Continental J. Fisheries and Aquatic Science 7 (1): 18 - 31, 2013 ISSN: 2141 – 4246
© Wilolud Journals, 2013 http://www.wiloludjournal.com
Printed in Nigeria doi:10.5707/cjfas.2013.7.1.18.31

NITROGEN AND MINERAL BUDGET OF NILE TILAPIA FRY FED RECYCLED FOOD WASTES MATERIALS SUPPLEMENTED WITH LYSINE AND METHIONINE IN A CLOSED RECIRCULATING FISH CULTURE SYSTEM

G. G. Bake[1,2], M. Endo[2], S. Satoh[2] , S.O.E Sadiku[1],T. Takeuchi[2]
[1]Department of Water Resources, Aquaculture and Fisheries Technology, School of Agric and Agric Technology, Federal University of Technology Minna P.M.B 65 Minna Niger state - Nigeria
[2]Department of Marine Biosciences, Tokyo University of Marine Science and Technology, 4-5-7 Konan, Minato, Tokyo 108-8477, Japan.

ABSTRACT
The study evaluated nitrogen and mineral budgets of Nile tilapia *Oreochromis niloticus* in a closedrecirculation system for 11 weeks, during which no water renewal was carried out. Fish (mean weight 1.2 ± 0.11 g) were fed with different levels of recycled waste materials supplemented with lysine (lys) and methionine (met), designated as D1 (0% FIW+SSW), D2 (57.90 % FIW+SSW + lys and met) and D3 (54.84% FIW+SSW only). The result showed that fish fed D2 diet produced higher ($P<0.05$) growth performance and feed efficiency than those fed the other diets. The biofilter used in the recirculation system effectively converted ammonia to nitrate, as such toxic ammonia and nitrite were negligible. However inorganic phosphorus was similar among the treatments. Phosphorus retention was significantly higher ($P<0.05$) in fish fed the D2 diet, while nitrogen retention did not differ significantly among fish fed the experimental diets. No significant difference was recorded in nitrogen loading among the treatments, but phosphorus loading was lower in D2 and was significantly ($P<0.05$) different from other treatments. There was no considerable variation in the solid wastes generated among all treatments; however, mineral composition confirmed that the solid wastes were composed of phosphorus, calcium, iron, zinc, manganese and copper; while nitrogen, magnesium, sodium and potassium were noted in the rearing water. The results of this study revealed that diet D2 made the least negative impact on the environment.

KEYWORDS: Food industry waste; Nitrogen loading; phosphorus loading; Recycled food waste; Soy sauce waste;

Received for Publication: 22/10/12 Accepted for Publication: 01/02/13
Corresponding author: gabbygana@yahoo.co.uk

INTRODUCTION

Aquaculture intensification requires improved production technology including efficient low-cost and low-pollution feeding system. It has been well established that in all fish diets, irrespective of species, protein is the most important dietary nutrient and fishmeal remains one of the major source of dietary protein (Kaushik, 1990).

Formulation of fish feeds containing high levels of conventional and non-conventional alternative protein source has become a major focus in aquaculture nutrition research. This has become imperative because of the ever-increasing cost and uncertain availability of fishmeal. Hence conventional plant proteins appear to be the most suitable alternatives for fishmeal in fish diets (Soybean, rapeseed, etc.). However, their scarcity as well as competition from other sectors for these conventional crops for livestock, human consumption and industrial use makes their cost too high, thereby placing them far beyond the reach of average farmers or aqua feed producers (Fasakin, 1999). In Japan, there is a basic law establishing a recycling-based society, with

emphasis on the minimization of associated biodegradable waste by-products by recycling them for effective re-use. With recent developments in technology, a concerted effort to recycle and re-use food waste is increasing.

Aquaculture intensification has resulted in culturing activities with a higher degree of feed input and waste output per rearing system. This waste output has adverse effect on the quality of the rearing water system, since the enriching nutrients originate from the feed added to the rearing system (Hardy and Gatlin III, 2002). It has been well established that nitrogen is associated with the most expensive component of feed. Regardless of species, protein is the most important dietary nutrient in all fish diets; and fishmeal remains one of the major sources of dietary protein (Kaushik, 1990).

Increasing concerns over the environmental impact of fish farming and incidence of disease have led to a progressive reduction in water exchange up to the development of nearly static systems with no water exchange where effluent discharge and the introduction and dissemination of pathogens are significantly reduced (Colt, 2006) The closed recirculation fish culture system provides an alternative means of intensive production that reduces environmental impacts and allows greater control of factors that might affect growth. It also allows direct treatment of wastes and its re-use, the ability to limit the release of antibiotics to the external environment, and control over water pH, temperature and chemistry. Such fine manipulation of the culture environment helps to optimize fish growth. However, one of the major problems with the closed recirculation system is related to the accumulation rate of suspended solids particularly very fine particles. Almost all wastes generated from aquaculture are derived from the addition of feed, uneaten feed or unavailable dietary nutrients in faeces and other metabolic products are primary contributors in fish farm effluents.

Accumulation of organic matter associated with aquaculture discharge can result in the development of reducing anoxic sediments, high sediment oxygen demand, production of hydrogen sulfide and other gases, and decrease in benthic fauna (Wu *et al* 1995; Hansen *et al* 1998). One way of reducing eutrophication problems is through the reduction of nutrients in aquaculture effluents. Thus, it is important to understand the cycle of nitrogen in aquaculture systems. As nitrogen is associated with the most expensive component of the feed, then the performance and efficiency of an aquaculture system can be evaluated through analysis of the conversion of nitrogen to fish biomass. Current studies documenting nitrogen dynamics in aquaculture primarily target pond systems (Boyd, 1985; Krom and Neori, 1989).

