OPTIMAL INCLUSION OF RECYCLED FOOD WASTE MATERIALS SUPPLEMENTED WITH LYSINE, AND METHIONINE IN THE PRACTICAL DIET OF NILE TILAPIA *Oreochromis niloticus* FRY

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ABSTRACT
This study evaluates the optimal inclusion of recycled food industry waste (FIW) and soy sauce waste (SSW) supplemented with lysine (Lys), and methionine (Met) in the diet of Nile tilapia Oreochromis niloticus fry. A control diet and four waste-amended formulations were administered to O. niloticus fry (initial weight 0.01 g; length 8.0 mm) for 84 days. The diets were designated as D1 (Fishmeal (FM) only), D2 (35.77% FIW + Lys and Met substituted FM protein in the diet), D3 (45.69% FIW/SSW + Lys and Met substituted FM protein in the diet), D4 (41.94% SSW+ Lys and Met substituted FM protein in the diet) and D5 (33.71% FIW/SSW only substituted FM protein in the diet). Each formulation was composed of 35% crude protein and 9.8% lipid. D2, D3 and D4 were supplemented with lysine and methionine. Nile tilapia fry fed D3 had the highest (P < 0.05) growth, feed utilization and body composition than those fed with the other experimental diets. Feed utilization and growth performance were lowest for D4 fed fish. Highest and lowest body compositions were observed in fish fed D1 and D2 respectively. The results revealed that judicious supplementation of recycled food wastes with lysine and methionine can effectively reduce FM utilization in the practical diet of Nile tilapia fry.

Keywords: alternative protein, proximate analysis, aquaculture, additives.

INTRODUCTION

Several research studies on fish nutrition have shown that high quality and quantity of protein in diets are essential for optimal production of fish especially in intensive aquaculture where the fishes depend mainly on artificial diets. With the ever-increasing production costs, the continuous decline and seasonal availability in the production of fishmeal (FM), it has become the most expensive macro- ingredient used in aqua-feeds. Reducing protein cost in fish diet formulation has necessitated the quest for cheaper and locally available plant and animal's protein sources to replace high cost FM and soybean that have other competing uses by man and other sectors (Jackson *et al.,* 1982; De Silva and Anderson, 1995; El-Sayed, 1999; Abdelghany A. E., 2003; El-Saidy and Gaber, 2003; Fasakin *et al.*, 2006).. Research has also shown that other alternative non FM protein sources are considered to be less suitable as FM replacements owing to indispensable amino acid profiles which may be markedly different to those required by many cultured teleosts (Lim and Akiyama, 1992). Hence, the use of non-FM protein sources in aquaculture may have necessitated the use of amino acid supplements to boost the amino acid profile of the feed to a level which matches the requirement of the target species.

Tilapia are one of the most cultivated fish species all over the world, the second most cultured fin fish after carps, there production is also on the increase (FAO, 2008). This increase in the production of tilapia may be attributed to their tolerance to stressors imposed in routine aquaculture practices, marketability and their ability to utilize nutrients from a wide variety of sources; including plants feed stuffs (Gonzales *et al.,* 2007). While the diversity of acceptable feedstuffs is an asset in culture, several challenges remain in feeding tilapia.
With the technological advancement most biodegradable waste are usually recycled in Japan hence a lot of recycled biodegradable waste (e.g. soy sauce waste, food industry waste) are on the increase and its effective reusability as been the major challenge. It is well established that alternative protein source substitution for FM in fish diets are usually inferior in essential amino acids (EAA) balance compared to FM (Takagi *et al.,* 2001; Cheng *et al.,* 2003), hence, the use of alternative protein sources may require the use of amino acid supplements to boost the amino acid content level of a given diet to match with the amino acid requirement of the target species. Much research

work has shown that supplementation of amino acids improves growth the performance of fish (El-Dahhar and El-Shazly, 1993; Wu *et al.,* 1997; Yamamoto *et al.,* 1998; Alam *et al.,* 2002; Biswas *et al.,* 2007).

In a previous study we demonstrated the suitability of recycled food waste in the diet of tilapia fry (Bake *et al.,* 2009). The main aim of the present study is to investigate and evaluate the effect of the optimal inclusion of recycled food waste materials (food industry waste and soy sauce waste) enriched with amino acids (lysine and methionine) supplement in the practical diet of Nile tilapia fry through their growth performance and nutrient utilization.

MATERIALS AND METHODS

Soy sauce waste: Soy sauce waste (SSW) was produced by Yamasa Corporation and processed by Nippon Formula Feed Mfg. Co., Ltd. Yokohama Japan. 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 26.1% and 11.9%, 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, and food waste residues discharged during processing, hotel waste, restaurant cooking waste, tofu waste and bread production waste. The FIW was processed by fry-cooking the waste with vegetable oil at a very low pressure and an initial temperature of between 80 - 100 °C held for 1 hour and later increased to between 100 - 110 °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 19.6% and 11.3%, respectively.

