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Evaluation of High-Level Inclusion of Recycled Food Wastes Supplemented with Lysine, Methionine and Arginine in the Diet of Nile Tilapia *Oreochromis niloticus* Juveniles

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Abstract

This study evaluates the use of high-level inclusion of recycled food waste materials: food industrial waste (FIW) and soy sauce waste (SSW) supplemented with lysine methionine and arginine (AA) to replace fishmeal (FM) in practical diets of Nile tilapia *Oreochromis niloticus* juveniles. Fish (mean initial weight 6.1±0.1 g) were fed five diets containing different levels of recycled waste materials (designated as FM, FIW+AA (dietary level: 70.5%), FIW+SSW+AA (65.0%), SSW+AA (56.5%) and FIW+SSW (60.0%) for 84 days. Growth, feed utilization and survival rate in fish fed the FIW+AA and SSW+AA diet were significantly lower than that in FM ($P<0.05$) whereas; no significant difference was obtained in these parameters between fish fed the FM and FIW+SSW+AA ($P>0.05$). Protein digestibility in fish fed the FM diet was significantly the highest ($P<0.05$), whereas it was lowest in FIW+AA. Whole body proximate composition of fish fed all diets showed that the level of recycled waste materials added influenced moisture and lipid contents. The result of this study indicates that a combination of FIW and SSW, supplemented with AA gives better growth performance when used to completely replace FM in the diet for juveniles tilapia.

Keywords: *Oreochromis niloticus*; Growth performance; Food industry waste; Soy sauce by-product

Formulation of fish feeds containing high levels of non-conventional alternative protein source has become a major focus in aquaculture nutrition research. This as become imperative because of the ever increasing cost and uncertain availability of fishmeal, which is primarily regarded as the only protein source in fish diet. Hence, conventional plant proteins (soybean, rapeseed etc.) appear to be the most suitable alternatives for fishmeal in fish diets. However, their scarcity and competition from other sectors for these conventional crops for livestock and human consumption as well as industrial use make their cost too high and put them far beyond the reach of average farmers or aqua feed producers (Fasakin et al. 1999).

In Japan, there is a basic law establishing a recycling-based society, with emphasis on the minimization of associated biodegradable waste by-products through recycling and effective reuse. With recent developments in technology, our ability to recycle and reuse food waste is increasing. There is an abundance of these recycled food waste materials (e.g. food industry waste (FIW), soy sauce waste (SSW)). In our previous studies we demonstrated the suitability of recycled food waste materials from FIW and SSW in the practical diet of Nile tilapia fry and inclusion of these recycled food wastes up to 58% enriched with lysine (lys), methionine (met) and arginine (arg). This was also reported by (Bake et al. 2009).

Therefore, the main purpose of this study was to further evaluate the effects of dietary high level inclusion of recycled FIW and SSW, supplemented with lys, met and arg on growth, feed utilization and body composition of juveniles Nile tilapia *Oreochromis niloticus*.

Materials and Methods

Ingredients

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 26.1% 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 ^{o}C maintained for 1 hour and later increased to between 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 19.6% and 11.3%, 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 63.5% and 11.8%, respectively.

Experimental diets

Based on the nutritional requirements of tilapia (NRC 1993), five isonitrogenous and iso-lipidic diets were formulated at 30% protein and 9.5% lipid, containing different sources of recycled food waste materials supplemented with lys, met and arg designated as FM representing the commercial fishmeal (control). FM was replaced with food waste at dietary levels of 70.5% (FIW+AA), 65.0% mixture of FIW and SSW (FIW+SSW+AA), 56.5% SSW (SSW+AA) and 60.0% mixture FIW and SSW (FIW+SSW) on a dry matter basis. FIW+AA, FIW+SSW+AA and SSW+AA were supplemented with mixture of lys, met and arg at the rate of 4.5%. These diets were fed to the fish 84 days (Tables 1 and 2).