The partial or complete replacement of fishmeal (FM) with alternative plant or animal protein ingredients generally results in an imbalance of amino acids, which can affect fish growth and protein utilization efficiency, resulting in higher N loading into the culture medium (Rumsey, 1993, Sajiura, 1998). Improvement of fish feed formulations through partial replacement of FM by ingredient low in P is essential for lowering P loading (Takeuchi *et al*., 1993; Sugiura, 1998; Cho and Bareau 2001). Hence, optimal replacement of FM without negative effect on the fish growth and culture medium is highly desirable to fish nutritionists. Tilapia are known as “aquatic chicken” because of their fast growth, good quality flesh, disease resistance, adaptability to a wide range of environmental conditions, ability to grow and reproduce in captivity, and feed on low trophic levels. Thus, they have become an excellent choice for aquaculture, especially in tropical and subtropical environments (El-Sayed, 2006). Due to the importance of these species, it is critical that feeds are both economically and environmentally sustainable in their culture environment.
Aquaculture wastes contain mainly P and nitrogen (N), which can contribute to excessive algae and macrophyte growth in the receiving waters (Pillay, 1992). Since the ultimate source of aquaculture waste is the feed fed to fish, one effective way to reduce the waste load of fish farm effluent is to improve mineral utilization, the feed quality, and achieve a reduction in excretion of P and N and total solids relative to fish growth (Lall, 1991). Management of aquaculture waste can be approached through improvements in nutrient utilization and feed formulation (Lall, 1991). Feed quality improvement by increase ingredient of dietary P and other nutrients has become one of the main strategies to reduce the harmful effects of nutrient loading on natural waters by the aquaculture industry. Nutrient budgets of a number of freshwater ponds (Boyd, 1985;

Avnimelech *et al*, 1986; Foy and Rosell, 1991a,b) and seawater ponds (Krom *et al*., 1985; Krom and Neori, 1989) have been reported. The waste loadings from tank culture systems have been quantified mainly for salmonid fish culture including, adult (Fivelstad *et al*., 1990) and juvenile stages (Watanabe, *et al* 1999).

In our previous studies we demonstrated the suitability of recycled food waste materials from food industry waste (FIW) and soy sauce waste (SSW) in the practical diet of Nile tilapia fry and inclusion of these recycled food waste up to 58% enriched with lysine (lys), methionine (met) and arginine (arg) was also achieved (Bake *et al* 2009; 2010 and 2011). Thus, the main objective of this study was to investigate the Nitrogen and mineral budget of Nile tilapia *Oreochromis niloticus* Fingerlings fed recycled food waste-based diets in a closedrecirculation fish culture system.

MATERIALS AND METHODS

Culture Facilities:

The experiment was carried out at the Laboratory of Fish Culture, Tokyo University of Marine Science and Technology, Japan using replicated closed recirculating aquaculture systems. The system consists of a 30-liter rectangular fiberglass aquarium, which is the culturing chamber, a sedimentary chamber for solid waste collection and the bio-filtration chamber, which harbors the bacterial community for nitrification. The culturing chamber was completely closed to the atmosphere and was equipped with a slide glass cover to the aquarium and can be opened during water addition to compensate for evaporation and also during other aquaculture rudimentary work. Water temperature was maintained at 28±0.5 ^{o}C using electric heaters placed in each tank. The aquariums were provided with continuous aeration through an air compressor and illuminated by overhead fluorescent lights to maintain a constant photoperiod of 12 h light and 12 h dark cycle (8:00-20:00) throughout the study. The sediment tank used was the TUF (Tokyo University of Fisheries) column system according to Satoh, *et al*. (1992).

This solid waste collector was attached to each aquarium and then connected to the bio-filter chamber. The solid waste was usually collected from sediment tank once a week. The EMEH 2213330 model bio-filter tank was used. Inside the bio-filter tank is the synthetic filter media, which were the bacterial carriers for nitrification; coral sand were placed on top of the filter media to buffer the pH of the water system, and foam layer was placed between the coral sand and the water outlet of the pump of EMEH cylinder. Each system had an independent recirculating system and the water flow maintained at 900 ml/min for each aquarium. Bacterial community for nitrification process was established in bio-filter tanks two weeks before the commencement of the feeding trial. After the establishment of the bacterial community in the bio-filter tanks, the systems were cleaned up and 33 liters of fresh dechlorinated tap water was supplied to each system without any water exchange throughout the experimental period. Distilled water was used for compensation of the loss due to evaporation or sampling for water quality analysis.

Diet formulation and preparation

Ingredients and diet formulation

Soy sauce waste: Soy sauce waste (SSW) was produced by Yamasa Corporation and processed by Nippon Formula Feed Mfg. Co. Ltd. After the fermentation of soybeans and soy sauce extraction, the residual cake, which is a waste product was collected, dried and recycled by dehydration to reduce the moisture to a low level. Crude protein and lipid contents of SSW were 26.13% and 11.93%, respectively.

Food industry waste: The food industry waste (FIW) used in this study was obtained from Nippon Formula Feed Mfg. Co. Ltd. It includes leftover food from convenience stores, food waste residues discharged during processing, hotel waste, restaurant cooking waste, tofu waste and bread production waste. Fry-cooking the waste with vegetable oil at a very low pressure processed the FIW and an initial temperature of between 80-100 ^{o}C maintained for 1 hour and later increased to 100 - 110 ^{o}C for about 30 minutes after which the product was allowed to cool off before grinding it into a powdered form. Crude protein and lipid contents of FIW were 19.60% and 11.34%, respectively.

Fishmeal: The fishmeal (FM) used in this experiment was obtained from Nippon Formula Feed Mfg. Co. Ltd. The crude protein and lipid contents of FM were 63.50% and 11.79%, respectively.

Experimental diets

Based on the nutritional requirements of tilapia (NRC 1993), three isonitrogenous and iso-lipidic diets were formulated at 35% protein and 9.5% lipid, containing different sources of recycled food waste materials supplemented with lys and met designated as FM representing the commercial fishmeal (control) containing different inclusion levels of recycled waste materials supplemented with lys and met and designated as D1 (0% inclusion of FIW+SSW), D2 (57.90 % inclusion of FIW+SSW supplemented with lys and met) and D3 (54.84% inclusion of FIW+SSW only) .

Fish and Feeding

The tilapia *O. niloticus* fry (average weight: 1.21± 0.27g) used for this experiment were obtained from pure–bred tilapia broodstock in the Fish culture laboratory of the Tokyo University of Marine Science and Technology Japan. Twenty fish were stocked in each 30-liter aquariums and fed for 79 days. Two replicates of each treatment were reared for each of the 3 diets and were hand fed 3 times daily at 80%of Japanese fisheries Agency feeding rate standard at 10:00, 14:00, and 17:00. Feeding rates were subsequently adjusted according to their growth rate per aquarium. Fish were denied feed 24 h prior to sampling. 10 fish were randomly sampled and weights were measured using a digital electronic weighing balance (AW220; Shimadzu Corporation, Kyoto, Japan) on a weekly basis. Totallengthandstandardlengthweremeasuredusingdigimaticcalipers(CD-20CP; Mitutoyo Corporation,Tokyo,Japan).