Fishmeal: The FM use in this experiment was obtained from Nippon Formula Feed Mfg. Co., Ltd. The crude protein and lipid contents of FM were 63.5% and 11.8%, respectively.

Experimental diets

Based on the nutritional requirements of tilapia (NRC 1993), and our previous work (Bake *et al.*, 2009), five isonitrogenous diets were formulated at 35% iso-nitrogenous diet and 9.8% lipid, containing 0, 47.7 -59.8% of different proportions of recycled food waste (FIW and SSW) + EAA (Lys + Met) as shown in Table 1. The experimental diets were designated as D1 0% inclusion of recycled food waste (fishmeal only) as the control, D2: 51% of FIW + Lys and Met (35.77 % of fishmeal protein was replaced with FIW+ Lys and Met), D3: 59.8% of recycled food waste, FIW and SSW + Lys and Met (45.69 % of fishmeal protein was replaced with FIW and SSW + Lys and Met), D4: 47.7% of SSW + Lys and Met (41.94% of fishmeal protein was replaced with SSW + Lys and Met) and D5: 51.6% of FIW and SSW only (33.71% of fishmeal protein was replaced with FIW and SSW no Lys and Met). D5 was formulated to evaluate the effect of additional EAA in D2, D3 and D4. All the ingredients were mixed in a domestic feed mixer together with mineral and vitamin premix dissolved in a small quantity of distilled water. During the mixing small quantity of distilled water was added to enhance its pelletability. The diets were pelleted using a laboratory pelletizer (AFZ12M, Hiraga-Seisakusho, Kobe, Japan) and dried using vacuums freeze drier (RLE-206, Kyowa Vacuum Tech., Saitama, Japan). The pelleted diets were then crumbled using a pestle and mortal. The diet was then screened using a 500µ mesh and stored at 4 °C until use.

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

Diet code	D1	D2	D3	D4	D5
Fishmeal*[1]	551.2	354.0	299.5	320.0	365.0
Soy sauce waste	0.0	0.0	284.0	447.0	258.0
Food industry waste	0.0	485.0	284.0	0.0	258.0
α-starch	173.8	30.0	30.0	30.0	30.0
Vitamin premix*[2]	30.0	22.5	22.5	22.5	22.5
P-free mineral mixture*[3]	30.0	22.5	22.5	22.5	22.5
$Ca(H_2PO_4)_2 \cdot H_2O$	30.0	22.5	22.5	22.5	22.5
Soybean oil	35.0	0.0	0.0	7.0	0.0
Lysine	0.0	15.0	15.0	15.0	0.0
Methionine	0.0	15.0	15.0	15.0	0.0
Cellulose	172.5	33.5	5.0	98.5	21.5

Fishmeal*[1]: Anchovy fish meal from Chile

*2 Composition (mg/100g): Thiamin HCl 6, riboflavin 10, pyridoxine HCl 4, cynocobalamin 0.01, ascrobic 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

Experimental system and fish

Newly hatched Nile tilapia *Oreochromis niloticus* larvae were obtained from purebred stock and experimented at the Laboratory of Fish Culture, Tokyo University of Marine Science and Technology. The feeding experiment was conducted using 13-days old tilapia fry having initial standard length (SL) 8.0 ± 0.0 mm and wet body weight of 0.01 g at the onset of exogenous feeding (Lu *et al*., 2002).

Freshwater supply was supplied to the system consisting of 15 aquariums of approximate capacity of 30 L through filtered dechlorinated tap water at a flow rate of 250-300 ml min^{-1}. Water temperature was maintained at 28.0 ± 0.5 °C using electric heaters. The aquariums were 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 aquariums were provided with continuous aeration through an air compressor. The water quality parameters in the system were monitored weekly and the ranges were: dissolved oxygen 6.30-7.25 mg/l, total ammonia nitrogen 0.1-0.25 mg/l, and pH 6.8-7.2. No critical values were detected for nitrite and nitrate.

Each treatment was carried out with three replications and sixty fish per tank were maintained in the 30 l- aquariums with a flow through system. Feed was manually administered. Fish were fed 6 times daily at 30% (on dry basis) of body weight at 7.00 h, 9.00 h, 11.00 h, 13.00 h, 15.00 h, and 17.00 h. Feeding rates were subsequently adjusted according to their growth rate per aquarium. Fish were starved for 24 hours prior to sampling. Five fish were randomly sampled; weights were measured using a digital electronic weighing balance (AW220, Shimadzu Corporation, Kyoto, Japan) on weekly basis. Total length and standard length were measured using digimatic caliper (CD-20CP, Mitutoyo Corporation, Tokyo, Japan).

