AMERICAN UNIVERSITY OF BEIRUT

CONVERTING POST-CONSUMER FOOD WASTE INTO FISH FEED

by

NIVIN ABDALLAH NASSER

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science to the Department of Nutrition and Food Sciences of the Faculty of Agriculture and Food Sciences at the American University of Beirut

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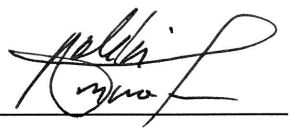

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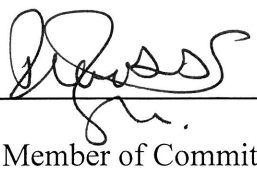

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AN ABSTRACT OF THE THESIS OF

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More than 40% of human food produced is wasted and much of it in restaurants. Hence, food waste remains a serious environmental challenge facing the world today, with sizable quantities generated and disposed of in landfills. Accordingly, exploring solutions to mitigate the detrimental impacts of such waste becomes vital. Aquaculture, a rapidly growing industry in rural areas of developing nations, offers the potential of using this waste productively to partially replace commercial feed, which is often absent or expensive.

The first experimental setup consisted of two 8-week feeding experiments performed to evaluate the suitability of using restaurant food waste to supplement commercial feed (CF) in the aquaculture of Nile tilapia *Oreochromis niloticus*. In the first experiment, five feeding regimens in which CF was substituted by waste-based feed at 0, 25, 50, 75 and 100% of daily offering were evaluated. Results show that 25% of the CF can be replaced with waste-based feed without any significant effect on survival, growth, feed conversion, hepatosomatic, viscerosomatic indices, hemoglobin, hematocrit and total plasma protein. In the second experiment, seven feeding treatments were evaluated in which daily offerings of CF were alternated with waste-based feed in 6-day cycles. Results suggest that replacement between 25% and 33% is feasible without significantly affecting survival or growth. Again, no significant differences were observed in growth, feed conversion ratio, VSI, hemoglobin, hematocrit and TPP. Findings suggest that around 25% replacement of CF with Lebanese restaurant waste-based feed can be utilized in the culture of *O. niloticus* thus improving financial returns of farmers while reducing the environmental impact of food waste.

The present work also assessed the effect of using post-consumer food waste as feed on the amino acid and fatty acid profiles of Nile tilapia, *Oreochromis niloticus*. Whole body protein proportion decreased, and amino acid composition changed significantly with increase of WBF in the regimen. Fatty acid composition of the fingerlings fed with increasing percentages of WBF showed a significant increase in saturated fatty acids and monounsaturated fatty acids. Conversely, the proportion of omega-3 and omega-6 poly-unsaturated fatty acids decreased significantly with increasing WBF fraction. At 25% replacement of commercial feed with WBF, there was limited effect on amino acid and fatty acid profiles of the fish and no significant effect on growth. Accordingly, farmers could replace 25% of the commercial feed they use with WBF and still maintain

good growth and nutritional quality of their fish while reducing the environmental impact of restaurant generated food waste and improving profits of their operations. A third experiment was performed to evaluate the suitability of using restaurant food waste to supplement commercial feed (CF) in the aquaculture of rabbitfish, *Siganus rivulatus*. Five feeding regimens were adopted in which commercial feed was substituted with waste-based feed at 0, 25, 50, 75 and 100% of daily offering. Results suggest that up to 73.2% of the commercial feed can be substituted by waste-based feed with no significant effects on survival, growth rate, feed conversion ratio, or hepatosomatic and viscerosomatic indices of the fish. There were no statistical differences among treatments with 0, 25 and 50% replacement in terms of whole-body protein and lipid content or in hematological parameters. However, at 75% substitution, there was a clear decrease in growth, whereas at 100% replacement the fish showed complete mortality. Therefore, the present study demonstrates a potential solution to mitigate food waste from landfills by utilizing it as a partial replacement of commercial fish feed. This in turn decreases the cost incurred in aquaculture production.

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CHAPTER I
INTRODUCTION

The number of undernourished people in the world has been on the rise since 2014, and it was estimated to reach 821 million in 2017 (FAO *et al.* 2018). With food waste contributing significantly to greenhouse gas emissions (FAO 2014), and suitable agricultural land and fresh water resources being inefficiently used, food waste has become a major environmental, financial, and ethical concern. Thus, it is crucial that we produce more food sustainably while reducing food waste. Hence, why not use food waste to produce nutritive protein in marine environments. This approach would increase food production, reduce food waste, use non-traditional agriculture land and salt water and reduce greenhouse gas emissions in landfills.

Human food waste is comprised mainly of food left uneaten on a plate in a restaurant, at home or food purchased but not consumed. Food waste often includes non-nutritive components such as egg shells, pineapple skin, apple cores, and bones. It also has a significant amount of protein, lipids and carbohydrates. In other words, food waste includes quality food along the value chain suitable for human consumption but eventually ending up unconsumed and/or discarded (Gustavsson *et al.* 2011). In the hospitality sector (hotels, restaurants, cafeterias, pubs, catering services, etc.), one of the main reasons for food waste is standardized portion size which more often than not exceeds the needs of individuals, leading to wasted leftovers (Gunders 2012, Monier *et al.* 2010).

Aquaculture is the fastest growing sector of agriculture, increasing by 5.8% yearly between 2005 and 2014 (FAO 2016). Approximately 50 million tons of the 74 million tons produced in 2014 were fish, crustaceans and other species that are offered feed. Average feed conversion ratio in aquaculture is about 1.5

(Cao *et al.* 2015) which means that fed aquaculture requires *circa* 75 million tons of prepared feed annually. This amounts to approximately 250 – 300 million tons of fresh ingredients. If only 10% of this feed can be replaced by food waste, that would save 25 to 30 million tons of fresh ingredients a year, enough to produce an extra 5 million tons of fish with no extra use of agricultural land or freshwater resources. Furthermore, the recycled waste would not end up in landfills, thus reducing major environmental issues.

In the present work, we collected plate food waste from local (Lebanese) restaurants and analyzed them for crude nutritional content. We processed the food waste into fish feed whilst ensuring water stability, attractability and palatability of the manufactured feed. Next, we assessed to what degree we can substitute a commercial fish feed with the processed waste without affecting survival, growth parameters, hematological indices and nutritional characteristics of the fish. The dietary regimen was tested on an omnivorous freshwater fish (*Oreochromis niloticus;* Tilapia) and an herbivorous marine fish (*Siganidae*; rabbitfish). The experiments performed are listed in the following chapters.

CHAPTER II
EXPERIMENT ONE

Effect of using post-consumer food waste as feed on growth parameters and hematological indices of Nile tilapia, *Oreochromis niloticus*

A. Introduction

Aquafeed, one of the major factors limiting the growth of aquaculture production in many developing countries (FAO 2016), often amounts to more than 60 percent of aquaculture production costs (Chong 1993). Although most aquaculture is of species that feed low on the food chain where no pelleted feed is essentially needed (De Silva 2000, Tacon and De Silva 1997), growth of the industry is increasingly dependent on intensification and the use of manufactured feed that often incorporates fishmeal in the formulation. Manufactured commercial feed tends to be expensive, especially in countries that must import it or at least import the main ingredients to manufacture it. Therefore, sustainable expansion of the aquaculture industry to produce affordable and healthy protein in developing nations will depend in part on replacing or supplementing expensive commercial feeds with locally sourced and produced feeds. Preferably, these feeds would be nutritious, digestible, less expensive than fishmeal based commercial feeds, more sustainable and have a smaller environmental footprint.