Water sampling and Analytical procedure

Water quality was measured throughout the experimental period. To determine the quality of the rearing water, samples were obtained from each aquarium before feeding using clean, plastic bottles. Water temperature and dissolved oxygen in the system were monitored daily in the morning by DO meter (HQ 30d, HACH Company, Colorado, USA). Water samples were taken once a week from the surface water near the center of each aquarium for subsequent analysis for pH, ammonia-nitrogen (NH_4-N), nitrite–nitrogen (NO_2-N), nitrate–nitrogen (NO_3-N), phosphorus, coloration and turbidity. Concentration of these elements in rearing water was analyzed with reagents of water analysis (HACH Company, Colorado, USA) using the following methods for each element, ammonia nitrogen: Nessler method, nitrite nitrogen: Diazotization method, nitrate nitrogen: Cadmium reduction method and Phosphorus: Ascorbic acid method and analyzing them under ultra violet and visible light spectrophotometer (UV-1200, Shimadzu Corporation, Kyoto, Japan). Data collected from NH_4-N, NO_2-N and NO_3-N were used to determine the total dissolved nitrogen.(APHA, AWWA and WEF 2005).

Sample collection and Biochemical analyses

The experimental diets, fish body samples, solid waste materials, the sludge and the rearing water were subjected to chemical analysis at the end of the experiment. Fish were weighed individually at the beginning of the experiment and subsequently once a week using an electronic balance (EB-3200D, Shimadzu Corporation, Kyoto, Japan). The average of total weight of the fish in each tank was used as a unit of observation for analysis. Upon termination of the experiment, 10 fish from each aquarium were randomly selected for the chemical analyses of the whole body. Whole body samples were pooled from 5 fish per aquarium and minced by a centrifugal mill (Retsch ZM 200, Germany) fitted with a 0.25-mm screen. The homogenate was collected and kept at -20^{O}C until analysis. The remaining 5 fish per tank were freeze-dried, crushed and then mixed using (Millser IFM-700G IWATANI) and kept for mineral analysis. Feces were collected once a week from the sedimentary tank (TUF) water column (Satoh, *et al.* 1992). Fecal matter samples from each treatment were centrifuged (Hitachi Himac CF 15R) at 6000 *g* for 10 min and then freeze-dried and the corresponding dry weight of each sample was recorded and then the dried samples were kept for subsequent analyses. At the end of the experiment, the fish were scooped out of each nitrification tank and emptied into its rearing tank and the filter media was washed into the rearing water and the sludge was allowed to settle for 2h. The supernatant was returned to the rearing tank and the concentrated sludge was centrifuged (Hitachi himac CF 15R) at 6000 *g* for 10 mins and then freeze-dried and the corresponding dry

weight of each sample was recorded and then the dried samples were kept for subsequent analyses. Proximate analysis and lipid analysis were done according to the methods detailed in Takeuchi (1988) and Folch *et al.*(1957). Closed vessel digestion with microwave heating was done to determine the mineral composition of the experimental diets fish body samples and solid waste materials. Dried samples (0.3g) were mixed with 6ml of concentrated sulfuric acid in a closed Teflon vessel (85ml) and digested by using a microwave oven (model 7295, O.I. Analytical, Texas USA) at 175 ºC for 15 mins. 10ml of 35% hydrogen peroxide was added to the processed mixture and was heated by microwave at 150 ºC for 5 min. The digested solution was then used for the mineral analysis. The concentration of each element was measured by Inductively Coupled Plasma Atomic Spectrophotometer.

Evaluation of growth parameters

Growth Rate, Food Efficiency and Sedimentation of each element in tanks: substance balance (culture water, sedimentation, fish, and bubble-separated substances)

Specific growth rate of wet body weight (SGR %) = $(\text{In } W_t - \text{In } W_0) / t \times 100$……(1)

Feed efficiency (FE %) = $(Wt - W_0)/ \text{TFI} \times 100$ ……(2)

NRF (%) = $(W_t \times W_{nt} - W_0 \times W_{n0})/ \text{TFI} \times \text{DDC} \times 100$……...……(3)

$$ARW\ (\%) = \sum_{n=1}^{8} \left\{ \frac{\left(CER_t \times VR_t\right) - \left(CER_0 \times VR_0\right)}{\left(TFI \times DDC\right)} \right\} \times 100 \text{ ……..……...(4)}$$

ARSW (%) = (TSW x CESW)/ (TFI x DDC) x 100…….(5)

Where:

NRF = nutrient retention of fish fed experimental diet. (%)

Wt = Final body weight of the fish at the end of the experiment (g)

W_0 = Initial body weight (g)

W_{Nt} = Final body content of each element in the fish at the end of the experiment (mg/g)

W_{N0} = Initial body content of each element in the fish at the beginning of the experiment. (mg/g).

TFI = total feed in take (g)

DDC = Content of each element in the experimental diet (mg/g)

ARW = accumulation rate of rearing water (%)

CER_t= concentration of each element in the rearing tank at the end of week t (mg/L)

VR_t = volume of rearing water at the end of week t (L)

CER_0 = concentration of each element in the rearing tank at the beginning of week t (mg/L)

VR_0 = volume of rearing water at the beginning of week t (L)

ARSW (%) = accumulation rate of the solid waste

TSW = Total solid waste removed from the system (g)

CESW = content of each element in the solid waste (mg/g)

Statistical analyses

Data were analyzed via one-way analysis of variance (ANOVA) using Statistica 6.0 (Stat-Soft, Inc., USA). Differences between treatments were compared by (Tukey's) test. Level of significance was tested at $p<0.05$.

RESULTS

Chemical analysis of the experimental diets

All the formulated diets used in the present study were iso-nitrogenous and iso-lipidic. Table 2 shows the proximate composition and mineral content of the formulated experimental diets. The proximate composition of the experimental diets were similar, however, the moisture content was slightly lower in D1 than the other treatments. Furthermore there was no much differences in the mineral contents of the experimental diets except for zinc that was higher in D1 than the other treatments.