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

Diet code	FM	FIW + AA	FIW + SSW + AA	SSW + AA	FIW + SSW
Fishmeal*[1]	453.0	150.0	150.0	150.0	235.0
Soy sauce waste	0.0	0.0	325.0	562.0	300.0
Food industry waste	0.0	705.0	325.0	0.0	300.0
α - Starch	200.0	35.0	35.0	35.0	35.0
Vitamin premix*[2]	40.0	20.0	20.0	20.0	20.0
P-free mineral mixture*[3]	40.0	20.0	20.0	20.0	20.0
$Ca(HPO_4)_2.H_2O$	40.0	20.0	20.0	20.0	20.0
Lysine	0.0	15.0	15.0	15.0	0.0
Methionine	0.0	15.0	15.0	15.0	0.0
Arginine	0.0	15.0	15.0	15.0	0.0
Soybean oil	47.0	0.0	0.0	15.0	0.0
Chromic oxide	5.0	5.0	5.0	5.0	5.0
Cellulose	180.0	0.0	55.0	125.0	65.0
Cost of diet (¥/ kg) *[5]	31.08	10.03	13.00	9.47	8.54

*[1] Anchovy fishmeal from Chile.

*[2] Composition (mg/100g): Thiamine 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 K_3 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 *[5] Cost of the feeding stuffs at the prevailing market prices in Japan (April 2009):

1 USD = ¥ 91.00

Table 2: Proximate and fatty acid composition of the experimental diets

Diet code	FM	FIW + AA	FIW + SSW+AA	SSW + AA	FIW + SSW
Proximate composition					
Moisture (%)	3.85	4.31	4.22	4.91	4.70
Crude protein (% dry basis)	31.35	31.03	31.34	30.87	31.66
Starch (% dry basis)	25.42	34.65	30.32	21.54	26.23
Crude lipid (% dry basis)	9.21	9.24	9.25	9.44	9.25
Ash (% dry basis)	12.83	9.10	9.72	10.08	11.39
Fatty acids (g/100g dry basis)					
14:0	0.44	0.59	0.40	0.65	0.31
16:0	0.58	0.96	0.87	0.84	0.76
16:1n-7	0.79	1.03	0.91	1.07	0.80
16:3n-6	0.15	0.17	0.15	0.07	0.01
16:3n-3	0.06	0.05	0.05	0.05	0.06
18:0	0.71	0.90	0.77	0.79	0.67
18:1(OA)	1.01	1.43	1.21	1.23	1.00
18:2n-6(LA)	1.81	1.74	1.92	2.05	1.92
18:3n-6	0.22	0.24	0.32	0.32	0.25
18:3n-3(LNA)	0.16	0.08	0.09	0.08	0.13
18:4n-3	0.24	0.13	0.13	0.12	0.13
20:0	0.35	0.43	0.34	0.33	0.34
20:1	0.02	0.02	0.01	0.02	0.02
20:2n-6	0.04	0.02	0.02	0.01	0.02
20:3n-6	0.02	0.11	0.02	0.01	0.01
20:4n-6(AA)	0.08	0.11	0.11	0.11	0.11
20:3n-3	0.08	0.02	0.05	0.03	0.04
20:4n-3	0.06	0.05	0.05	0.05	0.06
20:5n-3(EPA)	0.07	0.03	0.03	0.03	0.04
22:0	0.15	0.13	0.14	0.13	0.14
22:1	0.04	0.01	0.01	0.02	0.01
22:4n-6	0.01	0.01	0.03	0.03	0.02
22:5n-6	0.01	0.02	0.03	0.09	0.02
22:5n-3	0.26	0.14	0.18	0.13	0.25
22:6n-3(DHA)	0.14	0.09	0.10	0.08	0.10
Σ Monoene	1.86	2.50	2.15	2.34	1.83
Σ Saturate	2.23	3.01	2.53	2.76	2.23
Σ n-3	1.07	0.59	0.67	0.58	0.81
Σ n-6	2.34	2.43	2.60	2.68	2.36
Σ n-3HUFA	0.62	0.33	0.40	0.32	0.49