Biochemical analyses

About 15 g of initial samples and 10 g of final samples from each aquarium were pooled separately and then homogenized using a mincing machine and an auto homogenizer (ED-3, Nihonseiki Kaisha Ltd., Tokyo, Japan). The experimental diets and fish body samples were subjected to chemical analysis. Proximate analysis and lipid analysis were carried out using standard methods (Takeuchi, 1988; Folch *et al*., 1957). For fatty acid profile analysis, crude lipid was saponified using 50% ethanol to prepare methyl esters with 7% boron trifluoride in a methanol solution (BF_3-methanol), and the fatty acid profile was determined using gas liquid chromatography (GC-14A, Shimadzu Corporation, Kyoto, Japan). Amino acids were determined according to the method described by Simpson *et al.* (1976) using an amino acid auto analyzer (JLC-500V, JEOL Nippon Electric, Tokyo, Japan). After adding 0.5 mL of concentrated HNO_3, the samples were digested using an MLS-1200. MEGA microwave digestion system (Milestone s. r. l., Bergamo, Italy). Concentration of each element was measured by inductively coupled plasma atomic emission spectrophotometer, ICP-AES (SPS 7800, SII Nanotechnology Inc., Chiba, Tokyo, Japan) except for phosphorus, which was quantified by visible light spectrophotometer (UV 265 FW, Shimadzu Corporation, Kyoto, Japan) at 750 nm.

Evaluation of growth parameters

Growth performance and diet nutrient utilization were analyzed in terms of body weight gain (WG), feed intake (FI), feed efficiency (FE), specific growth rate (SGR). The following formulae were used:

Weight gain (WG) (%) = (final body weight (g) − initial body weight (g)) / initial body weight (g) × 100.

Feed efficiency (FE) = weight gain (g) / feed amount (g).

Specific growth rate (SGR) (%) = (ln final body weight (g) - ln initial body weight (g)) / 84 days × 100.

Feed intake (FI) = feed amount (g) / fish / day.

Protein efficiency ratio (PER) = body weight gain (g) / protein intake (g).

Protein retention (PR) (%) = protein gain (g) / protein intake (g) × 100.

Statistical analyses

Data were analyzed using one way analysis of variance (ANOVA) using Statistica software (version 6.0, Stat-Soft. Inc., Oklahoma, USA). Differences between treatments were compared by Tukey's test level of significance at $P < 0.05$.

RESULTS

All the formulated diets used in the present study were iso-nitrogenous. The proximate composition of the experimental diets shows that protein and lipid contents of the diets were similar, while the moisture and starch contents were slightly lower in D1 and D4 than the others treatments. Except for D1 and D5 the constitutional amino acid contents of the experimental diets were similar, as a result of lysine and methionine supplementation (Table 2). The fatty acid contents of all experimental diets were similar except in D1, which had higher n-3 and n-3 HUFA contents (Table 3). There was no feed rejection during the experimental period, although the acceptability of the diets varied among the experimental diets.

Table 2: Proximate composition of the diets and constitutional amino acid content of the experimental diets fed *Oreochromis niloticus* fry

Diet code	D1	D2	D3	D4	D5	NRC(1993) requirement
Proximate composition						
Crude protein	36.41	36.85	36.98	36.78	36.11	
Starch	13.88	15.08	16.63	13.40	16.37	
Crude lipid	9.49	9.61	9.46	9.59	9.47	
Ash	12.67	12.08	12.38	12.48	14.95	
Moisture	3.75	4.76	4.36	4.34	4.24	
Essential amino acid						
Arginine	2.82	2.31	2.69	2.60	2.78	4.2
Lysine	2.41	3.45	3.31	3.47	1.95	5.1
Histidine	1.43	1.29	1.30	1.37	1.39	1.7
Phenylalanine	1.62	1.22	1.74	1.81	1.77	3.8
Leucine	3.00	2.40	3.14	3.11	3.59	3.4
Isoleucine	1.53	1.35	1.47	1.34	1.52	3.1
Methionine	1.16	2.37	2.33	2.35	0.89	3.2
Valine	1.70	1.38	1.55	1.63	1.61	2.8
Threonine	1.54	1.35	1.62	1.34	1.65	3.8
Tryptophan	0.38	0.21	0.24	0.28	0.25	1.0
Non-essential amino acid						
Taurine	0.41	0.30	0.24	0.27	0.27	
Alanine	2.88	2.55	2.46	2.61	2.58	
Glycine	2.87	2.95	2.82	2.45	2.73	
Glutamic acid	5.11	4.39	4.65	4.41	4.84	
Serine	2.67	2.37	2.30	2.66	2.78	
Aspartic acid	3.43	2.77	2.88	2.41	3.30	
SUM	34.95	32.66	34.74	34.07	33.92	