Growth and feed conversion

It was observed that there was no feed rejection by the fish fed the experimental diets, and they vigorously ingested the experimental diets. The change in weight per week and growth performance data of Nile tilapia fry fed the experimental diets for 79 days are summarized in Table 3. Table 3 shows that fish fed D2 had the highest values of all growth performances and was significantly different from the other treatments (P<0.05). On the other hand fish fed D1 had the lowest final body weight (22.99±0.05 g) but was not significantly different from D3 (P>0.05). The SGR, FE and FI efficiency ratio of the tilapias fed experimental diets followed the same pattern as the final body weight.

Retention and loading of nitrogen and phosphorus

Fig. 1 shows the phosphorus and nitrogen retention of Nile tilapia fed experimental diets for 79 days. D2 had the highest phosphorus retention and was significantly different from D1 and D2 (P<0.05), while the nitrogen retention was similar among the treatments. Fig. 2 shows the nitrogen loading of this study range between 23.15 – 27.42 kg/t among the treatments but they were not significantly different from each other. Phosphorus loading in this study ranges between 5.43 – 8.00 kg/t. D2 had the lowest phosphorus loading and was significantly (P< 0.05) different from the other two treatments, which were statistically similar

Solid waste generation and mineral composition of the faecal matter and sludge

Fig. 3 shows the total solid waste produced as fecal matter and sludge in the bio-filter tanks (biofilm) after washing the sludge with the rearing water. The solid waste collected through the use of the sediment tank (TUF), ranged between 33.45- 43.50 g, and was higher than the waste found in the bio-filter tanks. Furthermore, D2 had the lowest solid waste generation and was significantly lower than waste produced by D3 and D1. Waste generated in the bio-filter tanks ranged between 15.84 -19.00 g. There was no significant difference in the waste produced in the bio-filter tanks (total biofilm) among the treatments.

Table 4 showed the mineral composition of both the solid waste (fecal matters) and the bio film. The concentration of phosphorus and calcium were higher in the solid waste while the concentration of nitrogen, potassium and iron were higher in the biofilm. Other elements tend to have little or no variation among the waste constituents in each of the treatment.

Percentages of Nitrogen and minerals in the, rearing water, solid wastes biofilm and *O.niloticus* body

Table 5 showed a detail composition of each element in the rearing water, solid wastes, biofilm and the fish body fed the experimental diets, from the result, Nitrogen retained in the fish body ranges between 42.86±1.03% - 48.94±0.94%, while phosphorus ranges between 45.32±3.54% – 56.21±0.65%. 3.62±0.88% - 5.51±0.59 of Nitrogen and 28.21±0.08 - 35.68±4.67 of phosphorus was removed as solid waste. Furthermore 2.53±0.29 – 3.49±0.34% of nitrogen and 4.98±0.04 – 6.78±0.47 of phosphorus was removed in the biofilm chamber among the 3 treatments. Rearing water budget showed that 36.50±0.45% - 40.46±1.51% of Nitrogen and 7.38±0.32% - 11.43±1.36 of phosphorus was accumulated in the rearing water among the treatments respectively.

DISCUSSION

All the fish in this study showed normal growth with high FE. The specific growth rate gradually declined with the culture period and weight gain. The FE was above 100% for all the experimental fish, indicating the diets were of high quality and the fish adequately utilized the nutrients.

Retention efficiencies of P and N are of paramount important for evaluation of feed quality and these may change depending on fish weight and amount of feed consumed (Lall 1991; Cho *et al*, 1994). The retentions of P and N in this study were high and vary within the treatments. The high retention of P among the treatments may be attributed to the addition of highly available P source to the diets. This agrees with Satoh *et al*, (1998) and Sarker *et al* (2005) who reported that fish need additional soluble P especially when the ingredients are low in P content. Furthermore the higher retention of P by the fish fed D2 than the other treatments may also be resulted from the supplementation of lys and met. This also agrees with the finding of Hernandez (2005) and Satoh, *et al* (2002) who found that supplementation of Amino acid (AA) improves P

retention. In the context of this study there was no significant difference in the N retention among all the treatments, however nitrogen retention was high and was comparable to the results of Quillere, *et al*. (1993) Siddiqui *et al*. (1988) and El-Sayed (1990). This study agrees with the report of Tacon (1995) as to those differences in feed nutrients retention by fish can be related to differences in the diet compositions, chemical form of minerals in a fish diet, the quantity of minerals, the current physiological state of fish (rate of gain, live weight, and age), and the design of the experiment. The high P and N retentions obtained in this study was accompanied with low P and N loading. This was particularly the case in fish fed D2. Therefore in the context of this study the increased in P and N retention by the fish fed D2 diet and the lowered loading might have induce better growth and FE under recirculation culture conditions.

It has been reported that sludge production in a culture system varied from 3.00 to 4.00 kg with the dry matter of 8.00% to 12.00%, while the input feed is between 2.00–3.00 kg (Rakocy, 1995), however during long storage of fish in the culture system, dry matter of sludge exceeds 40.00% (Bergheim and Forsberg, 1993). In the context of our study the waste generation was lower than the above estimation. Suspended solids showed no significant trends, which could be due to the small size of the tanks and the relatively large biomass of fish present. The amount of solids produced per unit biomass significantly decreased with increasing nutrient density as compared with the beginning of the experimentindicating that nutrient density of the diet had an effect on the quantity of solids accumulated in the system. This result agrees with Kiaerskou (1991) who also found a smaller amount of solid waste produced in a system with fish fed low pollution diets. The amount of solid waste in a closed recirculating fish culture system are usually in particulate and usually consumes oxygen during biological degradation which will decrease the availability of oxygen for fish in culture (Davidson and Summerfelt, 2005). A decrease in nitrification is generally associated with increased organic loading (Bovendeur *et al*., 1990) due to the fact that the biodegradable organic matter in recirculating aquaculture systems generally supports growth of heterotrophic bacteria which compete with the autotrophic nitrifiers, especially in fixed film bioreactors (Zhu and Chen, 2001;Davidson and Summerfelt, 2005; Chen *et al*., 2006). Small quantities of unionized ammonia can be toxic for epithelial tissues and disturb the elimination of protein metabolites across gills. In the context of this study, such solid waste produced during the experimental period was small to cause any of the above problems in the rearing water (Fig 3) This suggests that the removal of the solid waste once a week was very effective to check the effect of solid waste accumulation in the rearing tank.