Experimental conditions and fish rearing

The experiment was carried out at the Laboratory of Fish Culture, Tokyo University of Marine Science and Technology, Japan. A freshwater flow-through system with filtered and dechlorinated tap water at 9 *l* /h in each aquarium was used for the study. This consists of fifteen 30 *l*-aquaria (triplicate experiments) and water temperature was maintained at 28 ± 0.5°C using electric heaters. The aquariums were provided with continuous aeration through an air blower. Illumination was supplied by overhead fluorescent lights to maintain a constant photoperiod of 12 h light and 12 h dark cycle (8:00-20:00) throughout the experiment. The water temperature and dissolved oxygen in the system were monitored weekly using DO meter (HQ 30d, HACH Company, Colorado, USA). Concentration of ammonium in rearing aquaria was analyzed with reagents of water analysis (HACH Company, Colorado, USA) using ultra violet and visible light spectrophotometer (UV-1200, Shimadzu Corporation, Kyoto, Japan) bi-weekly.

The tilapia *O. niloticus* juveniles (average weight: 6.1± 0.1g) used for this experiment were obtained from pure-bred tilapia broodstock, held at the laboratory. Twenty fish were stocked in each aquaria with a flow through system for 84 days.

Sample collection and biochemical analyses

Fish were weighed individually at the beginning of the experiment and subsequently bi-weekly 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, 8 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 (ZM 200, Retsch Technology GmbH, Haan, Germany) fitted with a 0.25mm-screen. The homogenate was collected and kept at -20°C until analysis. The TUF (Tokyo University of Fisheries) column system was used for faecal collection (Satoh *et al*. 1992). After feeding, uneaten feed was siphoned out and the faecal collector was installed an hour later. Faeces were collected during the last two weeks of the experiment and pooled samples were freeze-dried and kept for later analyses. The indigestible marker employed was Cr_2O_3.

The experimental diets were subject to chemical analysis. Proximate analysis and lipid analysis were carried out according to the methods of Takeuchi (1988) and 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). The fatty acid profile was determined using gas liquid chromatography (GC-14A, Shimadzu Corporation, Kyoto, Japan). An amino acid auto analyzer (JLC-500V, JOEL Ltd., Tokyo, Japan) was used to determine the constitutional amino acids of the diets in accordance with the method described by Simpson et al. (1976). The samples for analysis of minerals were digested in nitric acid using the microwave digestion system (MLS-1200 mega, 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 weight gain (WG), feed efficiency (FE), specific growth rate (SGR), feed intake (FI) protein efficiency ratio (PER) and apparent protein digestibility. The following formulas were used:

WG (%) = (final weight (g) - initial weight (g)) / initial weight (g) × 100

FE (%) = weight gained (g) / feed fed (g) ×100

SGR (%) = ((ln final weight (g) - ln initial weight (g)) / feeding period (day) ×100

FI (mg/fish/day) = dry feed (mg) fed / number of fish / feeding period (day)

PER = wet body weight gain (g) / protein intake (g)

Apparent protein digestibility (%) = 100 – ((Cr_2O_3 in diet (%) / Cr_2O_3 in feces (%)) × (N in feces (%) / N in diet (%)) × 100)

Statistical analyses

Data were analyzed using one-way analysis of variance (ANOVA) using Statistica 8.0 (Stat-Soft, Inc., Oklahoma, USA). Differences between treatments were compared by Tukey's test. Level of significance was tested at $P<0.05$.

Results

Over the 12-week feeding period, no significant differences were observed in the water-quality indices between the experimental treatments. The water-quality parameters were within the acceptable range for Nile tilapia culture (Balarin and Hatton 1979). The water temperature ranges from 27.5-28.3 °C, Dissolved oxygen from 6.1-7.4 mg/ *l*, pH from 6.8-7.6 and ammonia from 0.22-0.28 mg/ *l*.