Recent research about novel aquaculture feeds focused mainly on replacing fishmeal with plant based by-products mainly soybean meal because of its protein content, availability and affordability (Fontainhas-Fernandes *et al.* 1999, Robinson and Li 1994). However, soybean meal supply faces a lot of competitive demand for both human and livestock food, which may contribute to future increases in price and limit availability for fish farmers and aquafeed producers (Fasakin *et al.* 1999). Furthermore, many plant-protein sources contain growth inhibitors and anti-nutritional factors and lack some essential amino acids

(El-Sayed 2004, Hasan 2000, Monzer *et al.* 2017, Wu *et al.* 1999). Other aquafeed research tended to focus on using non-traditional protein sources such as plant seeds, leaves, and agro-industrial byproducts in aquafeed production (Ali *et al.* 2003, Balogun and Fagbenro 1995, El-Sayed 2004, Lima *et al.* 2012, Metwally and El-Gellal 2009, Nguyen *et al.* 2009, Richter *et al.* 2003). This often decreases costs of aquafeed production, decreases the pressure on unsustainable natural resources, and maintains stability of aquafeed production. A neglected source of inexpensive and readily available nutrients is recycled human food leftovers that could be used to partially replace traditionally used raw materials in aquaculture diets (Al Ruqaie 2007, Bake *et al.* 2009, Cheng *et al.* 2014, Cheng *et al.* 2015, Choi *et al.* 2016, Mo *et al.* 2015). Restaurant food wastes generally consist of food ingredients suitable for human consumption and should not be problematic for omnivorous fish species such as tilapia.

Tilapia, *Oreochromis niloticus*, is one of the most intensively aquacultured fresh water finfishes (FAO 2016). Their ability to tolerate stressors in aquaculture systems such as high stocking density and wide ranges of water quality parameters, breed easily, and grow fast, made them excellent candidates for global aquaculture. Tilapia are omnivorous, feeding on a range of foods varying from zooplankton to commercial fish feeds (Gonzales *et al.* 2007, Olaosebikan and Raji 1998, Sala and Ballesteros 1997) and are thus excellent candidates to be offered unconsumed human food. However, growth of tilapia aquaculture is predicted to be in equatorial developing countries where access to commercial feeds is difficult and expensive. Local feed production is rare and thus locally produced post-consumer food waste cannot easily be incorporated into imported commercial fish feed in the areas where it is most needed.

The present work evaluates the use of post-consumer food waste (plate food waste) as a supplement or partial substitute for commercial fish feed in

tilapia aquaculture. Instead of incorporating the food waste into the feed, we decided to investigate whether locally produced feed using post-consumer food waste could be substituted for commercial fish meal-based diets on a temporal regimen. Accordingly, we performed two experiments to assess the effects of partial replacement of commercial fish feed with locally produced waste-based feed on survival, growth, feed conversion, hematology and proximate analysis of Nile tilapia, *Oreochromis niloticus*, fingerlings.

B. Materials and Methods

1. Waste collection and processing

Plate food waste (post-consumer waste) was collected daily over a period of 5 weeks between the months June and July 2016 from two local Mediterranean/Lebanese food restaurants in Beirut, Lebanon. Every day after collection, the food waste was manually inspected to remove any non-organics then ground and dried at 70 ^{o}C in a forced-air oven until moisture content of 6 ± 1%. The dried waste was milled to an average particle size of 50 µm and stored in vacuum bags at room temperature. Before starting the fish feeding experiments, an equal portion from each of the stored samples was taken and all feeds mixed together. Samples from the blend were test mixed with various quantities of gelatinizing and binding ingredients such as wheat flour and cellulose (Vitacel R 200® J. Rettenmaier & Söhne GmbH & Co. KG, Rosenberg, Germany) to determine optimal composition for water stability when used as fish feeds. The mix with a 4:1 (dried food waste: cellulose) ratio had good water stability for at least 10 min and was used in the present experiment. Dried-milled food waste, warm water and cellulose at the prescribed ratio were mixed in a dough mixer and extruded in a fish feed extruder (Model BD-GP50, Henan Bedo Machinery Equipment Co., Ltd). Pellets were then dried in a forced-air oven at 40°C to a

moisture content of 8 % and stored at -20°C. The commercial diet used was a 36 % protein, 6 % lipid commercial tilapia diet (Rangen Inc., Buhl, Idaho, USA). The commercial feed and the waste-based feed were ground to equal sizes of approximately 3 mm crumbles.

2. Proximate composition

Moisture, ash, total nitrogen, crude protein, lipid, fiber, carbohydrate, energy, carbohydrate to lipid ratio, protein to energy ratio, as well as protein solubility were determined for both fish diets. Moisture content was measured as the weight difference between moist and dry samples after placing in an oven at 105°C until constant weight. Ash content was determined by combusting the samples in a muffle furnace at 500°C for 9h (AOAC 2000). Feed samples were analyzed for protein content using a nitrogen analyzer (Thermo Finnigan/ FlashEA® 1112 elemental analyzer) with aspartic acid as the calibration standard. Crude protein was calculated by multiplying total nitrogen by 6.25 (Albanese 2012). Protein solubility was determined as described by Araba and Dale (1990). Briefly, a sample (1.5 g) was placed in 75 mL of 0.2% (w/v) KOH solution for 20 min at room temperature. The sample was then centrifuged for 15 min at 2,700 rpm and the supernatant analyzed for protein content using the Kjeldahl method. Protein solubility, expressed as a proportion of the total, was calculated by dividing the protein content of the KOH-extracted solution by the protein content of the original sample. Lipid content was quantified using a reflux extractor ANKOM XT ^{15} Fat Analyzer (ANKOM Technology Corporation, Macedon, NY, USA) and a solvent mixture of petroleum ether: diethyl ether (9:1) (AOCS 2005). Crude fiber was determined using ANKOM ^{200} fiber analyzer (ANKOM Technology Corporation, Macedon, NY, USA). The nitrogen free extract of the diets was determined according to Castell and Tiews (1980) by subtracting the

sum of crude protein, crude lipid, moisture, ash and fiber from total content. Gross energy content of the feed samples was measured using a Parr Model 6200 bomb calorimeter (Parr Instruments Co., Moline, IL, USA). Digestible energy (DE) content was calculated from standard physiological fuel values as 16.72, 17.62 and 37.62 KJ/g for protein, carbohydrate and lipid, respectively (Garling and Wilson 1976).

3. Fish growth

The studies were performed at the aquaculture research laboratory of the American University of Beirut (AUB), Lebanon. Institutional animal care approval was obtained prior to commencing the experiment. Nile tilapia, *Oreochromis niloticus* fingerlings spawned at the lab were used in experiments 1 and 2. Swim-up fry were collected from $1m^3$ holding tanks, stocked into 52-L aquaria of an indoor recirculating system and sex reversed using methyl testosterone treated Tilapia starter feed (Rangen Inc., Buhl, Idaho, USA). The fish were then offered a 36% protein and 6% crude lipid commercial feed (Rangen Inc., Buhl, Idaho, USA) twice daily until the beginning of the experiments.

Water was aerated using a regenerative blower and submerged air diffusers. Temperature was maintained at 27 ± 1 °C using submerged heating elements. Measurements of temperature and dissolved oxygen were performed daily using a handheld YSI Model 85 dissolved oxygen (DO) meter (YSI Inc., Yellow Springs, OH, USA). Total ammonia nitrogen (TAN), nitrite nitrogen and pH were measured weekly using a Freshwater Aquaculture Test Kit, Model FF-3 (HACH Company, Loveland, CO, USA). Photoperiod was maintained at 14:10 h (light: dark) and 30% of the water in each system was changed weekly to maintain optimal water quality. In both experiments, fish were offered feed at 5 percent of total body weight divided over 4 portions per day divided over 12 hours (7:00 h,

11:00 h, 15:00 h and 19:00 h), 6 days a week. Fish were group-weighed biweekly after a day of fasting and ration adjusted accordingly to 5 % of the average total biomass of the largest treatment. The ration was increased by 10 % on weeks when biomass was not measured. Both experiments were terminated after 8 weeks.

4. Experiment 1

The first experiment was performed in a system consisting of fifteen 180-L fiberglass tanks connected to a biological filter and settling tank. The fish were size-sorted by hand and 20 fish 2.0 g ± 0.54 and 5.0 cm ± 0.42 each (mean ± SD) were randomly stocked into each tank. Fish were offered one of five feed regimens with three replicate tanks per treatment. Commercial feed (CF) was offered always in the morning, followed by Waste Based Feed (WBF) when applicable. For example, in treatment 1 (100 % CF), the 4 portions were commercial feed. In treatment 2 (75 % CF, 25 % WBF), first 3 portions of the day were CF and the fourth was WBF. Treatments were: 1- Experiment1 treatment 1 (E1T1): 100 % commercial feed (CF); 2- Experiment 1 treatment 2 (E1T2): 75 % CF and 25 % waste-based feed (WBF); 3- E1T3: 50 % CF 50 % WBF; 4- E1T4: 25 % CF, 75 % WBF; 5- E1T5: 100 % WBF.