Bergheim and Asgard (1993) reported that although feed is ultimately the main source of nutrients released into the rearing water, the amount and form that the nutrients takes depend not only on the feed properties but also on the type of culture system used, thus environmental impact of diets, water discharged and sludge composition are highly variable. Our result tends to agree with this assertion. P and Ca concentrations tend to be higher in the solid waste compared with the biofilm. While N and Zn, were higher in the biofilm.

Result from this study also showed that the mineral concentration of the solid waste was higher in phosphorus, calcium, iron, zinc, manganese and copper than the rearing water, while nitrogen magnesium, sodium and potassium were higher in the rearing water. These results aggress with the findings of Endo *et al*. (1999, 2002)

In conclusion this study reveals that a combination of recycled food waste supplemented with AA gave us a higher growth performance than fishmeal based diet and they also produced lower waste loads, in particular of P, to the system thus recycled food waste can be used as ingredient in the diet of Nile tilapia with less environmental impact in a simple closed recirculation systems. From this study based on the performance and nutrient loading D2 gave the best result hence a judicious combination of recycled food waste can effectively be used to reduce fishmeal in fish diet without much environmental impact. However there is still need to evaluate the use of this waste in a closed recirculation system on a longer term

ACKNOWLEDGMENTS
This study was carried out with the financial support provided by a Grant-in-Aid for Scientific Research (A) from Japan Society for the Promotion of Science (No. 20248021).

REFERENCES

American Public Health Association, (2005). American Waterworks Association, and Pollution Control Federal 16th ed., APHA, Washington, DC, pp. 1268.

Avnimelech, Y., Weber, B., Hepher, B., Milstein, A. and Zorn, M. (1986). "Studies incirculated fish ponds: organic matter recycling and nitrogen transformations".Aquaculture and Fisheries Management 17: 231-242

Bake, G. G., M. Endo, A. Akimoto and T. Takeuchi (2009) Effect of reducing fishmeal content by using recycled food waste materials supplemented with lysine, methionine and arginine for a practical diet of Nile tilapia *Oreochromis niloticus* fry. The Japanese Society of Fisheries science Autumn meeting 30th September-3rd October 2009 Iwate prefecture Citizen's Cultural Exchange Center, Aiina Japan. . Book of Abstract Pp.37.

Bake G.G., Endo M., S.Satoh. S.O.E. Sadiku and Takeuchi T. (2011). Nutrient influx, Water quality and growth performance of Nile tilapia fry fed Recycled Food Waste Based Die in a Closed Recirculating Fish Culture System. *Nigerian Journal of Technological Research* (6) 22-31

Bake G.G., Sarker. M.S.A, Endo M., Akimoto A. and Takeuchi T. (2012). Evaluation of high-level inclusion of recycled food wastes materials supplemented with lysine, methionine and arginine in the practical diet of Nile tilapia (*Oreochromis niloticus*) *International Journal of Aquaculture and Fisheries Science*. Vol. 2 (2) 73-88.

Bergheim, A., Forsberg, O.I., (1993). Attempts to reduce effluent loadings from salmon farms by varying feeding frequencies and mechanical effluent treatment, in production. In: Barnable, G., Kestemont, P. (Eds.), Environment and Quality, Special Population vol. 18. European Aquaculture Society, Ghent, Belgium,pp. 115–124.

Bhukaswan, T.; Tonguthai,K.; Maclean, J. L. (ed). The Second International Symposium on Tilapia in Aquaculture. International Center for Living Aquatic Resources Management. Manila, Philippines

Boyd, C.E. (1985) Chemical budgets for channel catfish ponds.*Transactions of the American Fisheries Society*, 114:291-298.

Bovendeur, J., Zwaga, A. B., Lobee, G. J., Blom, H. J., (1990). Fixed-Biofilm Reactors in Aquacultural water recycle systems: Effects of Organic Matter Elimination on Nitrification Kinetics, *Wat. Res., Vol. 24, No. 2, pp. 207 – 213*.

Chen, S., Ling, J., Blancheton, J.,(2006). Nitrification kinetics of biofilm as affected by water quality factors.*Aquacultural Engineering*. 34, 179-197.

Cho C.Y., Hynes J.D., Wood K.R., Yoshida H.K. (1994): Development of high-nutrient-dense low-pollution diets and prediction of aquaculture waste using biological approaches. *Aquaculture* 124, 293–305.

Colt, J., (2006). Water quality requirements for reuse systems. Aquacultural Engineering, 34, 143–156.

Davidson, J., Summerfelt, S.T. (2005). Solids removal from a coldwater recirculating system - comparison of a swirl separator and radial-flow settler. *Aquacultural Engineering, 33*, 47-61.

El-Sayed, A.M., (1990).Long-term evaluation of cottonseed meal as a protein source for Nile tilapia *Oreochromis niloticus* L. *Aquaculture* 84, 315– 320.

El-Sayed, A.-F. M. (2006): Tilapia Culture. CAB International, Wallingford, UK, 277 p.

Endo M., Takeuchi, T., Yoshizaki, G., Toyobe M., Kanki , Suzuki K., Oguchi M., Kibe S. (1999): Studies on the development of closed ecological recirculating aquaculture system (CERAS) IV. Along term feeding experiment with *Oreochromis niloticus*in the fish-rearing closed tank. CELSS J., 12: 17-24.

Endo M., Takeuchi, T., Yoshizaki, G., Satoh S., Omori K., Oguchi M., Nakajima A. (2000): Studies on the development of closed ecological recirculating aquaculture system (CERAS) VI. Phosphorus form and mineral budgets in the fish- rearing closed tank during a long-term feeding experiment with tilapia. *CELSS J*., 13: 19-26

Fasakin EA, Balogun AM, Fasuru BE (1999) Use of duckweed, *Spirodelapolyrrhiza* L. Schleiden, as a protein, feedstuff in practical diets for tilapia, *Oreochromis niloticus*L. *Aquacult Res* 30: 313-318.

