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Effects of Incorporating Spent Bleaching Clay from Palm Oil Refining in the Diets of African Catfish, *Clarias gariepinus*

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Abstract

Spent palm oil bleaching clays contain 20 to 30% adsorbed oil that cannot be recovered economically. A feeding trial was conducted to determine the potential use of this energy-rich oil-laden by-product from palm oil refining in African catfish diets. Seven practical diets containing 0, 5, 10, 15, 20, 25 or 30% SBC were formulated and fed to triplicate groups of African catfish (mean initial weight of 17.9 ± 0.1 g) for 6 weeks. All diets were formulated to be isonitrogenous (35% crude protein) and isolipidic (12% crude lipid). Growth, feed utilization efficiency, survival, body composition, and hematocrits of catfish fed up to 30% SBC were not significantly different ($P > 0.05$) compared to fish fed the control diet without any SBC. Bone ash tended to slightly increase at dietary SBC levels of 15% and above. Hepatosomatic index of fish fed SBC incorporated diets were significantly lower compared to fish fed the control diet but preliminary histological examination did not reveal any structural abnormalities in the liver cells. Total solids and total suspended solids concentration in tank water increased significantly concomitant to the increasing dietary levels of SBC fed to African catfish. It was concluded that African catfish can be effectively used as a biological agent for the economical extraction of the adsorbed oil in SBC from palm oil refining.

Introduction

Spent bleaching clays (SBC) are by-products from the refining of vegetable oils and animal fats. During the refining process, bleaching is carried out to remove impurities and pigments to produce the desired color and flavor acceptable to the refined oil buyer. Bleaching earth used in edible oil refining are mainly bentonites, which are complex alumino-silicates belonging to the montmorillonite group of clays. The physical and chemical properties of these bentonites had been described by Hertrampf and Piedad-Pascual (2000) and the bleaching process described by Gunstone and Norris (1983). Depending on its activity and particle size, the oil retention in used or spent bleaching clays ranges from 20 to 75% of the spent earth weight (Gunstone and Norris 1983; Wong 1983).

Twenty seven million tons of crude palm oil (CPO) was produced in 2003 (Oil World 2004). In palm oil refining, the quantity of bleaching earth used is usually 0.5 to 2.0 % of the

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weight of CPO (Young 1987). This would give rise to an estimated annual generation of 135,000 to 540,000 tons of SBC globally. After use, these spent bleaching clays may contain 20 to 30% adsorbed oil that cannot be recovered economically. World production of oils and fats was estimated at 124 million tons in 2003 (Oil World 2004) and the amount of SBC produced from the refining industry would have been substantial. SBC is currently regarded as a waste product and usually discarded in landfills (Wong 1983) or burnt in incinerators to produce energy.

The objective of the present study was to use African catfish (*Clarias gariepinus*, Clariidae) as a biological agent for the economical extraction of the adsorbed oil in SBC from palm oil refining. Austreng (1978) was the first researcher to incorporate SBC from the refining of marine oils in the diets of rainbow trout, *Oncorhynchus mykiss* (Salmonidae). Up to 30% dietary inclusion of SBC had no significant effects on growth, mortality, fat content, fatty acid composition, nutrient digestibility, or organoleptic properties of rainbow trout. About 27 years later, we reported new information on the practicality of using SBC from palm oil refining as a cheap source of lipid in the diets of red hybrid tilapia (*Oreochromis* sp., Cichlidae) (Ng and Low 2005). As far as we know, no other nutritional evaluation of the potential of using this energy-rich oil-laden SBC from edible oil refining in fish diets have been reported. The incorporation of this currently discarded by-product in the diets of African catfish will greatly contribute to reducing the impact of rising feed costs in the culture of this species in many tropical countries.

Materials and Methods

Seven isonitrogenous (35% crude protein) and isolipidic (12%) practical diets were formulated (Table 1). Dietary protein was provided by fish meal (23% of total protein) and solvent-extracted soybean meal (12% of total protein). The control diet contained no SBC and six diets containing 5, 10, 15, 20, 25 or 30% SBC, respectively, were also formulated. SBC containing 24.7% residual oil and CPO were obtained from a local palm oil refinery. With increasing dietary SBC, the inclusion of CPO was correspondingly decreased to account for the residual oil present in SBC. We have previously shown that palm oil products can be successfully used in the diets of African catfish (Ng et al. 2003, Ng et al. 2004). The experimental diets were prepared following the procedure described by Ng et al. (2003). All pelleted diets were stored in an air-tight polyethylene bag in a freezer at -20°C until needed.

