.58 ^{bc}	72.18±3.44 ^b	70.58±0.63 ^{bc}	75.71±1.76 ^a	68.29±0.26 ^c	73.17±2.67 ^{ab}	59.50±1.21 ^d	60.64±1.62 ^d
AFI	68.19 ±0.94	70.23±7.70	68.22±0.94	65.23±2.30	68.35±1.53	65.69±1.42	69.08±2.13	65.77±2.20
SGR	2.32±0.02 ^{bc}	2.33±0.06 ^{bc}	2.31±0.10 ^{bc}	2.40±0.03 ^a	2.27±0.02 ^c	2.26±0.06 ^{ab}	2.09±0.03 ^d	2.11±0.03 ^d
FCR	1.04±0.02 ^{bc}	1.03±0.12 ^{bc}	1.03±0.02 ^{bc}	0.86±0.04 ^a	1.00±0.02 ^{cd}	1.11±0.05 ^{ab}	1.16±0.06 ^c	0.92±0.02 ^{de}
PER	2.89±0.06 ^{bc}	2.87±0.34 ^{bc}	2.87±0.05 ^{bc}	3.22±0.15 ^a	2.78±0.06 ^{cd}	3.10±0.14 ^{ab}	2.39±0.10 ^e	2.56±0.06 ^{de}

All values on the same row with different superscripts are significantly different ($P < 0.05$). INWT = Initial Weight; FNWT = Final Weight.

under standard condition; temperature (27.5–29.5°C), dissolved oxygen (4.5–4.8mg/l), and pH (7.3–8.0) as described by Aderolu & Akpabio (2009). The weekly weight gain of the fish was done by bulk-weighing the feed, and also the weekly feed intake also recorded. Fish were treated in accordance with the guidelines of the local ethics committee.

The following growth and nutrients utilization parameters were calculated thus;

Mean Weight Gain (MWG)g = Mean Final Body Weight-Mean Initial Body Weight

Specific Growth Rate (SGR) = $[(\text{Ln}w_2 - \text{Ln}w_1) / t \times 100]$

Where: w_1 is the initial fish weight (g), w_2 is the final fish weight (g), t =Experimental period.

Feed Conversion Ratio (FCR) = Total Feed Intake (g) / Wet Weight Gain (g)

Protein Efficiency Ratio (PER) = Fish Weight Gain/Protein Intake

Where: Protein Intake is Total Feed / Protein Content of Feed.

Average feed intake (AFI) = Feed intake for Experimental period/ Number of days in the period.

Blood samples of fish taken at random from each tank were collected using a 2ml syringe in both syringe and EDTA bottles for haematological assay at Bioassay Diagnostic Laboratory, Cele-Egbe, Ikotun, Lagos where haemoglobin (Hb), red blood cells (RBC), white blood cells (WBC), packed cell volume (PCV) and cholesterol (chol) level were analysed using standard formula described by Joshi et al. (2002).

All data collected were subjected to analysis of

variance (ANOVA). Comparisons of means among diets were carried out by Duncan Multiple Range test (Duncan 1955) at significant level of 0.05. All computations were performed using statistical package SPSS 18.0 (SPSS Inc., Chicago, IL, USA).

Results

The results of the growth performance and nutrients utilization of *C. gariepinus* are shown in Table 2. There was no significant difference ($P > 0.05$) in the mean initial weight of the fish, but the weight gain record across the graded inclusion level of the RH reflected a significant decrease with increasing fibre level. There was significant improvement in growth on the addition of activated charcoal to the diet of experimental fish; especially at the 2.5 and 5.0% inclusion of the test ingredient. The highest mean weight gain (MWG) was recorded in the diet with 2.5% rice husk plus activated charcoal (75.71±1.76g) while the least value (59.50±1.21g) was recorded in the diet with 7.5% rice husk. The specific growth rate (SGR) follows similar pattern as that of the MWG, with the highest value of 2.40±0.03 %/day recorded by fish fed 2.5% RH plus AC, while the lowest was recorded in the diet with 7.5% rice husk (2.09±0.03%/day). There was significant improvement in the protein efficiency ratio (PER), with the highest value recorded in the diet that contained 2.5% RH plus AC (3.22±0.15) while the lowest value was recorded in the diet that contained 7.5% RH (2.39±0.10). Although the average feed intake (AFI) did not record any significant difference ($P > 0.05$) among the test diets, the feed conversion ratio (FCR)

Table 3. Hematological parameters of *Clarias gariepinus* juveniles fed different experimental diets.