Table 3: Fatty acid composition of the experimental diets fed *Oreochromis niloticus* fry

Diet code	D1	D2	D3	D4	D5
Fatty acids					
14:0	0.26	0.29	0.16	0.33	0.42
16:0	0.30	2.03	1.82	1.95	1.99
16:1n-7	1.52	0.28	0.24	0.28	0.38
16:3n-6	0.37	0.02	0.02	0.06	0.03
16:3n-3	0.09	0.02	0.07	0.03	0.03
18:0	0.50	0.57	0.45	0.38	0.33
18:1 (OA)	1.68	2.35	2.18	1.40	1.74
18:2n-6 (LA)	1.54	1.39	2.10	2.54	1.78
18:3n-6	0.21	0.16	0.21	0.24	0.24
18:3n-3 (LNA)	0.06	0.05	0.03	0.04	0.06
18:4n-3	0.01	0.03	0.01	0.00	0.00
20:0	0.03	0.02	0.03	0.03	0.03
20:1	0.14	0.06	0.09	0.10	0.03
20:2n-6	0.02	0.02	0.02	0.01	0.02
20:3n-6	0.01	0.02	0.03	0.03	0.03
20:4n-6 (ARA)	0.00	0.06	0.05	0.06	0.05
20:4n-3	0.12	0.03	0.05	0.02	0.04
20:5n-3 (EPA)	0.30	0.17	0.15	0.15	0.22
22:0	0.02	0.00	0.02	0.02	0.02
22:1	0.08	0.05	0.06	0.04	0.01
22:4n-6	0.04	0.02	0.03	0.01	0.02
22:5n-6	0.04	0.03	0.02	0.02	0.04
22:5n-3	0.06	0.04	0.05	0.05	0.04
22:6n-3 (DHA)	0.66	0.43	0.41	0.47	0.39
Σ Monoenes	3.41	2.74	2.56	1.81	2.15
Σ Saturates	1.12	2.91	2.48	2.71	2.79
Σ **n**-3	1.29	0.76	0.77	0.76	0.78
Σ **n**-6	2.23	1.73	2.48	2.98	2.22
Σ **n**-3HUFA	1.14	0.67	0.66	0.69	0.69

Values are given on a percentage dry basis

At the end of the feeding trial, the final body weight and other feed performances of tilapia fry clearly showed differences among the experimental diets (Fig. 1). Table 4 shows that fish fed D3 had the highest values of all growth performances, on the other hand fish fed D4 had the lowest final body weight (9.49 ± 0.05 g) and percentage WG (94800 ± 500%). When D3 was compared with the control there was a significant difference ($P < 0.05$), while D5 also did better than the control. There was no significant difference in growth performance between D1 and D2.

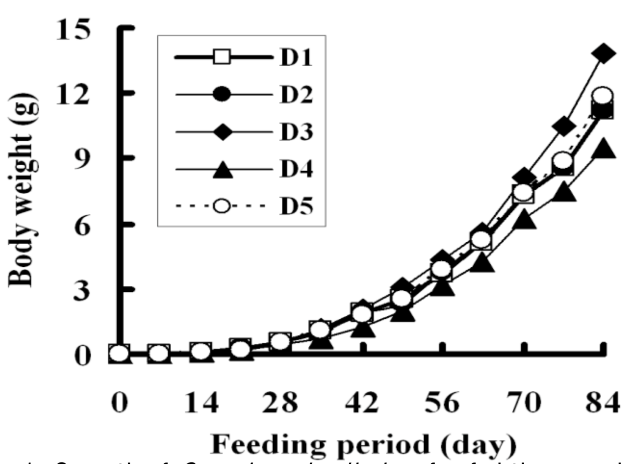

Fig. 1: Growth of *Oreochromis niloticus* fry fed the experimental diets for 84 days

Table 4: Growth performances of *Oreochromis niloticus* fry fed the experimental diets for 84 days