African catfish fingerlings obtained from a local fish hatchery were acclimatized in a 1000L fiberglass tank and fed a commercial catfish pellet (34% crude protein; KT Feeds, Thailand) for four weeks. One week prior to starting the feeding trial, all fish were fed the control diet. At the start of the experiment, 10 fish (mean weight of 17.9 ± 0.1 g) were stocked into each of 21 aquaria. The description and operation of the aquaria system were described in Ng et al. (2003) which basically consisted of a static water system with a series of 100L aquaria with aerated water. About 75% of the water in each aquarium was changed daily. Each experimental diet was fed to randomly assigned triplicate groups of fish at 4% of their body weight per day, separated into two equal feedings (1000 hours and 1700 hours). This feeding rate was close to the maximum daily ration estimated from the amounts consumed during the acclimation period. Fish were batch-weighed by aquarium once every week, and the daily ration adjusted accordingly. The feeding trial lasted 6 weeks.

Table 1. Ingredient formulation of experimental diets (g•100g^{-1} dry diet)

Ingredients	Spent bleaching clay inclusion (%)						
	0	5	10	15	20	25	30
Fish meal[1]	31.77	31.77	31.77	31.77	31.77	31.77	31.77
Soybean meal[2]	26.44	26.44	26.44	26.44	26.44	26.44	26.44
Spent bleaching clay[3]	0.00	5.00	10.00	15.00	20.00	25.00	30.00
Crude palm oil	7.89	6.65	5.41	4.17	2.94	1.70	0.46
Corn flour	3.50	3.15	2.79	2.44	2.09	1.73	1.38
Vitamin premix[4]	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Mineral premix[5]	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Dicalcium phosphate	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Carboxymethylcellulose	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Butylated hydroxytoluene	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Cellulose	22.38	18.98	15.57	12.16	8.75	5.34	1.94

[1] Danish fish meal contains (g•100g^{-1} dry weight): 72.4 crude protein, 11.0 crude lipid, 11.8 ash.

[2] Solvent-extracted soybean meal contains (g•100g^{-1} dry weight): 45.4 crude protein, 2.2 crude lipid, 8.2 ash.

[3] Spent bleaching clay contains (g•100g^{-1} dry weight): 0.2 crude protein, 24.7 crude lipid, 67.9 ash.

[4, 5] Composition according to Ng et al. (2003).

At the start of the experiment, 10 fish were sacrificed, weighed and kept frozen at -20°C until analyzed for whole-body composition. At the end of the experiment, all fish were killed and individually weighed. The caudal peduncle from five to six fish was severed, blood collected with a heparinized capillary tube, and hematocrits determined by the microcentrifugation method. The liver was removed and weighed to determine hepatosomatic index. Two whole liver tissues were selected randomly and fixed for 48 h at room temperature in Bouin's solution for histological analysis. Embedding was carried out in paraffin wax. Serial sections were then made through the whole liver, stained with hematoxylin-eosin and examined under a light microscope. Trunk and caudal vertebrae were removed after cooking the fish for a few minutes in a microwave oven. The bone samples were then washed in distilled water, dried, and ashed. The remaining fish from each aquarium were pooled and stored frozen at -20°C until analyzed for whole-body composition. Prior to analysis, fish were blended, dried and ground into powder. The moisture, crude protein, lipid, ash, and crude fiber contents of diet ingredients, experimental diets and fish were determined by standard AOAC methods (AOAC 1997). Moisture was determined by oven drying at 100°C to constant weight; crude protein determined indirectly from the analysis of total nitrogen by the Kjeldahl method; crude lipid determined by extraction with methanol and chloroform; ash determined from weighed moisture-free samples in a porcelain crucible placed in a muffle furnace at 550°C for 6 h; fiber content determined using acid-base digestion.

On the last week of the feeding trial before the first feeding at 1000 hours, water in each aquarium was sampled using a siphon positioned in the middle of the water column. Water samples were analyzed for total solids (TS) and total suspended solids (TSS) concentration within 2 hours after collection. A 100-ml portion of the water sample was poured into a pre-weighed beaker, placed in an oven at 100°C and evaporated to dryness. The difference in beaker weight was used to calculate the TS concentration (mg•L^{-1}) in the water. This represents both the dissolved and suspended particulate organic and inorganic matter. To estimate the concentration of TSS, another 100-ml portion of the water sample was vacuum-filtered through a pre-weighed membrane filter (0.65 µm, Millipore) that was subsequently dried in an oven. TSS concentration (mg•L^{-1}) was determined by the dry weight of the residue retained on the filter.