5. Experiment 2

The second experiment was performed in a recirculating system consisting of 21 indoor glass aquaria, 52-L (58 × 30 × 30 cm; L x W x H), connected to a biological filter and settling tank. Fish were size-sorted by hand and 23 fish 2.3 g ± 0.58 and 5.3 cm ± 0.49 each (mean ± SD) were randomly stocked into each tank. The fish were offered one of seven feed regimens with three replicate tanks per treatment as follows: 1- Experiment 2 treatment 1 (E2T1): 6 days CF; 2- Experiment 2 treatment 2 (E2T2): 5 days CF, 1 day WBF; 3- E2 T3: 4 days CF, 2

days WBF; 4- E2 T4: 3 days CF, 3 days WBF; 5- E2 T5: 2 days CF, 4 days WBF; 6- E2 T6: 1 day CF, 5 days WBF; 7- E2 T7: 6 days WBF.

6. Fish growth and performance parameters

Fish were starved for 24 h prior to harvest and data collection. The fish were anaesthetized with a MS 222 solution (Western Chemical Inc., Washington, USA), group weighed, and individual weight and length measured to calculate final Fulton's condition indices (K). Fulton's condition index (K), survival rate reported in %, feed conversion ratio (FCR) (Bake *et al.* 2009) and specific growth rate (SGR) (Hopkins 1992), were calculated according to Eqs. (1), (2), (3), and (4) respectively.

$$K = \frac{M}{L^3} \times 10^5 \quad (1)$$

where M is mass (g) and L is length (mm)

$$S = \left(\frac{No.\ of\ fish\ at\ end\ of\ experiment}{No.\ of\ fish\ at\ beginning\ of\ experiment} \right) \times 100 \quad (2)$$

$$FCR = \frac{total\ mass\ of\ feed\ provided}{final\ biomass - initial\ biomass} \quad (3)$$

$$SGR\ (\%) = \frac{[\ln \text{final weight (g)} - \ln \text{initial weight (g)}]}{\text{Feeding period (day)}} \times 100 \quad (4)$$

7. Fish hematology and proximate composition

Blood samples were collected from the caudal arch using heparinized 1 mL syringes with 20-gauge 4 cm needles. At least four samples were taken from each tank. Hematocrit (Hct) was determined by filling heparinized capillary tubes with blood and centrifuged at 10,000 x g for 5 min using a microhematocrit centrifuge. Hematocrit values were reported as % volume using a microhematocrit

reader (Morris and Davey 2001). Total plasma protein (TPP) (g/dL) was determined with a veterinary refractometer (RHC-200ATC, Westover Scientific, Inc., Mill Creek, WA, USA) using the plasma obtained from the microhematocrit tubes (Alexander and Ingram 1980). Hemoglobin (Hb) (g/dL) was determined by the Cyanmethemoglobin method using Drabkin's reagent (Sigma, St Louis, MO, USA) (Larsen and Snieszko 1961). All types of hemoglobin were first converted to methemoglobin and then to cyanmethemoglobin, which was then measured photometrically at 540 nm wave length.

The fish used for hematology were weighed prior to removing and weighing their liver and viscera. The hepatosomatic (HSI) and viscerosomatic (VSI) indices, reported as a percentage, were calculated as per Eqs. (5) and (6), respectively.

$$HSI = \frac{Liver\ Mass\ (g)}{Body\ Mass\ (g)} \times 100 \quad (5)$$

$$VSI = \frac{Viscera\ Mass\ (g)}{Body\ Mass\ (g)} \times 100 \quad (6)$$

At least 4 fish per tank were pooled, macerated and dried in an oven at 95°C to constant weight to determine moisture content. The dry samples were ground and stored for proximate analysis. Proximate analysis of samples of initial fish and "final" fish were analyzed using the procedures described previously for feeds.

8. Statistical analysis

Statistical analysis of the data was performed using SAS (V.9.2, SAS Institute Inc., Cary, NC, USA). Simple ANOVA was used to compare means of survival, growth parameters, feed conversion, hematological parameters and proximate composition of fish among treatments. Student Newman-Keuls (SNK)

means separation test was used to determine significant differences among treatment means. Significance level was set to 5%.

C. Results

Proximate compositions of the commercial and waste-based feeds used in the present work are summarized in Table 2.1. The commercial feed had almost twice the protein content of the waste-based feed (40.7% *vs.*18.9%) whilst the waste-based feed contained around four times more lipids than the commercial feed (22.2% *vs.* 5.2%). Although both feeds were nearly iso-energetic (*circa* 20 kJ/g of diet), they differed in the amount of digestible energy as well as ash and fiber contents.

1. Experiment 1

Survival was 100% in all treatments. Moreover, fish in all treatments exhibited an increase in weight and length during the experimental period. However, a significant difference in growth was observed among treatments (Table 2.2; Fig. 2.1).

Mean values of the final body weight (FBW), final length (FL), K (Fulton's condition index at harvest), FCR, SGR, HSI and VSI show significant differences among treatments ($P < 0.05$). Fish in treatments E1T1 and E1T2 grew significantly better in in both length and weight than fish in other treatments. Growth was negatively correlated with amount of waste-based feed offered in the regimen. Similar results were observed in the health indices and feed conversion. Fish in E1T5 exhibited the greatest FCR (2.30 %/d ± 0.10) and least SGR (2.20 ± 0.00), whilst HSI and VSI increased as the amount of WBF in the regimen increased (Table 2.2).

Table 2.1 Proximate composition of restaurant-waste based feed (mean ±SD) used to supplement diets of *Oreochromis niloticus* fingerlings and *Siganus rivulatus* juveniles for 8 weeks

Proximate Composition	Commercial Feed	Waste-Based Feed
Dry matter (%)	93.4 ± 0.4	93.7 ± 0.6
Ash (%)	7.5 ± 0.26	5.5 ± 0.46
Crude protein (%)	36.7 ± 0.57	18.9 ± 0.4
Lipid (%)	5.2 ± 0.33	22.2 ± 4.80
Fiber (%)	4.6 ± 0.1	15.3 ± 0.7
NFE (%) [†]	35.4	31.8
CHO:L ratio[‡]	7.6	2.1
P:E (g/MJ)[§]	2034.0	901.7
Protein solubility (%) [¶]	9.3 ± 1.70	8.3 ± 0.87
Gross energy (MJ/kg)	20.01 ± 0.16	20.96 ± 0.19
Digestible energy (MJ/kg) [‖]	14.99	17.11
CP/DE (g/MJ)[††]	27.15	11.05

[†]Nitrogen Free Extract (%) = 100 – (crude protein % + crude lipid % + moisture % + ash % + fiber %)

[‡]Carbohydrates to lipid (CHO: L) ratio = weight % in CHO: weight % in lipid

[§]Protein to energy (P: E) (g MJ^{-1}): Crude protein %: energy

[¶]Protein solubility (%) = Protein in KOH/protein in sample x 100 %

[‖]Digestible energy (16.72, 17.62 and 37.62 KJ/g for protein, carbohydrate and lipid, respectively; (Garling and Wilson 1976)