Fivelstad, S., Thomassen, J.M., Smith, M., Kjartansson, H. and Sando, A.B. (1990). Metabolite excretion rates from Atlantic salmon (*Salmo salar* L.) and Arctic charr (*Salvelinus alpinus* L.) reared in single pass land-based brackish water and sea water systems. *Aquacultural Engineering* 9: 1 – 21.

Folch J, Lee M, Sloane-Stanley GH (1957) A simple method for the isolation and purification of total lipids from animal tissues.*J Biol Chem*226: 497-509.

Food and Agriculture organization of the United Nations FAO (2003) Review of theState the World Agriculture. FAO Fisheries Circular 886.FAO, Rome, Italy.

Foy, R.H.; Rosell, R. (1991a).Loadings of nitrogen and phosphorus from a Northern Ireland fish farm. Aquaculture, 96:17–30.

Foy, R.H.; Rosell, R. (1991b).Fractionation of phosphorus and nitrogen loadings from a Northern Ireland fish farm.*Aquaculture*, 96:31–42.

Gonzales M J, Hutson H A, Rosinski E M, Wu V Y, Powless F T, Brown B Paul (2007). Evaluation of Fish meal –free diets for first feeding Nile tilapia, *Oreochromis niloticus*. *J Appl Aquaculture* 19(3): 89-99.

Hansen, K.H., Angelidaki, I., Ahring, B.K., 1998. Anaerobic digestion of swine manure: inhibition by ammonia. *Water Res*. 32, 5–12.

Hardy RW, Gatlin DM III (2002): Nutritional strategies to reduce nutrient losses in Intensive aquaculture. In: Cruz-Suárez LE,Ricque-Marie D, Tapia-Salazar M, Gaxiola-Cortés MG, simoes N (eds). Avances en Nutrición Acuícola VI Memorias del VI Simposium.Internacional de Nutrición Acuícola. Cancún, Quintana Roo, México. 23–34.

Kaushik, S.J. (1990): Use of alternative protein sources for the intensive rearing of carnivorous fish. In:Mediterranean Aquaculture (eds R. Flos, L. Tort and P. Torres), pp. 125–138. Ellis Horwood Limited

Kiaerskou J. (1991): Production and economics of low pollution diets for the aquaculture Industry: In Cowey, C.B., Cho, C.Y. (Eds.), Nutritional Strategies and Aquaculture Waste. Proceedings of the First International Symposium on Nutritional Strategies in Management of Aquaculture Waste. University of Guelph, Guelph, Ontario Canada. pp 65-76

Krom, M.D. & Neori, A. (1989).A total nutrient budget for an experimental fishpond with circularly moving seawater.*Aquaculture* 83: 345–358.

Lall S.P. (1991): Digestibility, metabolism and excretion of dietary phosphorus in fish. In: Cowey, C.B., Cho, C.Y. (Eds.), Nutritional Strategies and Aquaculture Waste. Proceedings of the First International Symposium on Nutritional Strategies in Management of Aquaculture Waste. University of Guelph, Guelph, Ontario,

Canada, pp. 21–35.

Lu J, Yoshizaki G, Sakai K, Takeuchi T (2002) Acceptability of raw *Spirulina platensis* by larval tilapia *Oreochromis niloticus.Fish Sci* 68: 51-58.

National Research Council, NRC (1993) *Nutrient Requirements of Fish*.NationalAcademy Press, Washington, DC, 114pp.

Pillay, T.V.R. (1992). Aquaculture and the Environment.Fishing Book News, London.

Quillere, I., Marie, D., Roux, L., Gosse, F., Morot-Guadry, J.F.,(1993). An artificial productive ecosystem based on a fish/bacteria/plant association: 1. Design and management. *Agric.Ecosyst. Env*iron. 47, 13–30.

Rakocy, J.E., (1995). The roles of plant crop production in aquacultural wastewater. Aquacultural Engineering and waste management, proceedings from the aquaculture Expo VIII and Aquaculture in the Mid-Atlantic Conference, Washington DC, June 24–28,

Rumsey, G.L., (1993). Fishmeal and alternate sources of protein in fish fee. *Fisheries* ds 18, 14–19.

Redner, R.; Stickney, R.R. (1979).Acclimation to ammonia by *Tilapia aurea*. Tran Am. *Fish Soc* 108:383-388.

Sarker MSA, Satoh S, Kiron V. (2005): Supplementation of citric acid and amino acid- chelated trace element to develop environment-friendly feed for red sea bream, Pagrus major. *Aquaculture* 248, 3–11.

Satoh, S., C.Y. Cho, and T. Watanabe (1992) Effect of faecal retrieval timing on digestibility of nutrients in rainbow trout diet with the Guelph and TUF faeces collection systems. *Nippon Suisan Gakkaishi*, 58, 1123–1127.

Satoh,S, Ishida R, Takeuchi T, Watanabe T, Seikai T (1998): Necessity of mineral supplement to fish meal based red sea bream feed. *Suisan Zoshoku* 46, 535–540

Siddiqui, A.Q., Howlader, M.S., Adam, A.A., (1988). Effects of dietary protein levels on growth, feed conversion and protein utilization in fry and young Nile tilapia, *Oreochromis niloticus*.*Aquac.Eng*. 11, 1 –22.

Sugiura, S.H., (1998). Development of low-pollution feeds for sustainable aquaculture.Ph.D Thesis, University of Washington, Seattle, WA.

Tacon, A.G.J., (1995). Application of nutrient requirement data under practical condition: special problems of intensive and semi intensive fish farming systems. *Appl. Ichthyol*. 11, 205– 214.

Takeuchi, T., Hoshi, M., Satoh, S., Watanabe, T., Takashima, Y., Kawamata, T., (1993).Effects of dietary digestible energy and available phosphorus contents on total amount of nitrogen excretion from carp.*Suisan Zoshoku* 41, 359–365.

Takeuchi T (1988) *Laboratory work- chemical evaluation of dietary nutrients*. In: Watanabe T (ed). Fish Nutrition and Marine Culture.Japan International Cooperation Agency, Tokyo. pp. 179-233.

Twarowska, J.G., Westerman, P.W. and Losordo, T.M., (1997). Water treatment and waste characterization evaluation of an intensive recirculating fish production system. *Aquacult. Eng*.16, pp. 133–147.