The proximate composition and fatty acid profile of the experimental diets is shown in Table 2. There were no variations in the protein and crude lipid content among the experimental diets. FIW+AA diet had the highest starch content while SSW+AA diet had the lowest. Moisture was lower in FM diet compared to the other experimental diets. The fatty acid profile composition shows that FIW+AA was higher in monoenes and saturates while FM and FIW+SSW diets had lower monoenes, however saturates n-6 composition did not vary significantly among the experimental diets.

Table 3 shows the amino acids profile of the experimental diets except for the amino acids that were supplemented and higher in FIW+AA, SSW+AA and FIW+SSW+AA. There was no major variation in the composition of the essential amino acids among the different experimental diets.

Table 3: Constitutional amino acid of the experimental diets (g/100g)

Diet Code	FM	FIW+AA	FIW + SSW+ AA	SSW+AA	FIW+SSW	NRC(1993) requirement
Essential amino acid						
Arginine	2.20	3.64	3.48	3.30	2.32	4.2
Lysine	2.03	2.49	2.57	2.62	1.42	5.12
Histidine	1.34	0.81	0.83	0.82	1.06	1.72
Phenylalanine	1.26	1.10	1.36	1.51	1.54	3.75
Leucine	2.49	2.90	2.79	2.61	3.14	3.39
Isoleucine	1.19	1.00	1.09	1.13	1.27	3.11
Methionine	1.12	2.03	2.00	1.97	1.02	3.22
Valine	1.32	1.01	1.11	1.15	1.32	2.8
Threonine	1.20	0.88	1.25	1.50	1.42	3.75
Tryptophan	0.22	0.10	0.17	0.22	0.20	1
Non-essential amino acid						
Taurine	0.32	0.22	0.22	0.33	0.28	
Alanine	2.24	1.55	1.71	1.78	2.06	
Glycine	2.23	1.98	1.89	1.83	2.24	
Glutamic acid	4.84	3.79	3.31	2.83	3.94	
Serine	3.15	2.88	2.92	2.90	3.34	
Aspartic acid	2.67	2.48	2.31	2.49	2.72	
SUM	29.81	28.84	29.01	28.98	29.28	

The result of the growth performance and nutrient utilization is presented in Table 4 and Fig 1. Significant differences were observed among tilapia fed FM, FIW+AA, SSW+AA, FIW+SSW+AA and FIW+SSW diets (P<0.05). Tilapia fed FM diet showed the highest final body weight and percentage weight gain, while tilapia fed FIW+AA and SSW+AA showed the lowest values. No significant differences was observed for survival rate between tilapia fed FM diet and FIW+SSW+AA (P>0.05), and were significantly higher than tilapia fed other experimental diets (P<0.05). Tilapia fed FM diet had the highest SGR value though it was not significantly different from those fed FIW+SSW+AA diets, but significantly different from those fed other experimental diets (P<0.05). Tilapia fed SSW+AA had the lowest SGR value, and not significantly different from tilapia fed SSW+AA, FIW+ AA and FIW+SSW (P>0.05). FE values were similar between tilapia fed FM and FIW+SSW+AA, which were significantly better than those fed FIW+AA and SSW+AA and FIW+SSW diets. PR and PER followed the same pattern, being similar in FM and FIW+SSW+AA performing significantly higher than the other experimental diets (P<0.05). FM diet displayed significantly higher protein digestibility compared with the other experimental diets (P<0.05), whereas FIW+AA diet had the lowest and was significantly different from others.