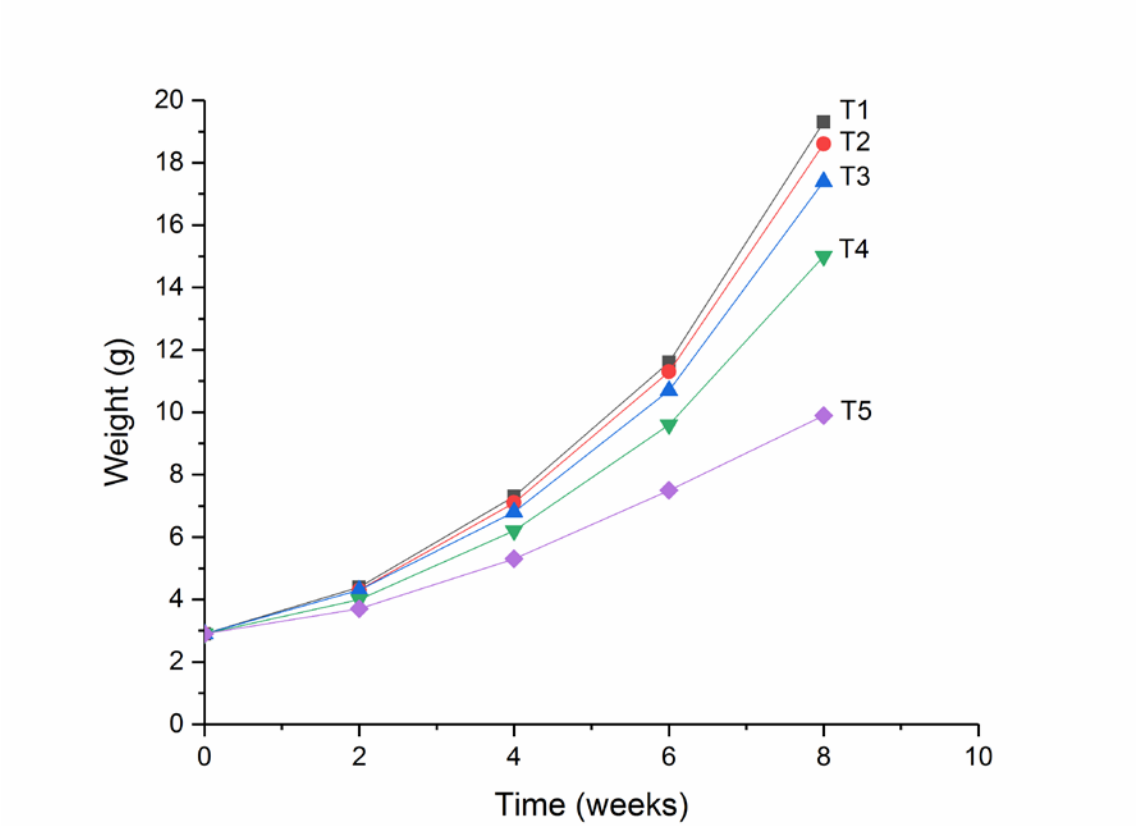
[††]Crude protein to digestible energy (National Research Council 1993)

Table 2.2 Survival (S; %), final body weight (FBW; g), final length (FL; cm), Fulton's condition index at harvest (K), feed conversion ratio (FCR), specific growth rate (SGR; % per day), and hepatosomatic (HSI; %) and viscerosomatic (VSI; %) indices of *Oreochromis niloticus* fingerlings fed with increasing proportions of waste-based feed (WBF). Values in the same column with different superscript are significantly different from each other based on SNK mean separation test ($P < 0.05$)

Treatment	S	FBW	FL	K	FCR	SGR	HSI	VSI
CF 100% (E1T1)	100^{a}	19.3^{a}	10.30^{a}	1.75^{a}	1.03^{d}	3.30^{a}	0.99^{c}	9.02^{c}
WBF 25% (E1T2)	100^{a}	18.6^{a}	10.17^{a}	1.76^{a}	$1.10^{c, d}$	3.23^{a}	1.17^{c}	10.15^{c}
WBF 50% (E1T3)	100^{a}	17.4^{b}	9.91^{b}	1.77^{a}	1.20^{c}	3.10^{b}	1.49^{b}	11.47^{b}
WBF 75% (E1T4)	100^{a}	14.9^{c}	9.30^{c}	1.83^{b}	1.37^{b}	2.93^{c}	2.05^{a}	13.80^{a}
WBF100% (E1T5)	100^{a}	9.9^{d}	7.92^{d}	1.94^{c}	2.30^{a}	2.20^{d}	2.30^{a}	15.06^{a}
PSE*	-	0.40	0.08	0.02	0.03	0.03	0.001	0.005

*PSE: Pooled standard error

Figure 2.1 Growth in average individual body weight (g) of juvenile *Oreochromis niloticus* fed with increasing % of waste-based feed (WBF) over 8 weeks; T1: 100% commercial feed (CF); T2: 75% CF and 25% waste-based feed (WBF); T3: 50% CF 50% WBF; T4: 25% CF, 75% WBF; and T5: 100% WBF



There were no significant differences among treatments in hemoglobin and hematocrit (Table 2.3). Mean hemoglobin values ranged between 13.07 and 14.84 g/dL whilst Hct varied between 22.30 and 25.53 %. Total plasma protein values were significantly different among treatments with E1T5 having the greatest mean (9.76 ± 1.62 g/dL). Whole-body proximate compositions reported as a proportion of dry weight of fish are also summarized in Table 2.3. Both protein content and lipid proportion varied significantly as treatments changed. As the proportion of waste-based feed offered to the fish increased, protein fraction of fish tissue decreased significantly, and lipid proportions increased significantly. Both moisture and ash content of the fish decreased as the proportion of WBF in the diet increased.

2. Experiment 2

All fish in all treatments survived for the duration of the experiment. However, growth varied significantly with treatment (Table 2.4; Fig. 2.2). Mean final body weight (FBW), final length (FL), K (Fulton's condition index at harvest), FCR, SGR, HSI and VSI were significantly different among treatments ($P < 0.05$). Both final weight and final length of the fish decreased as the proportion of waste-based feed offered to the fish increased. Growth in treatment E2T2 were similar to treatment E2T1 but fish growth started decreasing as WBF in the regimen increased beyond 1 day a week. When fish were offered only WBF (treatment E2T7) they had the smallest FBW (7.8 ± 2.52 g) and FL (7.48 ± 0.77 cm) of all treatments and the greatest FCR (2.20 ± 0.10) (Table 2.4). Feed conversion and HSI and VSI also changed significantly when WBF offered to the fish increased beyond two days out of six. HSI increased as WBF in the regimen increased but then decreased significantly when the fish were only offered WBF with no commercial feed supplementation (Table 2.4).

Table 2.3 Hemoglobin (Hb; g/dL), hematocrit (Hct; %), total plasma protein (TPP; g/dL), and proximate content (moisture, ash content, protein, and lipid) as percent of dry matter of *Oreochromis niloticus* fingerlings fed with increasing dietary proportions of waste-based feed (WBF). Values in the same column with different superscript are significantly different from each other based on SNK mean separation test ($P < 0.05$)

Treatment	Hb	Hct	TPP	Moisture	Ash	Protein	Lipid
CF 100% (E1T1)	13.45^{a}	23.54^{a}	3.64^{c}	75.97^{a}	15.34^{a}	68.95^{a}	20.30^{e}
WBF 25% (E1T2)	14.00^{a}	23.62^{a}	3.61^{c}	73.85^{b}	14.07^{a}	63.26^{b}	25.12^{d}
WBF 50% (E1T3)	13.07^{a}	25.53^{a}	3.87^{c}	70.87^{c}	12.53^{b}	55.71^{c}	31.52^{c}
WBF 75% (E1T4)	14.23^{a}	22.30^{a}	5.16^{b}	67.53^{d}	10.71^{c}	48.77^{d}	38.79^{b}
WBF 100% (E1T5)	14.84^{a}	24.04^{a}	9.76^{a}	64.22^{e}	9.08^{d}	44.05^{e}	41.97^{a}
PSE*	0.53	0.65	0.24	0.24	0.50	0.79	0.79