Data collected from the feeding trial were tested statistically for significance ($P < 0.05$) using the one-way analysis of variance (SAS Institute, Cary, North Carolina). Differences between means were assessed by Duncan's multiple range test.

Results

The experimental diets were similar in protein, lipid and energy concentration as expected from the ingredient formulations used (Table 2). Diet ash content increased from 8.3 to 29.6% with increasing dietary levels of SBC. All experimental diets were highly palatable and were immediately consumed by the African catfish fingerlings during the feeding trial. All fish were active and appeared healthy at the end of the experiment, and there were very few fish mortality (Table 3).

Table 2. Proximate composition (% dry weight) of the experimental diets

Component	Spent bleaching clay inclusion (%)						
	0	5	10	15	20	25	30
Moisture	14.4	14.5	12.9	14.0	12.6	12.4	14.1
Crude protein	35.7	35.9	35.8	35.6	35.8	35.8	35.9
Crude lipid	11.3	11.2	11.5	11.4	11.6	11.8	11.7
Ash	8.3	12.2	14.5	18.8	22.9	25.7	29.6
Crude fiber	18.4	16.6	13.6	9.1	6.5	4.2	1.5
NFE[1]	26.3	24.1	24.6	25.1	23.1	22.5	21.3
Metabolizable energy[2]	14.6	14.2	14.4	14.4	14.2	14.2	14.0
Protein energy[3]	40.8	42.1	41.6	41.3	42.0	42.3	43.0
P:E ratio[4]	24.4	25.2	24.8	24.7	25.1	25.2	25.7

[1] Nitrogen-free extract = 100 – (crude protein + crude lipid + crude fiber + ash).

[2] Metabolizable energy in kJ/g, calculated based on 16.7 kJ/g protein; 37.7 kJ/g lipid; 16.7 kJ/g carbohydrate (Garling and Wilson 1976).

[3] Protein energy = (energy in protein /metabolizable energy) x 100%.

[4] P:E ratio = Protein to energy ratio in mg protein/kJ metabolizable energy.

All catfish fingerlings showed excellent growth performance and increased in weight from about 18 g to 60 g at the end of the 6-week feeding trial. Growth of African catfish fed diets with the highest dietary inclusion of SBC at 30% did not show any significant difference ($P > 0.05$) compared to fish fed the control diet without any added SBC (Table 3). No significant effect of dietary SBC inclusion on feed conversion ratios, protein efficiency ratios or net protein utilization efficiencies were observed (Table 3).

There was no evident trend as to the influence of SBC on whole-body composition of catfish (Table 4). Increasing dietary SBC at 15% and above tended to slightly increase the bone ash content but most of these differences were not significant statistically. The hepatosomatic index (HSI) of fish fed the SBC incorporated diets were all significantly lower compared to the HSI of fish fed the control diet (Table 4). A preliminary evaluation of liver histology under the light microscope did not reveal any obvious structural abnormalities (data not shown). Liver parenchyma in fish fed SBC added diets were well organized and the hepatocytes well compartmented similar to that observed in fish fed the control diet. Hematocrits of fish fed the various diets were not significantly different (Table 4).

Total solids concentration in the aquarium water increased significantly concomitant to the increasing dietary levels of SBC fed to African catfish (Table 5). The concentration of TSS, representing suspended particulate organic and inorganic matter in the water, increased markedly from 11 to 69 mg•L^{-1} when SBC increased from 0 to 30% in the diets fed to the respective groups of catfish.

Table 3. Growth performance, feed utilization efficiency and survival of African catfish fed increasing dietary levels of spent bleaching clay for 6 weeks[1]

Parameter	Spent bleaching clay inclusion (%)							
	0	5	10	15	20	25	30	S.E.M.[2]
Final weight (g)	60.2	61.4	65.8	58.0	61.4	58.2	60.8	0.9
Weight gain (%)[3]	234.9	245.4	266.3	228.0	241.2	226.8	238.1	4.6
SGR (% day^{-1})[4]	2.86	2.95	3.09	2.83	2.92	2.81	2.90	0.03
FCR[5]	0.99	0.96	0.95	0.98	0.97	1.00	1.07	0.01
PER[6]	2.85	2.90	2.96	2.85	2.88	2.78	2.60	0.04
NPU[7]	46.7	46.0	45.4	45.8	45.8	43.2	41.2	0.6
Survival (%)	96.6	100.0	96.6	100.0	100.0	100.0	93.3	0.9

[1] Values are the mean of triplicate groups of 10 fish. Mean values in the same row with different letters are significantly different ($P < 0.05$). Average initial body weight of individual fish was 17.9 ± 0.1g.