Watanabe, T.; Jahan, P. Satoh, S., Kiron, V. (1999) Total phosphorus loading on to the water environment from common carp fed commercial diets. *Fisheries Science*, Tokyo, 65:712-716.

Wu, R.S.S., Lam, K.S., MacKay, D.W., Lau, T.C., Yam, V., (1994). Impact of marine fish farming on water quality and bottom sediment: a case study in the sub-tropical environment. Mar. *Environ. Res*. 38, 115–145.

Zhu, S., Chen, S., (2001). Impacts of Reynolds number on nitrification biofilm kinetics, *Aquacultural Engineering*, 24, 213-229.

Table 1Formulation of the experimental diets for *O. niloticus* fry(g/kg)

Ingredients	D1	D2	D3
Fishmeal [*1]	55.12	25.00	32.90
Soy sauce waste	0.00	28.95	27.42
Food industry waste	0.00	28.95	27.42
α Starch	16.37	4.00	4.50
Vitamin premix [*2]	2.00	2.00	2.00
P-Free Mineral mixture [*3]	4.00	3.00	3.00
$Ca(HPO_4)2.H_2O$	3.00	2.76	2.76
Soybean oil	3.10	0.00	0.00
Lysine	0.00	1.17	0.00
Methionine	0.00	1.17	0.00
Cellulose	16.41	0.00	0.00
Total	100.00	100.00	100.00

*1 Fishmeal: Anchovy fish meal from Chile

*2 Composition (mg/100g): Thiamin HCl 6, riboflavin 10, pyridoxine HCl 4, cynocobalamin 0.01, ascorbic acid 500, niacin 40, Ca-pantothenate 10, inositol 200, biotin 0.6, folic acid 1.5, p-aminobenzoic acid 5, vitamin K3 5, vitamin A acetate 4000 IU, vitamin D3 4000IU

*3 Composition (g/100g): NaCl 5.0, $MgSO_4 \cdot 7H_2O$ 74.5, $FeC_6H_5O_7 \cdot nH_2O$ 12.5; trace element mixture[*4] 5.0, cellulose 3.0

*4 Composition (mg/g): $ZnSO_4 \cdot 7H_2O$ 353, $MnSO_4 \cdot 5H_2O$ 162, $CuSO_4 \cdot 5H_2O$ 31, $AlCl_3 \cdot 6H_2O$ 10, $CoCl_2 \cdot 6H_2O$, KlO_3 3, cellulose 440

Table 2. Proximate and mineral compositions of the experimental diet

Diet code	D1	D2	D3
Moisture (%)	3.0	5.5	4.8
Crude Protein	35.1	37.8	36.9
Crude lipid	9.8	9.3	9.4
Ash	12.9	13.0	14.6
energy (Kcal/g)	19.2	18.5	18.5
N (mg/g)	57.9	64.1	62.0
P (mg/g)	19.3	17.1	17.3
Ca (mg/g)	28.9	29.4	32.0
Mg (mg/g)	3.2	2.1	2.5
Na (mg/g)	17.5	26.0	24.3
K (mg/g)	19.7	25.6	19.1
Fe (µg/g)	1454.9	1112.1	1223.1
Zn (µg/g)	261.8	184.6	200.0
Mn (µg/g)	87.4	72.4	74.5
Cu (µg/g)	38.4	59.6	59.3

* D1 (0% inclusion of FIW+SSW)
* D2 (57.90 % inclusion of FIW+SSW supplemented with lys and met)
* D3 (54.84% inclusion of FIW+SSW only)

Table 3 Growth performances of *O. niloticus* fry fed experimental diets for 79 days in a closed recirculating system

Diet code	Av. Body weight (g)		Specific growth rate (%)	Feed efficiency (%)	Total feed consumed (g/fish)	Biomass conversion (%)
	Initial	Final				
D_1	1.21±0.02[a]	22.99±0.24[bc]	3.79±0.06[b]	125.95±4.48[b]	17.30±0.42[b]	33.29±0.51[b]
D_2	1.21±0.03[a]	28.61±0.16[a]	4.11±0.01[a]	138.02±2.29[a]	19.85±0.21[a]	39.38±0.87[a]
D_3	1.21±0.04[a]	24.14±0.16[b]	3.85±0.06[b]	129.20±2.42[b]	17.75±0.21[b]	33.67±0.41[b]

*Values in the same column with different superscript letters are significantly different (P<0.05) from each other.

Table 4: Mineral composition of the faecal matter and sludge produced by *O. niloticus*tilapia fed experimental diet for 79 days

Solid waste			
	D1	D2	D3
N (mg/g)	24.66	25.24	25.91
P (mg/g)	37.50	33.46	26.37
Ca (mg/g)	86.68	92.54	75.87
Mg (mg/g)	0.84	0.39	0.27
Na (mg/g)	12.82	12.44	10.75
K (mg/g)	20.53	19.23	19.54
Fe (µg/g)	5847.77	5318.09	3939.85
Zn (µg/g)	880.95	721.95	701.44
Mn (µg/g)	320.53	234.90	245.19
Cu (µg/g)	148.97	170.71	175.17
Biofilm			
N (mg/g)	38.90	34.60	37.54
P (mg/g)	25.92	16.85	18.42
Ca (mg/g)	81.30	69.64	57.52
Mg (mg/g)	1.62	1.29	1.04
Na (mg/g)	12.38	13.43	13.17
K (mg/g)	23.57	22.92	22.37
Fe (µg/g)	6747.22	4424.56	4461.64
Zn (µg/g)	894.06	630.24	588.97
Mn (µg/g)	340.39	240.84	286.05
Cu (µg/g)	145.65	156.17	146.87

Table 5: Percentages of Nitrogen and minerals in the, rearing water, solid wastes biofilm and *O.niloticus* body fed experimental diets for 79 days