Parameter	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	Diet 7	Diet 8
CHOL (mg/dl)	120.00±7.07 ^a	91.00±16.97 ^{abc}	106.00±11.31 ^{ab}	109.00±4.24 ^{ab}	80.00±22.62 ^{bc}	102.50±4.95 ^{abc}	71.00±19.80 ^c	82.50±9.19 ^{bc}
PCV%	25.00±2.83	23.00±5.66	17.00±1.14	24.50±0.71	21.00±1.14	23.50±7.78	17.50±2.12	21.50±2.12
HB g/dl	8.25±0.92	8.15±1.77	6.80±0.28	8.10±0.28	6.95±0.50	7.65±2.47	5.80±0.71	7.05±0.78
WBC	14500±707.11 ^b	11000±4242.64 ^b	9000±2828.43 ^b	13000±1414.21 ^b	12500±707.11 ^b	10000±4242.64 ^b	13500±707.11 ^b	11000±1414.21 ^b
RBC	5.85±0.35	6.15±0.91	6.05±0.35	5.95±0.07	5.55±0.70	6.05±1.06	5.15±0.21	5.55±0.21

All values on the same row with different superscripts are significantly different ($P<0.05$).

had significant difference ($P<0.05$) across diets, with diet 4 having the best value for FCR (0.86 ± 0.04).

Graded levels of rice husk resulted in reduction in cholesterol, PCV and Hb levels in the fish fed the different experimental diets, but the inclusion level of activated charcoal at each level of the RH tested resulted in a non-significant increase ($P>0.05$) in the levels of cholesterol, PCV and Hb respectively (Table 3). The values of WBC and RBC did not show any significant differences ($P>0.05$) amongst the test diets, however diets 1 and 2 recorded the highest values for WBC (14500 ± 707.11) and RBC (6.15 ± 0.91). The least values for WBC and RBC were recorded with fish fed diets 3 (9000 ± 2828.43) and 7 (5.15 ± 0.21), respectively (Table 3).

Discussion

The fish fed diet 4 had the best growth and nutrient utilization performance compared to control and other diets with graded rice husk inclusion; this could be attributed to higher fiber load as a result of inclusion effect of RH. This is similar to the result recorded when unprocessed rice husk was fed to *C. gariepinus* at different graded levels by Aderolu & Oyedokun (2009). On the other hand the fish fed diet with activated charcoal recorded superior growth rate than their counterpart on same RH inclusion. This improved nutrient utilization and growth performance was noticed up to 5% inclusion level of RH supplemented with AC. This increased growth performance and the enhanced nutrients utilization capacity of the AC fed groups despite the increased fibre load could be supported by the findings of Kutlu

et al. (2001) when they fed Broiler chicken with AC and Thu et al. (2009) when they fed Tiger fish with graded levels of bamboo charcoal. The above authors attributed the improved growth and nutrient utilization to increase availability of certain macronutrients, particularly proteins, leading to a better nutrient accumulation for the animals in line with the assertion of Van et al. (2006). Also, the enhanced growth of nutrient enhancing microbes along the gastro intestinal tract of the fish and possibly the thinner and lighter surface area of the intestinal villi of animal fed activated charcoal (Casparly 1992) could also result in greater absorption of available nutrients (Mekbungwan et al. 2004; Jahan et al. 2014).

The improved FCR, SGR and PER were similar to previous studies when activated charcoal was included in the feed of chicken as reported by Samanya & Yamauchi (2001), Moe et al. (2009) and Jahan et al. (2014) on *Pangasius hypophthalmus* catfish.

The increased weight gain might also be due to the fact that the activated charcoal has the capacity to reduce toxins in diets by adsorbing it and thereby preventing its absorption from the intestine (Anjaneyulu et al. 1993). When the activated charcoal was added to diets containing aflatoxins or T-2 toxins, reduction in feed intake and body weight gain of chicken were reported ameliorated (Anjaneyulu et al. 1993; Edrington et al. 1997). Activated charcoal have been widely used as an adsorbent or as a detoxicant in modern veterinary and medical science (Hoshi et al. 1991; Jindal et al.

1994). Recently, Mekbungwan et al. (2004) reported that the wooden charcoal and vinegar compounds (WCVC) could activate the intestinal function both at villus and cellular levels and it also increase the feed efficiency of piglets. Moreover, improved feed conversion ratio and activated morphological changes of intestinal villi were observed in chickens fed WCVC supplement diets (Samanya & Yamauchi 2001).