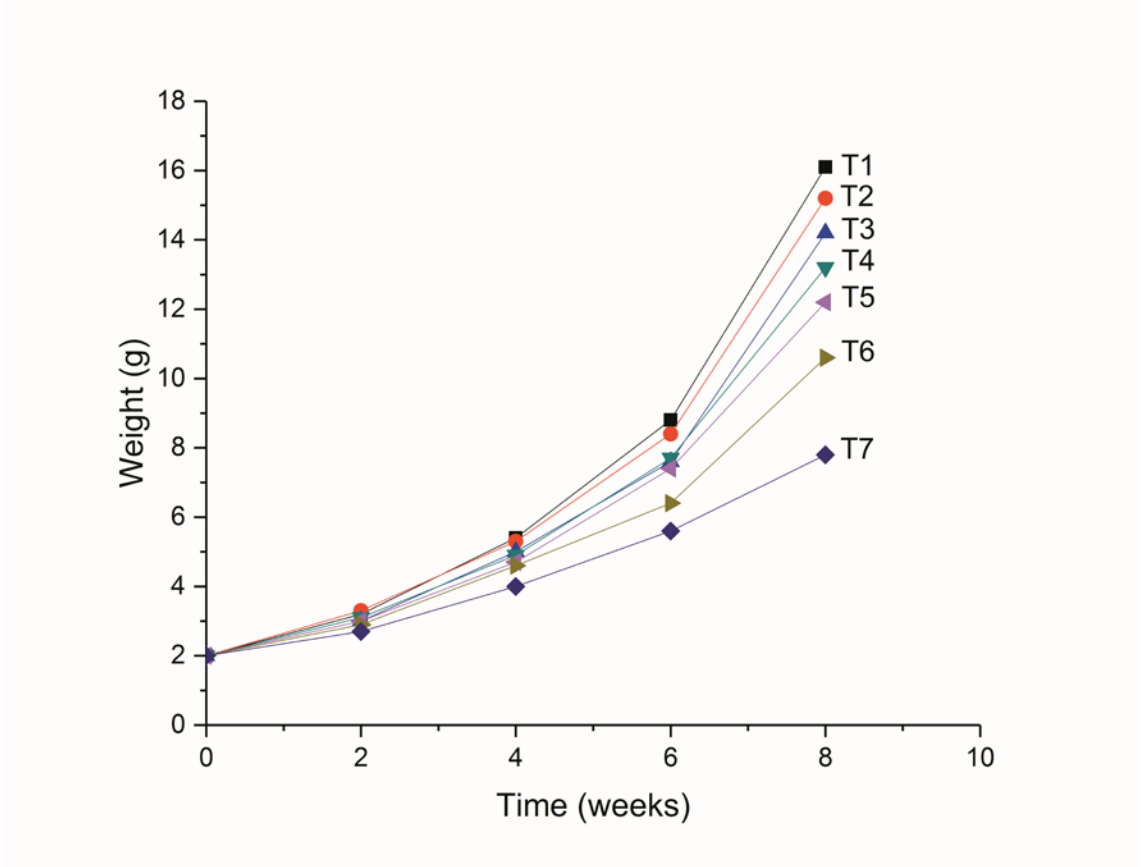
*PSE: Pooled standard error

Table 2.4 Survival (S; %), final body weight (FBW; g), final length (FL; cm), Fulton's condition index at harvest (K), feed conversion ratio (FCR), specific growth rate (SGR % per day), hepatosomatic (HSI; %) and viscerosomatic (VSI; %) indices of *Oreochromis niloticus* fingerlings offered CF or WBF by alternating daily feeding. Values in the same column with different superscript are significantly different from each other ($P < 0.05$)

Treatment CF: WBF (day: day)	S	FBW	FL	K	FCR	SGR	HSI	VSI
6:0 (E2T1)	100	16.1^{a}	9.77^{a}	$1.70^{b, c}$	0.97^{e}	3.50^{a}	0.98^{c}	10.20^{d}
5:1 (E2T2)	100	$15.2^{a, b}$	9.76^{a}	1.63^{d}	$1.03^{e, d}$	$3.40^{a, b}$	0.96^{c}	10.22^{d}
4:2 (E2T3)	100	14.2^{b}	9.46^{b}	$1.66^{d, c}$	$1.10^{e, d, c}$	$3.30^{b, c}$	1.63^{b}	$11.40^{d, c}$
3:3 (E2T4)	100	13.2^{c}	9.04^{c}	$1.75^{b, a}$	$1.17^{d, c}$	$3.23^{b, c}$	2.37^{a}	11.90^{c}
2:4 (E2T5)	100	12.2^{c}	8.79^{c}	$1.76^{b, a}$	1.20^{c}	3.17^{c}	2.14^{a}	13.08^{b}
1:5 (E2T6)	100	10.6^{d}	8.52^{d}	$1.68^{d, c}$	1.50^{b}	2.87^{d}	2.20^{a}	13.28^{b}
0:6 (E2T7)	100	7.8^{e}	7.48^{e}	1.80^{a}	2.20^{a}	2.30^{e}	1.79^{b}	15.63^{a}
PSE*	-	0.37	0.09	0.02	0.04	0.05	0.001	0.004

*PSE: Pooled standard error

Figure 2.2 Growth in average individual body weight (g) of juvenile *Oreochromis niloticus* fed by altering between CF and WBF over 8 weeks; T1: 6 days CF; T2: 5 days CF, 1 day WBF; T3: 4 days CF, 2 days WBF; T4: 3 days CF, 3 days WBF; T5: 2 days CF, 4 days WBF; T6: 1 day CF, 5 days WBF; T7: 6 days WBF



Although no significant differences were observed among hemoglobin values of fish in the various treatments (12.95 to 14.62 g/dL; Table 2.5), hematocrit and total plasma protein values were significantly different from each other among treatments with treatment E2T7 having the least Hct (20.43 ± 2.18 %) and most TPP (9.76 ± 1.63 g/dL). Whole-body proximate compositions reported as a proportion of dry weight of fish are also summarized in Table 2.5. Significant differences were observed in all proximate parameters with moisture, ash content and total proteins showing a significant decrease with increasing WBF in the regimen. Conversely, total lipids increased significantly as the proportion of WBF offered increased.

D. Discussion

Our results suggest that recycled food waste is an acceptable replacement for commercial feed for *O. niloticus* fingerlings up to 25 %. Using restaurant food waste in fish diets for eight weeks did not cause mortality or any discernible symptoms of disease. Also, fish did not reject feeds made completely from restaurant waste, suggesting that restaurant food waste was both attractive and palatable. The following discussion is written with the understanding that food waste used in the present work was sourced from two restaurants that serve primarily Lebanese food and does not represent food from all restaurants. However, preliminary work on food waste from several restaurants showed that the ingredient mix in waste was relatively similar among various restaurants and the main differences were spices, not proximate composition. Our main concern with the feed used in the present work is that it contained about 15% cellulose which was shown to affect digestibility of feed ingredients and growth of tilapia (Shiau *et al.* 1988, Amirkolaie *et al.* 2005). We believe that if a different binder is

Table 2.5 Hemoglobin (Hb; g/dL), hematocrit (Hct; %), total plasma protein (TPP; g/dL), and proximate content (moisture, ash content, protein, and lipid) as % of dry matter of *Oreochromis niloticus* fingerlings offered CF or WBF by alternating daily feeding. Treatments are CF: WBF on day: day basis. Values in the same column with different superscript are significantly different from each other based on SNK mean separation test ($P < 0.05$)

Treatment	Hb	Hct	TPP	Moisture	Ash	Protein	Lipid
6:0 (E2T1)	13.50^{a}	23.62^{a}	3.72^{d}	74.99^{a}	15.87^{a}	64.64^{a}	19.28^{f}
5:1 (E2T2)	12.95^{a}	$21.94^{a, b}$	3.75^{d}	$74.01^{a, b}$	14.92^{b}	63.61^{a}	21.83^{f}
4:2 (E2T3)	13.64^{a}	$21.96^{a, b}$	4.33^{d}	72.53^{b}	12.90^{c}	58.38^{b}	27.63^{e}
3:3 (E2T4)	13.43^{a}	21.38^{b}	5.99^{c}	68.83^{c}	12.08^{d}	52.72^{c}	31.50^{d}
2:4 (E2T5)	12.63^{a}	21.05^{b}	$6.63^{b, c}$	$69.14^{c, d}$	11.44^{e}	46.45^{d}	34.58^{c}
1:5 (E2T6)	14.62^{a}	20.95^{b}	7.27^{b}	$68.14^{c, d}$	10.93^{e}	44.62^{d}	38.35^{b}
0:6 (E2T7)	13.14^{a}	20.43^{b}	9.76^{a}	67.59^{d}	10.39^{f}	40.47^{e}	42.15^{a}
PSE*	0.65	0.54	0.23	0.56	0.19	0.76	0.90

*PSE: Pooled standard error

used, we could possibly replace more than 25% of the commercial feed with WBF.