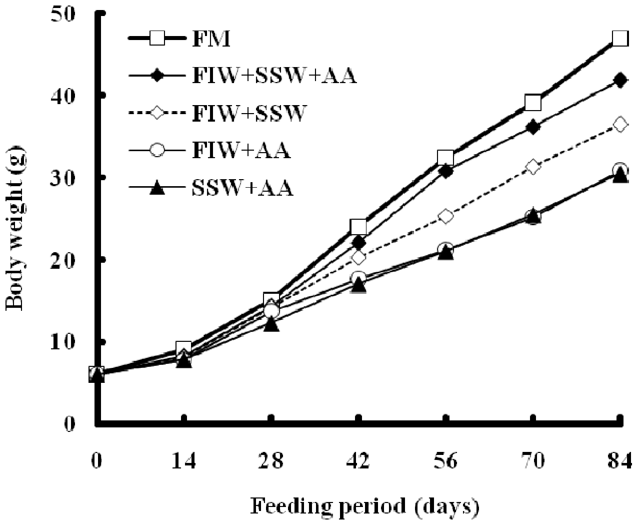

Figure 1: Growth of *Oreochromis niloticus* Juveniles fed the experimental diets for 84 days

Table 4: Growth performance of *Oreochromis niloticus* juveniles fed experimental diets for 84 days

Diet code	Av. body weight (g) Initial	Av. body weight (g) Final	Weight gain (%)	Survival rate (%)	Specific growth rate (%)	Total feed intake (g)	Feed efficiency	Protein retention (%)	Protein efficiency ratio	Protein digestibility (%)
FM	6.1±0.1	46.3±1.2[a]	699±96[a]	98.5±1.7[a]	2.43±0.42[a]	32.0±0.6[a]	1.41±0.03[a]	65.3±0.2[a]	4.47±0.16[a]	94.5±0.5[a]
FIW+AA	6.1±0.1	30.9±1.6[d]	405±36[d]	94.6±1.8[b]	1.92±0.23[c]	21.9±0.6[c]	1.14±0.05[b]	50.6±0.5[c]	3.58±0.37[d]	71.2±0.6[d]
FIW+SSW+AA	6.1±0.1	41.9±2.3[b]	584±24[b]	97.2±2.6[a]	2.29±0.38[a]	25.4±0.4[a]	1.32±0.04[a]	60.0±0.3[b]	4.15±0.18[ab]	84.2±0.5[bc]
SSW+AA	6.1±0.1	30.5±2.0[d]	400±15[d]	94.0±1.8[b]	1.91±0.23[c]	22.7±0.5[c]	1.10±0.02[b]	51.1±0.8[c]	3.58±0.59[d]	87.3±0.5[b]
FIW+SSW	6.1±0.1	36.3±1.3[c]	494±29[c]	94.6±1.8[b]	2.12±0.29[b]	23.2±0.5[b]	1.22±0.04[b]	55.3±0.3[bc]	3.65±0.18[c]	82.8±0.6[c]

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

Table 5: Proximate composition analyses of *Oreochromis niloticus* fed experimental diet for 84 days (%)

Component		Moisture	Protein	Lipid	Ash
Initial		75.6 ± 0.6	13.9 ± 1.3	5.2 ± 0.6	4.6 ± 0.4
Final*1	FM	73.7 ± 1.2^{c}	14.5 ± 1.1^{a}	6.3 ± 0.7^{c}	5.5 ± 0.2
	FIW + AA	72.5 ± 1.2^{a}	14.1 ± 1.1^{c}	7.8 ± 0.5^{a}	5.2 ± 0.3
	FIW + SSW + AA	72.7 ± 0.3^{ab}	14.4 ± 1.1^{ab}	7.4 ± 0.5^{b}	5.3 ± 0.1
	SSW + AA	73.3 ± 1.1^{bc}	14.3 ± 1.2^{b}	6.5 ± 0.4^{c}	5.4 ± 0.3
	FIW+ SSW	73.3 ± 1.2^{bc}	14.3 ± 1.1^{b}	7.0 ± 0.5^{b}	5.4 ± 0.2