Blood parameters afford a quick means for the assessment of fish response to feeds and its ultimate condition in culture systems, any abnormalities in fish due to deficiencies in diet can be detected by comparing the blood parameters of the affected fish with that of the control or with the 'reference' or baseline values if such are available (Owolabi 2011).

Plasma concentration of cholesterol was observed to increase significantly in the experimental fish with inclusion of activated charcoal in the present study. This is supported by the work of Jiya et al. (2014), who reported a rise in cholesterol level of Broiler fed contaminated aflatoxin feed with AC inclusion. Therapeutic effect of activated charcoal on urea, cholesterol, nitrogen and glucose concentrations in turkey poult fed aflatoxin added diet was also reported by Edrington et al. (1996).

The above result could be an indication and probably suggests a general increase in lipid mobilization as opined by Kori-Siakpere (2011). This may not be equally unconnected with the fact that activated charcoal has the capacity to make available energy resident in the feed.

The non-significant differences in the RBC, PVC and HB are in line with the findings of Prvulovic et al. (2008) when they studied the effect of hydrated aluminosilicate on performance and biochemical parameters of broiler chicken. From the present study, activated charcoal displayed the capacity to enhance growth through nutrients utilization in high fiber diet. Consequently, the inclusion of AC at 2.5% concentration in high fiber diet such as rice husk is advised for improved growth in juvenile of *C. gariepinus*.

Acknowledgments

We would like to thank Aarode O.O. for assisting in the statistical analysis of our data.

References

- Aderolu, A.Z. & Oyedokun G.T. 2009. Comparative utilization of biodegraded rice husk in the diets of *Clarias gariepinus*. *Journal of Fisheries and Aquatic Science* 3(5): 312-319.
- Aderolu, A.Z. & Akpabio, V.M. 2009. Growth and economic performance of *Clarias gariepinus* juvenile fed diets containing velvet bean, *Mucuna pruriens* seed meal. *African Journal of Aquatic Science* 34(2): 1-5.
- Ajiboye, O.O.; Yakubu, A.F. & Adams, T.E. 2012. A perspective on the ingestion and nutritional effects of feed additives in farmed fish species. *World Journal of Fish and Marine Sciences* 4(1): 87-101.
- Anjaneyulu, Y.; Rama Rao, P. & Gopal Naidu, N.R. 1993. Experimental aflatoxicosis and its amelioration by activated charcoal in broiler chicken-study on performance and haematology. *Journal of Veterinary and Animal Science* 24: 51-54.
- Belewu, M.A. 1998. Biodegradation of Sorghum stover by fungi and the feeding of resulting stover to ratio. 3rd Annual conference of Animal Science Association of Nigeria, Ikeja Lagos. 17-19.
- Caspary, W.T. 1992. Physiology and pathophysiology of intestinal absorption. *American Journal of Clinical Nutrition* 55: 2998-3000.
- Dafwang, I.I.; Shwaremen, E.B.N. 1996. Utilization of rice offal in practical rations for broiler chicks. *Nigerian Journal of Animal Production* 23(1): 21-23.
- Duncan, D.B. 1955. Multiple range and multiple F Test. *Biometrics* 11:1-42
- Edrington, T.S.; Kubena, L.F.; Harvey, R.B. & Rottinghaus, G.E. 1997. Influence of a super activated charcoal on the toxic effects of Aflatoxin or T-2 toxin in growing broiler. *Poultry Science* 76: 1205-1211.
- Edrington, T.S.; Sarr, A.B.; Kubena, L.F.; Harvey, R.B. & Phillips, T.D. 1996. Effects of hydrated sodium calcium aluminosilicate (HSCAS), acidic HSCAS and activated charcoal, on the metabolic profile and toxicity of aflatoxinB1 turkey poult. *Toxicology*