[2] Pooled standard error of means.

[3] Expressed as the percent of initial body weight at the end of 6 weeks.

[4] Specific growth rate (%/day) = 100 x (ln final – ln initial fish weight)/days.

[5] Feed conversion ratio = total dry diet fed (g)/ total wet weight gain (g).

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

[7] Net protein utilization (%) = 100 x [(final – initial fish body protein) / total protein intake].

Table 4. Whole-body composition (% wet weight basis), bone ash (% dry weight basis), hepatosomatic index, and hematocrit (%) of African catfish fed increasing dietary levels of spent bleaching clay for 6 weeks[1]

Component	Spent bleaching clay inclusion (%)							
	0	5	10	15	20	25	30	S.E.M.[2]
Whole-body								
Moisture	74.3	74.7	75.1	74.2	73.8	73.6	73.6	0.2
Crude protein	15.6	15.4	14.9	15.2	15.4	15.2	15.1	0.1
Crude lipid	5.4^{ab}	4.4^{b}	5.1^{ab}	5.5^{ab}	5.6^{ab}	5.9^{a}	5.6^{ab}	0.2
Ash	3.3	3.4	3.3	3.6	3.7	4.2	4.0	0.1
Bone ash	30.2^{ab}	27.8^{b}	30.7^{ab}	33.4^{a}	33.5^{a}	33.6^{a}	33.5^{a}	0.8
HSI[3]	1.18^{a}	0.80^{bc}	0.79^{bc}	0.88^{bc}	0.95^{b}	0.68^{c}	0.84^{bc}	0.06
Hematocrit	36.2	35.4	32.9	33.2	35.4	35.2	35.6	0.4

[1] Values are the mean of triplicate groups of 10 fish. Mean values in the same row with different letters are significantly different ($P < 0.05$). Initial whole-body composition was 76.3% moisture, 14.4% protein, 4.6% lipid and 4.0% ash.

[2] Pooled standard error of means.

[3] Hepatosomatic index.

Table 5. Total solids and total suspended solids in the culture water of African catfish fed increasing dietary levels of spent bleaching clay (SBC)[1]

SBC	Total solids (mg•L^{-1})	Total suspended solids (mg•L^{-1})
0	271.33 ± 14.77^{c}	10.67 ± 3.18^{d}
5	294.00 ± 14.18^{bc}	25.33 ± 0.67^{c}
10	270.67 ± 17.65^{c}	42.00 ± 2.89^{b}
15	275.00 ± 19.16^{c}	42.00 ± 6.81^{b}
20	338.33 ± 13.92^{ab}	43.00 ± 2.31^{b}
25	351.00 ± 4.36^{a}	46.33 ± 5.55^{b}
30	322.33 ± 36.96^{abc}	69.00 ± 3.06^{a}

[1] Values are the mean ± SD of three replicate determinations. Mean values in the same column with different letters are significantly different ($P < 0.05$).

Discussion

The present study demonstrated that SBC from palm oil refining could be included at 30% (dry weight basis) in the diets of African catfish without affecting growth performance and feed utilization efficiency. We recently reported similar high levels of spent palm oil bleaching clay that can be included in tilapia diets without adversely affecting growth performance (Ng and Low 2005). Austreng (1978) reported that dietary levels of up to 30% SBC from marine oils refining can be successfully used in the diets of rainbow trout. In the rainbow trout study, fish fed diets containing SBC showed a significantly higher frequency of discoloured livers compared to fish fed the control diet. Austreng (1978) attributed this observation to the fact that the residual oil in SBC might have been oxidized despite the addition of 700 mg/kg ethoxyquin. In the present study, all catfish livers appeared healthy despite slightly reduced organ weights in fish fed SBC-based diets. This reduction in the HSI was not previously observed in tilapia (Ng and Low 2005) even at the highest dietary level of SBC tested (40%) and its biological significance is currently not known. Nevertheless, since CPO contains about 48% saturated fatty acids, the residual oil found in SBC from CPO refining would be less prone to oxidation compared to marine oils which are highly unsaturated. Due to its higher oxidative stability, spent palm oil bleaching clay might be a more suitable diet ingredient for fish compared to SBC from the refining of marine oils or other vegetable oils which are more unsaturated. At the moment, there are no known anti-nutrients that have been identified in SBC. This might explain the lack of effect on the blood hematocrit values of African catfish in the present study and also in a previous study with hybrid tilapia (Ng and Low 2005) fed high levels of SBC. Keith and Bell (1986) reported that up to 4% spent canola oil bleaching clay could be included in the diets of mice and rats without adverse metabolic effects.