In experiment 1, the best growth responses were obtained in tilapia in treatments E1T1 and E1T2 (no replacement & 25 % replacement). Growth parameters (weight, length, final K, FCR, and SGR) showed no differences between treatments E1T1 and E1T2 indicating that 25 % replacement of CF with WBF can be efficiently used in feeding of cultured *O. niloticus* fingerlings. Similarly, in experiment 2, the best growth responses were obtained in tilapia in treatments E2T1 and E2T2 (6:0 & 5:1 days; CF: WBF). Growth parameters (weight, length, FCR, and SGR) showed no differences between treatments E2T1 and E2T2 indicating that replacing WBF with 1 out of 6 CF feeding days which corresponds to 16.7 % replacement can be efficiently used as a feeding regimen for cultured *O. niloticus* fingerlings. However, the data also suggests that the inflection point is somewhere between a one-day-a-week offering of WBF and a two-day-a-week offering of WBF which proposes that WBF could be offered more than one day out of six but less than two. This corroborates results of experiment 1 suggesting that a 25% replacement of commercial feed by waste-based feed does not reduce fish growth.

Treatments E1T5 and E2T7 showed the least growth over the experimental period indicating that WBF alone is not suitable for complete replacement of commercial feed for growing *O. niloticus* fingerlings. Typically, fish have specific nutritional and energy requirements for survival, healthy growth and metabolism. Nutritional requirements of *O. niloticus* fingerlings range between 30 and 35 % for protein depending on the protein source (Abdelghany 2000, De Silva and Radampola 1990, Wang *et al.* 1985), a minimum of 5 % lipid with an optimal range between 10 and 15 % combined with a protein to energy ratio (P/E) of 26.3

to 28.7 g/MJ in order to ensure enhanced growth and protein utilization efficiency (Shiau 2002, Fitzsimmons 2005, Webster and Lim 2006). The diet should provide adequate non-protein energy for survival and normal metabolism, or else, the energy needed will be provided by proteins at the expense of growth (National Research Council 1993). Although the diets used in both experiments (CF and WBF) had similar gross energy content, they differed significantly in protein and lipid content in addition to digestible energy (14.99 MJ/kg and 17.11 MJ/kg for CF and WBF, respectively). Although sufficient amounts of digestible energy were available to all experimental fish, the high lipid content of the waste-based feed could have affected the P/E ratio which could have influenced growth in both experiments.

Growth rate of *O. niloticus* decreased as substitution of commercial feed with waste-based feed increased. This could be attributed to a decrease in total protein offered to the fish or to a deficiency in essential amino acids, fatty acids, vitamins and/or minerals. However, we believe that the fish offered only commercial feed were getting more than their protein requirement for growth and the waste-based feed worked as a sparing supplement where expensive and essential amino acids were replaced by amino acids from waste. Growth could also have been affected by high lipid content of WBF. Du *et al.* (2005) suggested that an increase in lipid content beyond recommended ranges could adversely affect growth. In contrast to previous studies, Du *et al.* (2005) and Ayisi *et al.* (2017) suggest that protein content of the diets is more important in some fishes than the energy content as fish limit their feed intake based on the protein content and not energy. Hence, when the percentage of protein in the feed is low, the fish consume more feed up to a limit beyond which feed intake can’t be increased. In the present work, when commercial feed was replaced at more than 25 % by low

protein WBF, the diet did not supply the dietary protein requirement and provided excess lipids which affected growth. Alternatively, the significant increase in lipid to protein ratio could have affected growth as dietary increase in lipid content tends to affect the ability of fish to digest food and absorb nutrients as well as affect normal metabolic processes (Luo *et al.* 2005, Wang *et al.* 2005). In both cases, it appears that high lipid content of WBF affects growth.

Hepatosomatic and viscerosomatic indices are often an index of fat deposition in the body and are quite important in the study of fish metabolism, digestion and assimilation, production and secretion of digestive enzymes and carbohydrate metabolism (Mantel and Bliss 1983). Also, an increase in dietary lipid content is associated with body lipid retention and storage. In the present work, significant lipid deposition was observed in various sites such as the visceral cavity and muscle tissues and this probably affected HSI and VSI (Luo *et al.* 2010, Ochang *et al.* 2007). In both experiments, the lipid proportion in the fish increased and protein proportion decreased as the proportion of WBF in the regimen increased. In the first experiment, treatment E1T1 had the greatest protein and least lipid content, whereas treatment E1T5 showed the least protein and largest lipid content. As for the HSI and VSI, the lowest indices were reported in E1T1 whilst the greatest were reported for E1T5. These results are like those of other studies which reported least VSI and HSI in fish fed on high crude protein diets (Kumar *et al.* 2010, Lee *et al.* 2002, Nandeesha *et al.* 2002). In the second experiment, fish in treatments E2T1 and E2T2 had the greatest protein content and least lipid content, whereas E2T7 had the least protein and most lipid content. In both experiments, the fish offered a high-lipid diet deposited excess ingested lipids into adipose tissue rather than in the liver, probably by enhancing the ability of fatty acid uptake and triglyceride synthesis as described by (He *et al.* 2015).

HSI in E2T7 was similar to that of E2T3, indicating that with complete replacement of commercial feed and consequently a large increase in dietary lipid level, Nile tilapia didn't store excess lipids in their liver but rather could have experienced liver damage because of impaired dietary homeostasis in addition to a decreased growth rate. Storage of excess energy provided in the diet as lipid in adipose tissue is probably an evolved trait in wild fish that do not have constant access to energetic food. This would explain the increase in VSI with the increase in lipid and energy content in waste- based feed.

In experiment 1, regardless of the replacement with WBF, no significant differences in hematocrit were observed. Differences in hematocrit among treatments were observed in experiment 2 but although they were statistically significant, those differences were not great. Conversely, there were no significant differences in hemoglobin content of blood among treatments in both experiments. Hemoglobin and Hct are used to screen for nutritional deficiencies, specifically in micronutrients such as iron (Clauss *et al.* 2008). Present results suggest that enough iron and necessary proteins were being supplied by the WBF to maintain Hb levels, even if not sufficient for rapid growth.

Total plasma proteins showed a significant increase with increased replacement of CF by WBF in Nile tilapia fingerlings. Most plasma proteins are totally or partially formed hepatically (Coz-Rakovac *et al.* 2005, Coz-Rakovac *et al.* 2008) and the main fraction of fish blood protein is not albumin as in mammals but rather alpha (α) and beta (β) globulins that function as carriers of lipids in the blood (Larsson *et al.* 1976). Accordingly, total plasma protein is correlated with the amount of free fatty acids and cholesterol found in fish blood (Larsson *et al.* 1976). Hence, the increase in lipid proportion in the experimental fish with

increasing replacement of CF by WBF explains the increase in total plasma proteins since plasma proteins in fish act as lipid transporters in the blood.

E. Conclusion

In conclusion, replacement up to 25 % of commercial feed by our WBF didn't have negative effects on growth performance for *O. niloticus* fingerlings, as well as their proximate composition and hematological and biochemical indices. Analysis of amino acid and fatty acid profiles would help in better formulating feeds using restaurant waste. In any case, restaurant waste should be seriously considered a sustainable renewable source of raw material for aquafeed production that can replace other expensive, relatively unsustainable dietary ingredients.

CHAPTER III
EXPERIMENT TWO

Effect of using post-consumer food waste as feed on amino acid and fatty acid profiles of Nile tilapia, *Oreochromis niloticus*

A. Introduction

Aquaculture is the fastest growing sector of animal production with an estimated 5.8% annual growth between 2005 and 2014 (FAO 2016). It is an active industry and a major source of rationally priced, high quality protein and healthy lipids (omega-3 long chain polyunsaturated fatty acids). Aquafeed, being amongst the most pricey animal feed on the market because of the incorporation of huge amounts of expensive ingredients, is one of the major factors limiting the growth of aquaculture production in many developing countries (FAO 2016, Miles and Chapman 2007). Growth of the industry is also increasingly dependent on manufactured feed containing fishmeal and fish oil or oil grain meals, which either are unsustainable ingredients or needed for alternate animal husbandry. Demand for fishmeal is increasing (FAO 2016) but supply is limited and erratic (Yones *et al.* 2013), and soybean meal is becoming expensive as demand for poultry and terrestrial livestock production increases.