Fish budget (%)	D1	D2	D3	Rearing water budget (%)	D1	D2	D3
N	45.80±0.36	48.94±0.94	42.86±1.03	N	36.50±0.45	37.05±1.17	40.46±1.51
P	47.77±2.76	56.21±0.65	45.32±3.54	P	10.72±0.10	7.38±0.32	11.43±1.36
Ca	55.51±2.31	53.49±0.38	53.97±1.51	Ca	26.56±0.13	19.52±0.61	23.59±0.61
Mg	16.19±0.18	15.94±1.62	17.38±1.19	Mg	63.41±4.81	60.50±3.83	51.58±3.24
Na	19.24±1.17	18.17±0.43	18.21±0.75	Na	67.76±2.66	60.63±1.02	70.48±2.54
K	45.47±3.39	42.01±2.73	37.59±2.50	K	26.70±1.35	15.33±2.31	31.58±5.97
Fe	3.87±0.48	6.79±1.35	4.72±0.12	Fe	0.00±0.00	0.00±0.00	0.31±0.01
Zn	15.27±1.31	27.01±2.69	21.65±0.20	Zn	0.00±0.00	0.86±0.02	1.24±0.29
Mn	9.60±0.13	8.19±0.90	9.72±0.01	Mn	0.00±0.00	0.35±0.01	0.07±0.03
Cu	77.00±9.89	62.26±3.04	53.83±5.32	Cu	0.00±0.00	0.00±0.00	0.00±0.00

Solid waste budget (%)	D1	D2	D3	Biofilm budget (%)	D1	D2	D3
N	5.26±0.00	3.62±0.88	5.51±0.59	N	3.27±0.51	2.53±0.29	3.49±0.34
P	33.36±2.65	28.21±0.08	35.68±4.67	P	6.52±0.69	4.98±0.04	6.78±0.47
Ca	52.43±0.06	49.41±2.81	53.99±3.40	Ca	13.74±3.22	10.81±1.16	10.34±0.89
Mg	3.23±0.39	1.58±0.48	1.43±0.23	Mg	2.50±1.30	2.78±0.91	2.42±0.74
Na	9.03±0.15	4.27±0.69	5.79±0.02	Na	3.42±0.37	2.36±0.33	3.12±0.35
K	12.86±0.72	6.65±0.18	13.52±2.27	K	5.87±1.95	4.07±0.32	6.74±1.03
Fe	49.71±5.24	42.69±6.95	42.46±4.13	Fe	22.38±0.52	18.17±1.69	21.00±1.44
Zn	41.57±1.38	34.42±1.94	46.36±6.85	Zn	16.49±0.50	15.58±1.24	16.94±0.59
Mn	45.34±3.66	28.53±2.24	44.09±6.29	Mn	19.21±3.01	15.18±0.79	22.10±1.60
Cu	47.94±0.95	25.57±3.8	38.92±3.36	Cu	18.36±1.99	11.97±0.77	14.25±0.10

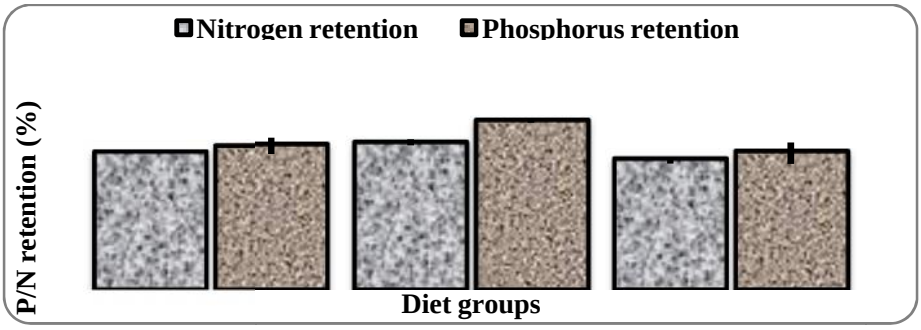

Fig.1 P/N retention of Nile tilapia fed experimental diet for 79 days in a closed recirculation system

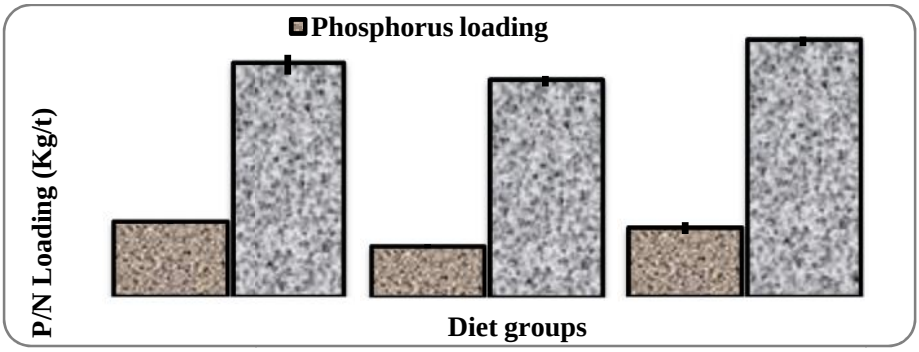

Fig.2 P/N loading of Nile tilapia fed experimental diet for 79 days in a closed recirculation system

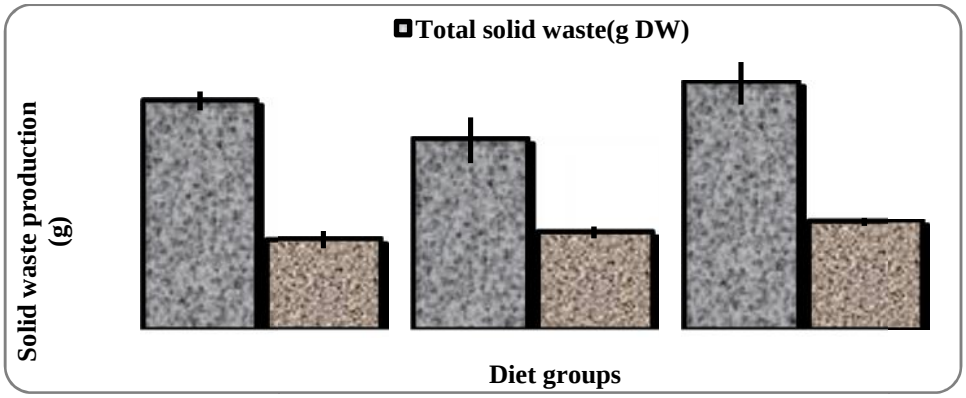

Fig. 3 Total Solid and biofilm waste of Nile tilapia fed experimental diet for 79 days in a closed recirculation system