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

The proximate carcass composition of the initial and final groups of the fish fed with the experimental diets is given in Table 6. Except for moisture, all the final carcass proximate compositions were higher than the initial. Fish fed FIW+AA diet had the lowest carcass moisture among the fish fed experimental diets and was significantly different from those fed other diets except FIW+SSW+AA (P<0.05). While fish fed FM had the highest moisture content, it was not significantly different from those fed SSW+AA and FIW+SSW diets (P>0.05). Carcass protein of the experimental fish fed changed significantly among the treatments, being the highest in FM diet which were similar to FIW+SSW+AA, but significantly different from those fed SSW+AA, FIW+AA and FIW+SSW (P<0.05) and lowest in FIW+AA which differed significantly from the others (P<0.05). Fish fed FIW+AA accumulated the highest carcass lipid composition and was different from those fed other experimental diets (P< 0.05). With exception of fish fed SSW+AA; fish fed FW had the lowest carcass lipid and was significantly different from those fed other experimental diets. There was no significant difference in the ash composition of all the fish fed experimental diet. Table 7 shows that there was no much remarkable variation in the constitutional amino acids composition of the whole body of the fish fed the experimental diets. However, showed that the fatty acid profile of the whole body of fish fed FIW+AA had higher monoenes and saturates, while fish fed FM were richer in Σn-3 (Table 8). There was no significant variation in Σn-6 among the experimental treatments.

Table 6: Constitutional amino acid of *Oreochromis niloticus* whole body fed experimental diets (g/100g dry basis)

	Initial	Final				
		FM	FIW + AA	FIW + SSW+ AA	SSW + AA	FIW + SSW
Essential amino acid						
Arginine	3.37	3.14	3.56	3.43	3.70	3.01
Lysine	3.97	3.62	4.32	4.56	4.40	3.64

Histidine	1.23	1.33	1.02	1.39	1.30	1.49
Phenylalanine	2.37	2.66	2.50	2.84	2.69	2.97
Leucine	3.26	3.81	3.04	3.15	3.77	3.29
Isoleucine	2.41	2.44	2.56	2.46	2.10	2.66
Methionine	2.85	1.56	2.41	2.50	2.39	1.49
Valine	2.66	2.80	2.07	2.82	2.36	2.06
Threonine	3.21	2.94	2.56	2.88	2.71	2.81
Tryptophan	0.71	0.53	0.45	0.45	0.46	0.55
Non-essential amino acid						
Taurine	1.08	1.28	1.37	1.40	1.39	1.18
Alanine	3.05	4.82	6.05	5.86	5.29	5.82
Glycine	4.21	4.60	5.88	5.57	6.51	6.53
Glutamic acid	7.83	6.95	5.13	5.15	5.09	5.69
Serine	3.42	3.71	2.56	2.90	2.53	2.69
Aspartic acid	5.24	4.74	3.91	4.09	3.90	3.75
Total	51.89	50.73	49.39	51.43	50.61	49.53

Table 7: Fatty acid composition of the whole *Oreochromis.niloticus* fed experimental diets (g/100g dry basis)

	Initial	Final				
		FM	FIW + AA	FIW + SSW+ AA	SSW + AA	FIW + SSW
Fatty acid						
14:0	1.26	0.69	0.96	1.10	0.90	1.02
16:0	4.77	4.37	5.85	5.26	4.56	5.32
16:1n-7	1.35	1.38	1.43	1.59	1.70	1.58
16:3n-6	0.17	0.06	0.14	0.08	0.06	0.07
16:3n-3	0.11	0.05	0.13	0.09	0.12	0.13
18:0	0.24	1.03	1.52	1.44	1.05	1.25
18:1(OA)	2.75	5.03	7.08	5.88	3.59	4.53
18:2n-6(LA)	1.00	0.72	1.69	3.27	0.97	
18:3n-6	0.74	3.01	3.25	1.48	3.70	2.55
18:3n-3(LNA)	0.05	0.31	0.03	0.34	0.34	0.19
18:4n-3	0.09	0.16	0.16	0.21	0.09	0.14
20:0	0.20	0.19	0.10	0.18	0.17	0.27
20:1	0.29	0.28	0.10	0.28	0.23	0.27
20:2n-6	0.25	0.03	0.07	0.06	0.14	0.16
20:3n-6	0.03	0.13	0.07	0.09	0.07	0.09
20:4n-6(AA)	0.03	0.11	0.16	0.05	0.14	0.12
20:3n-3	0.02	0.24	0.09	0.16	0.04	0.05