Traditionally, research to develop sustainable aquafeeds attempted to replace fishmeal with plant-based products such as soybean. However, the ingredients evaluated tended to be in demand for human and livestock consumption, which are causing price increases and limiting the availability for fish farmers and aquafeed producers (Fasakin *et al.* 1999). Furthermore, many plant-protein sources contain growth inhibitors and anti-nutritional components and lack some essential amino acids (EAAs) and fatty acids (El-Sayed 2004, Hasan 2000, Monzer *et al.* 2017). A neglected but readily available and inexpensive source of nutrients is recycled human food waste that could be used

to partially replace traditional aquafeeds (Bake *et al.* 2009, Cheng *et al.* 2015, Choi *et al.* 2016, Mo *et al.* 2015). Food waste generally consists of food ingredients suitable for human consumption and should not be problematic for omnivorous fish species such as Tilapia. However, the efficiency of a fish diet depends on providing the nutritional requirements of the species such as dietary protein and lipid content in addition to proper amino acid and fatty acid compositions. Moreover, amino acid and fatty acid profiles of fish body tissue typically resemble their dietary uptakes (Sargent *et al.* 2002, Wilson 2002) and thus the nutritional value of a fish changes with fish diet. In other words, fish are what they eat (Saoud *et al.* 2008a). Because a fish body profile tends to resemble that of its feed composition, farmers can modify diets to affect sensory and nutritional quality of aquacultured fish (Campos *et al.* 2006). Wild fish tissue typically contains important amounts of all essential amino acids (Thilsted *et al.* 2014) but aquacultured fish needs to be offered proper diets if their tissue is to have a healthy nutritional profile.

Lipids also play an important role in fish and human nutrition, serving as both a major energy source and providing essential fatty acids mainly ω3 PUFAs (EPA and DHA), and arachidonic acid (Sargent *et al.* 1999). These fatty acids are as essential for humans as for fish and are mainly supplied in the diet (Calder and Yaqoob 2009). Accordingly, aquaculture should strive to provide proper nutrition to fish to produce fish with good nutritional value for human consumers.

Tilapia, *Oreochromis niloticus*, is one of the most intensively aquacultured fresh water finfishes worldwide (FAO 2016) and production is expected to grow fast in tropical developing nations where access to commercial feeds is difficult and expensive. Accordingly, alternative, sustainable, locally available and nutritious feed ingredients need to be developed. A good candidate that meets these requirements is post-consumer food waste that can be

incorporated into commercial fish feed formulations or used to supplement expensive commercial fish feeds.

The present work evaluated the effect of using restaurant post-consumer food waste (plate food waste) as a partial substitute for commercial fish feed on production and amino acid and fatty acid profiles of Nile tilapia.

B. Materials and Methods

1. Waste collection, feed processing, and experimental diets

Plate food waste (post-consumer waste) was collected daily over a period of 5 weeks between the months June and July 2017 from two local Mediterranean food restaurants in Beirut, Lebanon. The food waste was manually inspected to remove any non-organics then ground and dried at 70 ^{o}C in a forced-air oven until moisture content of 6 ± 1%. The dried waste was milled to an average particle size of 50 µm and mixed with cellulose (Vitacel R 200® J. Rettenmaier & Söhne GmbH & Co. KG, Rosenberg, Germany) at 4:1 (dried food waste: cellulose) ratio and warm water and then mixed in a dough mixer and extruded in a fish feed extruder (Model BD-GP50, Henan Bedo Machinery Equipment Co., Ltd, China). Extruded pellets were dried in a forced-air oven at 40°C to a moisture content of 8 % and stored at -20°C. A commercial diet used as a control contained 35 g crude protein/ 100 g feed, and 5 g crude lipid/ 100 g feed (Rangen tilapia diet; Rangen Inc., Buhl, Idaho, USA). The commercial feed (CF) and the waste-based feed (WBF) were ground and sieved to equal sizes of approximately 3mm crumbles.

2. Composition of experimental diets

Moisture, ash, total nitrogen, crude protein, lipid, fiber, carbohydrate, energy, carbohydrate to lipid ratio, protein to energy ratio, as well as protein solubility were determined for both fish diets and reported in Table 2.1. Moisture

content was measured as the weight difference between moist and dry samples after placing in an oven at 105°C until constant weight. Ash content was determined by combusting the samples in a muffle furnace at 500°C for 9h (AOAC 2000). Feeds were analyzed for protein content using a nitrogen analyzer (Thermo Finnigan/ FlashEA® 1112 elemental analyzer) with aspartic acid as the calibration standard. Crude protein was calculated by multiplying total nitrogen by 6.25 (Albanese and Orto 2012). Protein solubility was determined according to Araba and Dale (1990) . Briefly, a 1.5 g sample was placed in 75 mL of 0.2% (w/v) KOH solution for 20 min at room temperature. The sample was then centrifuged for 15 minutes at 2,700 rpm and the supernatant analyzed for protein content using the Kjeldahl method. Protein solubility, expressed as a proportion of the total, was calculated by dividing the protein content of the KOH-extracted solution by the protein content of the original sample. Lipid content was quantified using a reflux extractor ANKOM XT ^{15} Fat Analyzer (ANKOM Technology Corporation, Macedon, NY, USA) and a solvent mixture of petroleum ether: diethyl ether (9:1) (AOCS 2005). Crude fiber was determined using ANKOM 200 fiber analyzer (ANKOM Technology Corporation, Macedon, NY, USA). The nitrogen free extract of the diets was determined according to Castell and Tiews (1980) by subtracting the sum of crude protein, crude lipid, moisture, ash and fiber from total content. Gross energy content of the feed samples was measured using a Parr Model 6200 bomb calorimeter (Parr Instruments Co., Moline, IL, USA). Digestible energy (DE) content was calculated from standard physiological fuel values as 16.72, 17.62 and 37.62 KJ/g for protein, carbohydrate and lipid, respectively (Garling and Wilson 1976).

Amino acid composition of the experimental diets was determined by liquid chromatography, using a cationic exchange resin column and ninhydrin

post-column derivation (Amino Acid Analyzer SYKAM: S4300/S2100/S5200) using a standard solution of amino acids as reference (Amino Acids Mix Solution; Prod. No. 79248; Sigma Aldrich). Samples were hydrolyzed with diluted HCl (6N) to extract and quantify free amino acids according to directive 98/64/EC of the European Community (European Union 1998). The amino acids were separated by ion exchange chromatography and determined by reaction with ninhydrin and photometric detection at 570 nm (440 nm for proline). For cyst(e)ine and methionine, samples were oxidized to cysteic acid and methionine sulphone, respectively, prior to hydrolysis. Tyrosine was determined in hydrolysates of unoxidized samples.

Lipid samples used for fatty acid analysis were extracted based on the method of Folch *et al.* (1957) using a 2:1 chloroform/methanol mixture (v/v). Fatty acids were trans-methylated using methanolic-BF3 after fat saponification then extracted using hexane (Morrison and Smith 1964). FAME analysis was performed on a Shimadzu GC-MS QP2010 Plus Ultra Gas Chromatograph Mass Spectrometer (Shimadzu, Kyoto, Japan), equipped with a 30-m DB-5MS capillary column (0.25 mm id, 0.25 µm film thickness; Agilent Technologies, Santa Clara, CA) using Helium as a carrier gas, with carrier gas flow rate to the detector at 1.20 mL/min. The injector temperature was set to 280 °C. GC temperature profile was as follows: initial temperature set at 70 °C, held for 2 min, then increased at the rate of 15 °C/min up to 190 °C and held for 1 min (ramp 1), then increased at 3 °C/min up to 210 °C and held for 8 min (ramp 2), then increased at 10 °C/min up to 270 °C and held for 2 min (ramp 3), and finally increased at the rate of 2 °C/min up to 310 °C and held for 3 min (ramp 4). Selective ion monitoring (SIM) was employed for enhanced sensitivity. Individual FAMEs were identified and quantified by comparing to results from a fatty acid methyl ester standard (FAME;

Part. No. CRM47885; Lot No. XA18271V; Supelco 37 Component FAME mix, Bellefonte, PA, USA).