In the present study, cellulose was included as an inert bulking agent and was assumed to have very minimal impact on the results, if any. However, it should be noted that high dietary cellulose had been reported to increase stomach emptying time, reduced growth and is known to reduce the availability of nutrients in some fish (Hilton et al. 1983). Any influence cellulose fiber has on nutrient digestibility and availability was not determined in the present study as this was not the main objective of the study. Even if the high levels of dietary cellulose had an effect on nutrient availability, this was not evident in the present study as excellent growth and feed utilization efficiency parameters were observed in catfish fed all the various experimental diets.

As far as we know, there is currently no other reported nutritional evaluation of the potential of using SBC from edible oil refining in fish diets. However, several researchers have conducted studies on the use of SBC in poultry diets. Blair et al. (1986) reported that up to 7.5% SBC from canola oil refining could be incorporated in broiler chicken diets with no negative effects on growth performance or health of the animals. Broiler chickens fed up to 10% SBC did not show any adverse effects on growth, feed efficiency, or mortality but at higher dietary levels of SBC (20-25%), the chickens had softer bones, ataxia, and hemorrhages in the brain (Waldroup and Ragland 1977). SBC from palm oil refining could be included in laying hen diets up to 10% without deleterious effects on egg productivity and quality (Al-Zubaidy 1992). Bentonite clay is routinely used as a binding and lubricating agent in the production of pelleted poultry diets.

The high ash content may limit the use of SBC since it will take up much of the non-nutritive bulk space in diet pellets. Therefore, despite results showing that high levels of SBC can be included in fish diets without deleterious effects, Hertrampf and Piedad-Pascual (2000) recommended no inclusion of SBC in starter diets, 3–7% in grower diets and 5-10% in finisher diets for fish. Silica, the main mineral in bleaching clay, is indigestible to fish. It is currently not known whether other minerals present in SBC are available to fish and deposited in tissues. In the present study, bone ash tended to increase slightly when dietary levels of SBC were 15% and above. In a previous study with African catfish, we observed no significant increase in bone ash or bone phosphorus content when catfish were fed diets incorporated with up to 8% of a montmorillonite-type clay (Ng et al. 2001). Blair et al. (1986) reported that bone ash of broiler chickens fed SBC from canola oil refining tended to increase slightly only when dietary levels are above 12%. In contrast, the addition of SBC up to 40% in the diet of tilapia did not significantly increase the bone ash content of fish (Ng and Low 2005).

The high silica (about 69% SiO_2) and mineral content of SBC may also limit its use in fish diets since the indigestible component will be discarded into the culture water through fecal matter. The significant increase in TS and TSS observed in this study (Table 5) would most likely be attributed to the increase in suspended mineral particles. It is currently not known how this biological discharge of clay particles into the water will affect the pond environment under commercial African catfish production conditions. Wallen (1951) determined the direct effects of montmorillonite clay turbidity on 16 species of fish and reported that significant fish mortalities only occurred at TSS concentrations above 175,000 $mg \cdot L^{-1}$. TSS concentrations usually measure less than 2,000 $mg \cdot L^{-1}$ in muddy pond waters. Therefore, pond water turbidity caused by the defecated clay particles is not likely to have any direct effects on African catfish which is known to prefer muddy habitats. However, muddiness can affect primary productivity by interfering with sunlight penetration into the pond water. The use of SBC in fish feeds might also be limited in countries that have strict standards in allowable limits for aquaculture effluents. The limit for TSS in effluent permits in the United States is about 50 $mg \cdot L^{-1}$ (Ozbay and Boyd 2003). In a recent study of TSS concentrations in channel catfish (*Ictalurus punctatus*, Ictarulidae) ponds in Alabama, USA, 46% of water samples collected exceeded this concentration (Ozbay and Boyd 2003).

In the production of tropical catfish diets, manufacturers are constantly faced with the need to reduce diet costs to match fluctuating and at times low ex-farm prices of African catfish. Since feed costs can account for more than 50% of the farm prices of catfish, there is great interest to reduce feed costs by using locally-available or alternative diet ingredients. In conclusion, SBC from palm oil refining is a potential diet additive in African catfish diets offering a cheap source of dietary lipid and energy. Further longer term feeding trials (up to marketable size fish) is needed especially in regards to safety and toxicological issues in the use of this waste product from edible oils refining.

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