- Letter 89: 115-122.
- Hoshi, K.; Yamamoto, T.; Takemura, N.; Sako, T.; Koyama, H. & Motoyoshi, S. 1991. Effects of activated charcoal on intraruminal lactic acid. Bulletin of Nippon Veterinary and Zoo technical College 40: 22-28.
- Jahan, R.; Quaiyum, M.A.; Jahan, N.; Akhter, T. & Sadiqul, I.M. 2014. Dietary added bamboo charcoal can evoke *Pangasianodon* growth and reduce ammonia from culture medium. International Journal of Fisheries and Aquaculture 6(7): 87-93.
- Jindal, N.; Mahipal, S.K. & Mahajan, N.K. 1994. Toxicity of aflatoxin B1 in broiler chicks and its reduction by activated charcoal. Research in Veterinary Science 56: 37-40.
- Jiya, E. Z.; Ayanwale, B.A.; Adeoye, A.B.; Kolo, P.S.; Tsado, D.N. & Alabi, O. J. 2014. Carcass yield, organoleptic and serum biochemistry of broiler chickens fed activated charcoal. Journal of Agricultural and Crop Research 2(5): 83-87.
- Joshi, P.K.; Bose, M. & Harish, D. 2002. Changes in certain haematological parameters in siluroid catfish, *Clarias batrachus* (Linn) exposed to cadmiumchloride. Pollution Resources 21(2): 129-131.
- Kori-Siakpere, O.; Ikomi, R.B. & Ogbe, M.G. 2011. Biochemical response of the African catfish, *Clarias gariepinus* (Burchell 1822) to sub-lethal concentrations of potassium permanganate. Annals of Biological Research 2(2): 1- 10.
- Kutlu, H.R.; Unsal, I. & Gorgulu, I. 2001. Effect of providing directory wood (oak) charcoal to broiler chicks and laying hens. Animal Feed science and Technology 90: 213-226.
- Mekbungwan, A.; Yamakuchi, K. & Sakaida, T. 2004. Intestinal villus histological alterations in piglets fed dietary charcoal powder including wood vinegar compound liquid. Anatomia, Histologia, Embryologia 33: 11-16.
- Moe, T.; Ishikawa, M.; Koshio, S. & Yokoyama, S. 2009. Effects of dietary bamboo charcoal on growth parameters and nutrient utilization of tiger puffer fish, *Takifugu rubripes*. Aquaculture Science 57: 53-60.
- Owolabi, O.D. 2011. Haematological and serum biochemical profile of the upside-down catfish, *Synodontis membranacea* Geoffroy Saint Hilaire from Jebba Lake, Nigeria. Comparative Clinical Pathology 20: 163-172.
- Oyenuga, V.A. 1968. *Nigeria Feeds and Feeding Stuffs*. Ibadan University Press. 3rd Edition.
- Prvulovic, D.; Kojic, D.; Grubor-Lajsic, G. & Kosarcic, S. 2008. The effects of dietary inclusion of hydrated aluminosilicate on performance and biochemical parameters of broiler chickens. Turkish Journal of Veterinary and Animal Sciences 32: 183-189.
- Quaiyum, M.A.; Jahan, R.; Jahan, N.; Akhter, T. & Sadiqul, I.M. 2014. Effects of Bamboo Charcoal Added Feed on Reduction of Ammonia and Growth of *Pangasius hypophthalmus*. Journal Aquaculture Research and Development 5: 269.
- Samanya, M. & Yamauchi, K. 2001. Morphology changes of the intestinal villi in chickens fed the dietary charcoal powder including wood vinegar compounds. Journal of Poultry Science 38: 289-301.
- Thu, M.; Koshio, S.; Ishikawa, M. & Yokoyama, S. 2009. Effects of Dietary Bamboo Charcoal on Growth Parameters, Apparent Digestibility and Ammonia Nitrogen Excretion of Tiger Puffer Fish, *Takifugu rubripes*. Aquaculture Science 57(1): 53-60.
- Van, D.T.T.; Mui, N.T. & Ledin, I. 2006. Effect of method of processing foliage of *Acacia mangium* and inclusion of bamboo charcoal in the diet on performance of growing goats. Animal Feed Science Technology 130(3-4): 242-256.
- Wing-Keong, N.G.; Hong-Aik, L.; Siew-Lang, L. & Che-Omar, I. 2002. Nutritive value of palm kernel meal pre-treated with enzyme or fermented with *Trichoderma koningii* (Oudemans) as a dietary ingredients for red hybrid tilapia, *Oreochromis* sp. Aquaculture Research 33(15): 1199-1207.
- Zhao, R.; Yuan, J.; Jiang, T.; Shi, J. & Cheng, C. 2008. Application of bamboo charcoal as solid-phase extraction adsorbent for the determination of atrazine and simazine in environmental water samples by high-performance liquid chromatography-ultraviolet detector. Talanta 76(4): 956-959.