Diet code	Body weight (g)		Weight gain (%)	Feed efficiency	Survival rate (%)	Specific growth rate (%)	Total feed intake (g)	Protein retention (%)	Protein efficiency
	Initial	Final							
D1	0.01 ±0.00	11.19 ±0.07^{c}	111800 ±7.02^{c}	1.32 ±0.61^{b}	98.2 ±0.7^{ab}	8.41 ±0.02^{c}	8.46 ±0.82^{ab}	48.7 ±1.3^{b}	3.20 ±0.06^{c}
D2	0.01 ±0.00	11.23 ±0.07^{c}	112300 ±7.29^{c}	1.33 ±0.21^{b}	98.4 ±0.8^{ab}	8.41 ±0.03^{c}	8.43 ±0.63^{b}	47.3 ±1.2^{c}	3.14 ±0.04^{c}
D3	0.01 ±0.00	13.79 ±0.03^{a}	137800 ±3.41^{a}	1.53 ±0.25^{a}	99.8 ±0.6^{a}	8.65 ±0.04^{a}	8.98 ±0.74^{a}	55.5 ±0.9^{a}	3.66 ±0.04^{a}
D4	0.01±0.00	9.49 ±0.05^{d}	94800 ±4.51^{d}	1.25 ±0.42^{c}	97.7 ±0.8^{b}	8.20 ±0.02^{c}	7.55 ±0.52^{b}	45.3 ±0.8^{c}	3.01 ±0.05^{d}
D5	0.01 ±0.00	11.86 ±0.06^{b}	118200 ±5.88^{b}	1.35 ±0.31^{b}	98.9 ±0.8^{ab}	8.47 ±0.03^{b}	8.73 ±0.64^{a}	51.3 ±1.5^{b}	3.38 ±0.07^{b}

Values in the same column with different superscript letters are significantly different (p<0.05) from each other. (n=3)

Survival of the fish fed experimental diets ranged from 97.7-99.8%. Survival rate of D3 was significantly higher than the others (P < 0.05). Protein retention and protein efficiency ratio of D3 also showed the highest values.

The whole body composition of the fish fed the experimental diets after 12 weeks is shown in Table 5. The crude protein content did not significantly differ among treatments but was higher than the initial protein content of the experimental fish. D1 had the lowest whole body lipid content. However, ash content did not differ significantly among all fish fed experimental diets (P < 0.05).

The fatty acid composition of the whole body lipid was similar although fish fed D1 were slightly higher in n-3 fatty acids. N-6 fatty acids, especially 18:2n-6 of all experimental fish increased compared with the initial fish (Table 6).

Table 7 shows that the constitutional amino acids of the whole body were similar except that the methionine content of D2, D3, and D4 was slightly higher than the level of D1 and D5.

The fish meal (FM) used in this experiment was higher in all the mineral content measured among the tested ingredients except sodium (Na) and copper (Cu), while SSW was higher in Na and Cu but lower in calcium (Ca), magnesium (Mg), potassium (K), phosphorus (P), manganese (Mn) and iron (Fe) than the other ingredients. FIW was lower in Na, Cu, and zinc (Zn) than the other ingredients used. The mineral contents of all the diets and the body mineral content were similar (Table 8). Except for Ca, initial body mineral contents were higher compared with mineral levels of the experimental fish.

Table 5: Body composition (wet basis) of *Oreochromis niloticus* fed the experimental diets for 84 days

Component (%)	Initial	Final*1				
		D1	D2	D3	D4	D5
Moisture	75.4	74.6±1.2^{a}	73.2±1.2^{b}	73.4±0.3^{b}	74.1±1.1^{a}	74.2±1.2^{a}
Protein	14.3	15.1±1.1^{a}	15.0±1.1^{a}	15.2±1.1^{a}	15.0±1.2^{a}	15.1±1.1^{a}
Lipid	5.8	6.03±0.7^{b}	6.5±0.5^{ab}	6.6±0.5^{a}	6.4±0.4^{ab}	6.5±0.5^{ab}
Ash	3.2	3.2±0.2^{a}	3.2±0.3^{a}	3.3±0.1^{a}	3.2±0.3^{a}	3.2±0.2^{a}

*1 Values in the same row with different superscript letters are significantly different (p<0.05) from each other (n=3)

Table 6: Fatty acid composition of lipids in whole body of *Oreochromis niloticus*