20:4n-3	0.16	0.05	0.19	0.10	0.08	0.11
20:5n-3(EPA)	0.50	0.62	0.47	0.50	0.53	0.59
22:0	0.11	0.01	0.06	0.03	0.05	0.03
22:1	1.00	0.35	0.44	0.23	0.14	0.20
22:4n-6	0.06	0.13	0.09	0.06	0.04	0.07
22:5n-6	0.08	0.09	0.04	0.11	0.06	0.08
22:5n-3	1.34	1.06	0.74	0.96	0.95	1.10
22:6n-3(DHA)	2.13	2.02	1.66	1.65	1.70	1.88
Σ Monoene	5.39	7.04	9.06	7.97	5.66	6.58
Σ Saturate	6.58	6.29	8.49	8.00	6.73	7.88
Σ n-3	4.41	4.51	3.47	4.02	3.85	4.18
Σ n-6	2.35	4.29	5.50	5.19	5.15	4.35
Σ n-3HUFA	4.15	3.98	3.15	3.38	3.29	3.72

Discussion

Results of our previous study demonstrated that 58% of FM in the diet of Nile tilapia fry could be replaced by recycled food waste (FIW and SSW) with judicious supplementation of AA when the dietary protein level was 40% (Bake et al. 2009). In the present study, there was no feed rejection during the experimental period, although the acceptability of the diets differed considerably among the treatments. The growth performance and nutrient utilization of this present study was lower than our previous studies (Bake et al. 2009, 2009). The result obtained from the present study indicates that *O. niloticus* juvenile fed FM gave a better growth performance and nutrient utilization than the other treatments. This may likely be due to the high inclusion level of the recycled food waste material (FIW and SSW) in the diets. This agrees with earlier reports (El-Sayed and Teshima 1991, Riche et al. 2001, Riche and Garling Jr. 2004, Ahmad 2008) suggesting that when alternative protein sources especially plant protein sources are very high in fish diet, palatability and attractiveness of the diets may be negatively affected. In general the reduced feed intake due to the unpalatability may lead to deterioration in growth performance and feed utilization. In the context of this study, the main protein sources used for the experimental diets were from FM and recycled food waste (FIW and SSW). As suggested by Watanabe et al. (1987) proper utilization of dietary protein is dependent on the good quality or amino acid balance of the protein sources. Based on the previous studies (Bake et al. 2009, 2009), the amino acid of the parent ingredients (FIW and SSW) required the supplementations of lysine, methionine and arginine to meet the minimum requirement of Nile tilapia, however the supplementation of both FIW and SSW with AA does not give us the desired result. This may likely be as a result of the inbalance in the amino acid profile of the parent ingredients as suggested by Boisen (2003); Peres and Oliva-Telas (2007) that pointed out that profound deleterious effect may occur in animals not only because of EAA deficiencies, but also AA imbalances.

The poor growth of Tilapia fed FIW+AA may also be attributed to the lower digestibility of the diet, since protein digestibility is a measure of protein availability by fish (Kenan and Yasar 2005). In the context of this study, the observed results showed that the fishmeal used had the digestibility value of a good quality fishmeal (Watanabe et al. 1983; Pike et al. 1990). From this it is evident that FIW cannot be used as a total or complete replacement of fishmeal even when enriched with deficient AA. Although SSW+AA had a high protein digestibility, the poor growth and nutrient utilization by Tilapia with this diet may be attributed to poor palatability and the attractiveness of the diet causing a reduction in feed intake by the fish. This agrees with the findings of Rodriguez-Serna et al. (1996), Fagbenro (1999), Francis et al. (2001), Siddhuraju and Becker (2003) who suggested that one of the major problem with alternative plant protein source in fish feeding is the changes in palatability and physical texture of a diet, considering the parental make up of the diet soy-sauce waste which mainly composes of soybean and wheat, which are plant origin.