Treatment	Initial	D1	D2	D3	D4	D5
14:0	1.14	0.78	0.76	0.83	0.85	0.84
16:0	4.97	5.21	6.02	5.51	5.06	5.71
16:1n-7	2.30	1.22	1.17	1.26	1.09	1.29
16:3n-6	0.52	0.20	0.12	0.15	0.57	0.14
16:3n-3	0.78	0.40	0.11	0.09	0.05	0.08
18:0	1.24	1.52	1.46	1.38	1.17	1.41
18:1 (OA)	6.32	3.63	4.56	4.58	3.11	4.67
18:2n-6 (LA)	0.17	1.85	1.63	1.53	2.04	1.69
18:3n-6	0.06	0.28	0.21	0.30	0.32	0.29
18:3n-3 (LNA)	0.05	1.08	0.53	0.80	1.06	0.80
18:4n-3	0.09	1.95	1.79	1.99	2.10	2.16
20:0	0.00	0.07	0.05	0.05	0.05	0.05
20:1	0.05	0.16	0.12	0.12	0.11	0.10
20:2n-6	0.34	0.15	0.13	0.17	0.17	0.16
20:3n-6	0.03	0.08	0.09	0.10	0.10	0.09
20:4n-6 (ARA)	0.31	0.23	0.21	0.22	0.26	0.22
20:3n-3	0.07	tr	Nd	tr	nd	tr
20:4n-3	0.14	1.85	1.60	1.67	1.71	1.67
20:5n-3 (EPA)	0.18	0.58	0.62	0.65	0.53	0.60
22:0	nd	nd	Tr	nd	tr	tr
22:1	0.06	0.12	0.08	0.10	0.11	0.09
22:4n-6	0.37	0.11	0.11	0.11	0.10	0.10
22:5n-6	0.05	0.13	0.10	0.11	0.12	0.11
22:4n-3	0.03	tr	Nd	tr	nd	tr
22:5n-3	0.96	0.36	0.33	0.41	0.44	0.30
22:6n-3 (DHA)	3.16	1.98	1.50	1.82	1.52	1.74
Σ Monoenes	8.73	5.12	5.93	6.05	4.43	6.16
Σ Saturates	7.35	7.58	8.29	7.77	7.13	8.01
Σ n-3	5.44	8.21	6.47	7.43	7.42	7.34
Σ n-6	1.84	3.02	2.59	2.68	3.67	2.80
Σ n-3HUFA	4.52	4.77	4.04	4.55	4.21	4.30

tr: trace, nd: not detected Values are given on a percentage dry basis

Table 7: Constitutional amino acid in whole body of *Oreochromis niloticus* fed experimental diet for 84 days (g/100g dry basis)

Treatment	Initial	D1	D2	D3	D4	D5
Essential amino acid						
Arginine	3.22	3.36	3.49	3.52	3.60	3.60
Lysine	4.58	4.44	4.27	4.54	4.37	3.20
Histidine	1.88	1.15	1.20	1.38	1.22	1.21
Phenylalanine	2.94	2.21	2.24	1.87	2.27	2.20
Leucine	3.74	3.89	3.92	3.40	4.03	3.86
Isoleucine	2.87	2.22	2.19	2.73	2.11	2.26
Methionine	1.89	1.84	2.71	2.86	2.78	1.84
Valine	2.54	2.32	2.36	2.05	2.49	2.33
Threonine	2.97	2.42	2.33	2.43	2.44	2.36
Tryptophan	0.74	0.58	0.53	0.59	0.52	0.56
Non-essential amino acid						
Taurine	1.57	2.11	2.14	2.27	2.17	2.26
Alanine	3.19	3.75	3.76	3.27	3.99	3.90
Glycine	3.29	3.24	4.36	4.02	4.77	5.11
Glutamic acid	7.12	7.47	7.55	7.38	7.84	7.59
Serine	4.62	4.23	3.29	3.93	3.39	2.36
Aspartic acid	5.12	5.42	4.42	5.93	5.85	4.92
Total	52.29	50.65	50.77	52.18	53.86	49.54

Table 8: Mineral contents of ingredients, diets and whole body of *Oreochromis niloticus* fed experimental diets for 84 days

	Ca (mg/g)	Mg (mg/g)	Na (mg/g)	K (mg/g)	P (mg/g)	Mn (µ/g)	Cu µ/g)	Zn (µ/g)	Fe (µ/g)
Ingredients									
FM	55.0	2.3	10.5	7.4	27.9	20.4	7.2	113.3	263.0
FIW	28.0	0.9	6.9	5.8	7.1	17.1	4.7	63.7	171.3
SSW	5.6	0.4	30.6	2.7	0.9	16.4	51.9	65.5	108.0
Diet									
D1	32.2	3.7	7.6	4.2	19.2	72.0	18.5	195.3	426.4
D2	29.5	3.9	7.1	4.5	17.8	77.3	18.8	217.9	308.7
D3	26.4	3.6	12.9	4.5	16.0	78.0	28.8	177.1	323.4
D4	21.5	3.4	16.3	3.2	14.6	76.3	33.6	189.0	312.1
D5	31.1	3.8	14.3	4.8	19.0	82.1	29.9	204.4	368.0
Whole body									
Initial	14.3	1.0	1.9	4.9	26.8	12.2	9.0	122.6	190.5
D1	17.6	0.4	1.1	2.4	22.2	2.6	1.9	98.0	109.4
D2	16.7	0.3	1.2	2.3	22.1	2.1	2.5	96.0	109.4
D3	16.4	0.3	1.0	2.8	22.8	2.6	2.4	105.7	109.0
D4	15.3	0.3	1.3	2.9	21.7	2.1	3.8	92.4	109.7
D5	16.8	0.3	1.3	2.8	22.4	2.3	2.4	99.7	107.8

FM: Fishmeal
FIW: Food industry waste
SSW: Soy sauce waste

DISCUSSION

Since fish are poikilothermic, their food requirement is related to activity and hence to the water temperature, which in the context of this experiment was maintained at 28 °C with the aid of water heaters; the dissolved oxygen ranged from 6.30 - 7.25 mg/l, pH ranged from 6.8 - 7.2. These water quality parameters were within the acceptable range for tilapia culture (Balarin and Hatton, 1979).

The results of this study indicates that recycled food waste (FIW and SSW) supplemented with limited quantity of lysine and methionine, can effectively be used to replace FM at level up to 48.3% in practical diets for tilapia fry without adversely affecting their growth performance. D3 had a better growth performance among all the tested diets, when compared to both the control D1 and D5 (Fig.1). This study agrees with the works of other researchers such as El-Dahhar and El-Shazly (1993); Wu *et al.* (1997); Alam *et al.* (2002); and Cheng *et al.* (2003) who have found that supplementing essential amino acid in fish diets usually improves the growth performance of the fish.

The growth performance and nutrient utilization in the present study was higher and better than the previous work reported by (Bake *et al*., 2009). This might be attributed to supplementation of lysine and methionine in the diet. The tilapia fry generally and easily accepted all the diets although acceptability differed among the treatments as indicated by the feed intake (FI). When plant materials are used in fish diets, one common problem is the reduced acceptability of the diet by the fish, which is related to palatability (Rodriguez-Serna *et al*., 1996; Fontaínhas-Fernandes *et al*., 1999). The higher FI by the fish fed D3 may be attributed to the balanced amino acid recorded by the diet; hence the higher intake of the fish fed the diet. Goda *et al*. (2007) reported that imbalance of amino acid reduce FI in fish. The lower FI by fish fed D4 might not be due to imbalance of amino acids. This is because the EAAs of the diets approximated the EAA requirement of tilapia as stated by Santiago and Lovell (1988). The lower palatability of D4 might be due to the non-palatability and lower intake of the SSW as a result of the production process of soy sauce. The SSW has a very strong offensive odor due to fermentation of soybeans by enzymes and the dehydration method used during the recycling process could not effectively remove it, by making the waste (cake) less palatable. Proper processing of feed ingredients usually increases the palatability of a diet, hence it is suggested that the texture and palatability or taste of experimental diets is related to the level of plant materials incorporated, this may affect the acceptability of the feed and consequently growth (Fagbenro 1999; Francis *et al*., 2001; Siddhuraju and Becker 2001).

The Weight gain (WG) of young fish is usually a reliable indicator of nutritional adequacy of the diet (Cho and Watanabe, 1988). The present study shows that supplementation of recycled food waste materials also affected the WG of the fish fed experimental diets. Differences in the WG among tilapia fry fed the experimental diets were significant; fish fed D3 showed the highest final body weight and percentage WG value, conversely, D2 was comparable to D1 in the final body weight gain, in our previous result D1 gave a better growth performance than D2 when it was not supplemented with amino acids. This result agrees with Murai *et al*. (1986), El-Saidy and Gaber (2003), which showed that additional supplementation of amino acids in diets of tilapia, improves the growth. Tilapia fry fed D4 showed the lowest values of body weight and percentage WG. This can be explained by the low FI due to the palatability of the diet. Another factor may be the high salt content during the production process, Hano *et al*. (2004) reported that one major constraint of using soy sauce cake for livestock feeding is the presence of a high salt content (usually about 5 - 7%). This may also affect the ability of the fish to effectively feed on the diet since for freshwater fish, high salt diets may compromise its osmoregulatory ability. Luh (1995) reported that soy sauce cake (waste) is rich in isoflavone, and when absorbed isoflavone can negatively affect the metabolism of the fish (Mambrini *et al*., 1999).

Reduction in feed conversion efficiency can be an indicator of amino acid deficiency (Wilson 1989). The feed efficiency (FE) and the specific growth rate (SGR) obtained from this study was higher than the values obtained in our previous report, carried out using tilapia fry fed recycled food waste (FIW and SSW only) (Bake *et al*., 2009). The higher FE and SGR values in this study may be the effect of supplementation of the recycled waste with lysine and methionine. In this study the higher FE of D3 indicates the superior quality of the diet over the other diets, while lower FE value of D4 may not be a result of amino acid deficiency but rather reduced feed intake resulting from lower palatability.