Fish fed the combination of the two recycled food waste (FIW and SSW) gave a better growth performance and nutrient utilization when compared to those fed FIW+AA and SSW+AA, agreeing with our earlier works Bake et al. (2009, 2009). This may be attributed to a more balanced amino acids profile of the diet; furthermore, addition of the AA to a combination of FIW+SSW enhances higher inclusion level in the diet and better growth performance than a combination of FIW+ SSW without AA supplementation. This also is in agreement with our previous findings (Bake et al. 2009, 2009) indicating that a proper combination of different ingredients in a diet gives a better amino acid balance in a diet than a single ingredient based diet. The higher protein efficiency ratio and protein retention in this present study compared with the previous (Bake et al. 2009) where fry tilapia was used may be attributed to the stage of fish used in this study. This implies that the juvenile tilapia could digest and assimilate recycled food waste materials (FIW and SSW) based diet more readily than the fry.

The proximate composition of whole body of the fish fed experimental diet in this present study agrees with those by Mohsen and Lovell (1990); Serrano et al. (1992); Yildirim et al. (2003) and Luo et al. (2005) in terms of the body fat content which was closely and inversely related to the body moisture content. Despite poor growth, the increase in the carcass lipid in the fish fed FIW+AA led us to conclude that tilapia have the ability to store significant quantities of lipid in carcass or viscera but may not utilize this energy source to improve its growth as proposed by Hanley (1991). The initial and final proximate body compositions and the fatty acids profiles of the juvenile tilapia fed high inclusion of recycled food waste (FIW and SSW) diets showed variational changes in their chemical contents reflecting more closely the contents of recycled food waste materials in the diet. The essential fatty acids (EFA) of tilapia, Σn-6 fatty acids in the diets are between 0.5-1.0%. This also showed that the EFA in recycled food waste materials (FIW and SSW) even at a higher inclusion level could meet the EFA requirement for normal tilapia growth (Takeuchi et al. 1983).

In this present study, we also observed that the dietary supplementation with arginine, lysine and methionine induced an increased of the concentrations of these three amino acids in the body. This agrees with D'mello (1993) and confirms that supplementation of EAA could improve the amino acid supply and balance as shown in other animals. Moreover, 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 0.22-0.32g/100g, while 1.08 g/100g in the initial fish body and ranged between 1.18-1.40g /100g in the final body of the fish fed the experimental diet for 12 weeks (Table 6). This reaffirms our previous (Bake et al. 2009) and Lu et al. (2002) suggesting that tilapia had the ability to biosynthesize taurine from methionine.

Production costs of the diets containing high-level inclusion of recycled food wastes control diets are summarized in Table 1. The control diets had the highest total cost of producing 1kg of feed. The most cost-effective diet in terms of production cost is FIW+SSW without EAA. Despite the better growth performance by FM diet, in a long term cost analysis the diets containing high-level inclusion of recycled food waste could be more profitable. The reduction in the feed cost per kilogram can facilitate the use of recycled food wastes in the diets. Moreover the fact that recycled food wastes can be available throughout the season, and no competition for humans use compared to the other conventional alternative proteins appears to be additional advantage.

In conclusion, this study showed that although none of the recycled food waste materials used, can singularly be used to completely replaced FM in the diet of *O. niloticus* juveniles, a proper combination of FIW and SSW supplemented with or without lys, met and arg can reduce the effect of bad odour in SSW which is highly digestible. The combined used of recycled food waste material FIW and SSW with or without EAA could be economical considering the availability and cost.

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