The results on the PER and PR indicated that supplementation of recycled food waste improves protein utilization. The present PER values compared favorably with 0.86 - 2.09 reported by Siddiqui *et al*. (1988) for tilapia with an initial weight of 0.84 g when fed for 98 days with 20 - 50% protein diets in tanks. Our results also show that D3 had the highest protein retention, and no significantly different from D1, D2 and D5 but D4 had the lowest PR. This also attests to the efficacy of the supplementation of D2, D3 and D4 (Table 4). The lower PR value of D4 implies that although tilapia fry fed D4 have the ability to ingest the diet it is likely that they could not completely assimilate it. Further research on the digestibility of recycled food waste (FIW and SSW) needs to be carried out.

Initial and final proximate body compositions, lipid profiles and amino acid profile of tilapia fry fed experimental diets showed that their chemical make up changed to reflect closely the contents of the experimental diets. Bendiksen *et al*. (2003) reported that the whole body proximate composition of the fish fed the experimental diets with the exception of moisture was higher than the initial, although differences in the body moisture are often accompanied by reciprocal changes in the whole lipid content. This present study showed that an increase in lipid content led to a decline in the body moisture and although the final body protein did not differ significantly between the fish fed the experiment diets, D3 had the highest protein content (Table 5).

One of the limiting growth factors in fish is protein, Wilson (1989) reported that the body protein usually accounts for 65-85% of the dry matter content of fish and the ability of fish like other terrestrial animals to synthesize protein at a rate which is required to promote growth from carbon skeleton is limited, 10 standard amino acids are reported to be essential for building new tissues (Mambrini and Guillaume, 1999; Lu *et al*., 2002). Table 2 compares the essential amino acids (EAA) composition of the diets with the minimum quantitative requirement for *O. niloticus* as reported by Santiago and Lovell (1988). Supplementation of D2, D3 and D4 by lysine and methionine enhanced the quality and quantity of amino acid in these experimental diets. The good growth of tilapia fry fed the diets in this study indicated that EAA values of these diets were sufficient despite being lower in tryptophan. The lowering of tryptophan in D2 to D5 might be a result of the original constituent make up of the parent ingredient materials (Table 7). Park *et al.* (2001) reported that taurine is an essential amino acid in marine fish (Japanese flounder). In the context of our study the taurine content of the diets ranged between only 0.22-0.44 g/100g, while 1.573 g/100g in the initial fish body and increased to a ranged between 2.11-2.27 g/100g in the final body of the fish fed the experimental diet for 12 weeks (Table 7). This is in agreement with Lu *et al.* (2002) that tilapia had the ability to biosynthesize taurine from methionine.

According to Coyle *et al*. (2000) essential fatty acids are those that are needed in the diet for optimum growth and survival. The body fatty acid composition of the fish generally reflected the trends that were observed in the fatty acid concentration of the diet treatment. In this context a comparable level to fatty acid composition of the whole body lipid of the experimental fish is required in the essential fatty acid of the diet for tilapia. All the diets were very rich in n-6 which according to Takeuchi *et al*. (1983) is optimal for tilapia. The diets also met the fatty acid minimum requirement for *O. niloticus* as specified by Takeuchi (1997). Considering the requirement of EFA in the diet is 0.5-1.0%, it is estimated that the content of EFA in the experimental diets might have met the requirement for tilapia growth.

The mineral composition of fish fed the experimental diets did not differ significantly. The contents of some elements in the diet met the requirements for active growth of tilapia e.g. macro elements (P: 5 mg/g, Mg: 0.5-0.7 mg/g, and Ca: 7 mg/g) and trace elements (Zn: 10 µg/g, Mn: 12 µg/g, and Cu 3-4 µg/g). The high Na content in D4 results from the high content of sodium chloride in the parental ingredient (SSW) (Table 8). The final mineral body content of the fish fed the experimental diets was similar across treatments. Except for Ca, the initial mineral content of the whole fish was higher than the final. The higher Ca in the final mineral content may be attributed to the ability of the experimental fish to effectively utilize the Ca in the diet or to directly absorb Ca from freshwater via the skin and gills (Millamena *et al.,* 2002).

CONCLUSION

The present study revealed that recycled food waste supplemented with lysine and methionine help in improving the growth, survival, nutrient retention without adverse effects on the fish fed these experimental diets. This present study shows that judicious supplementation of recycled food waste materials with lysine and methionine can effectively be used to reduce fish meal utilization in the practical diet of Nile tilapia fry. Further evaluation of recycled food waste based diet on growth of Nile tilapia in production trials for a longer duration should be investigated.

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