3. Experimental fish and feeding trial

The work was performed at the aquaculture research laboratory of the American University of Beirut (AUB), Lebanon. Nile tilapia *Oreochromis niloticus* fingerlings spawned at the lab were used in the experiment. Swim-up fry were caught from $1m^3$ holding tanks, stocked into 52-L aquaria in an indoor recirculating system and sex reversed using methyl testosterone treated Tilapia starter feed (Rangen Inc., Buhl, Idaho, USA).

The experiment was performed in a recirculating system consisting of fifteen 180-L fiberglass tanks connected to a biological filter and settling tank. Water was aerated using a regenerative blower and submerged air diffusers. Temperature was maintained at 27 ± 1 °C using submerged heating elements. Measurements of temperature and dissolved oxygen were performed daily using a handheld YSI Model 85 dissolved oxygen (DO) meter (YSI Inc., Yellow Springs, OH, USA). Total ammonia nitrogen (TAN), nitrite nitrogen and pH were measured weekly using a Freshwater Aquaculture Test Kit, Model FF-3 (HACH Company, Loveland, CO, USA). Photoperiod was maintained at 14:10 h (light: dark) and 30% of the water in each system was changed weekly to maintain optimal water quality. In both experiments described below, fish were offered feed at 5% body weight (BW) divided into four daily portions (7:00 h, 11:00 h, 15:00 h and 19:00 h), 6 days a week. Fish were group-weighed biweekly after a day of fasting and ration adjusted accordingly. Following the first weighing event, all tanks were offered feed at 5% BW of the tank with the greatest weight.

4. Experimental design

Fish were size-sorted by hand and 20 fish (2.0 g ± 0.54, 5.0 cm ± 0.42; mean ± SD) were randomly stocked into each tank. Fish were offered one of five feed regimens with three replicate tanks per treatment. Commercial feed (CF) was offered always in the morning, followed by Waste Based Feed (WBF) when applicable. For example, in treatment 1 (100 % CF), the 4 portions were commercial feed. In treatment 2 (75 % CF, 25 % WBF), first 3 portions were CF and the fourth was WBF. Treatments were: 1- T1: 100 % commercial feed (CF); 2- T2: 75 % CF and 25 % waste-based feed (WBF); 3- T3: 50 % CF 50 % WBF; 4- E1T4: 25 % CF, 75 % WBF; 5- T5: 100 % WBF.

5. Fish growth parameters, proximate composition and body profiles

At termination, fish were fasted for 24 hours prior to data collection. The fish were anaesthetized with MS 222 (Western Chemical Inc., Washington, USA), group weighed, and individual weight and length measured. At least 4 fish per tank were pooled, macerated and dried in an oven at 95°C for 24 hours. The dry samples were ground and stored for proximate analysis at −20°C. Proximate analysis of samples in addition to amino acid and fatty acid profiling of “initial” fish and “final” fish were analyzed using the procedures described previously for the feeds. All fish samples were analyzed for amino acid and fatty acid composition in duplicate.

6. Statistical analysis

Statistical analysis of the data was performed using SAS (V.9.2, SAS Institute Inc., Cary, NC, USA). Simple ANOVA was used to compare means of amino acid and fatty acid profiles of fish among treatments. Student Newman-Keuls (SNK) means separation test was used to determine significant differences among treatment means. Significance level was set to 5%.

C. Results

Survival and growth results for the present work are reported in Table 2.2. The results showed that 25% of the commercial feed could be substituted with restaurant waste feed without affecting survival and growth. The protein proportion of whole body of Nile tilapia decreased with an increase in the waste-based proportion of the regimen (Table 2.3). Fatty acid composition of Nile tilapia fingerlings showed a significant increase in SFAs and MUFAs and a significant decrease in ω-3 and ω-6 PUFA with the increase in WBF proportions.

Proximate compositions of the experimental diets showed that the commercial feed had almost twice the protein content of the waste-based feed, 40.7 *vs.* 18.9 %, respectively. Conversely, waste-based feed contained around four times more lipids than the commercial feed, 22.2 *vs.* 5.2 %, respectively. Both feeds were iso-energetic (20 kJ per g of diet) but differed in the amount of digestible energy (14.99 MJ/kg and 17.11 MJ/kg for CF and WBF, respectively) (Table 2.1). In addition, as the proportion of waste-based feed offered to the fish increased, protein content of fish tissue decreased significantly, and lipid content increased significantly (Table 2.3).

Both essential and nonessential amino acid profiles differed significantly between diets ($P < 0.05$; Table 3.1). Glutamic acid and aspartic acid were the most abundant amino acids with 5.90 ± 0.14 and 3.59 ± 0.17 %w/w in CF and 2.75 ± 0.32 and 1.49 ± 0.17 %w/w in WBF, respectively. Methionine and cysteic acid were the least abundant with 0.53 ± 0.09 and 0.75 ± 0.08 %w/w in CF and 0.30 ± 0.04 and 0.21± 0.06 % w/w in WBF, respectively.

The proportion of amino acids in whole body composition of Nile tilapia fingerlings decreased with an increase in waste-based feed in the regimen, except for Isoleucine that remained constant in all treatments (Table 3.2). Glutamic acid, aspartic acid, and glycine had the largest proportions amongst all measured amino

Table 3.1 Digestible amino acid composition of experimental diets (% w/w of diet) (mean ± SE)

Amino Acid (%w/w)	Commercial Feed (CF)	Waste Based Feed (WBF)	*P- value*
Essential			
Arginine	2.29 ± 0.149	1.03 ± 0.130	0.0031
Histidine	1.27 ± 0.070	0.55 ± 0.055	0.0013
Isoleucine	1.20 ± 0.035	0.51 ± 0.038	0.0002
Leucine	2.63 ± 0.041	1.08 ± 0.095	0.0001
Lysine	1.99 ± 0.027	0.89 ± 0.059	<0.0001
Methionine	0.53 ± 0.052	0.30 ± 0.021	0.0139
Tyrosine	0.94 ± 0.032	0.40 ± 0.009	<0.0001
Threonine	1.32 ± 0.033	0.59 ± 0.040	0.0001
Valine	1.54 ± 0.032	0.64 ± 0.038	<0.0001
Nonessential			
Cysteic Acid	0.75 ± 0.048	0.21 ± 0.032	0.0007
Aspartic Acid	3.59 ± 0.100	1.49 ± 0.098	0.0001
Serine	1.83 ± 0.038	0.66 ± 0.058	<0.0001
Glutamic Acid	5.90 ± 0.083	2.75 ± 0.185	<0.0001
Proline	1.77 ± 0.070	0.83 ± 0.110	0.0046
Glycine	1.77 ± 0.030	0.89 ± 0.072	0.0004
Alanine	1.74 ± 0.049	0.85 ± 0.081	0.0007
Phenylalanine	1.54 ± 0.095	0.60 ± 0.049	0.0009
Cysteine + Cystine	0.82 ± 0.052	0.30 ± 0.076	0.0052

Table 3.2 Amino acid composition of *Oreochromis niloticus* fingerlings fed increasing dietary proportions of waste-based feed (WBF). Values in the same row with different superscripts are significantly different from each other based on SNK mean separation test ($P < 0.05$)

Amino Acid (%w/w)	Treatment					
	CF 100% (T1)	WBF 25% (T2)	WBF 50% (T3)	WBF 75% (T4)	WBF 100% (T5)	PSE*
Essential						
Arginine	3.49[a]	3.05[a, b]	2.60[b, c]	2.04[c, d]	1.85[d]	0.16
Histidine	2.11[a]	1.84[b]	1.56[c]	1.40[c]	1.11[d]	0.05
Isoleucine	1.89[a]	1.58[a]	1.59[a]	1.36[a]	1.10[a]	0.16
Leucine	3.86[a]	3.47[a, b]	3.10[b, c]	2.72[c]	2.25[d]	0.13
Lysine	3.84[a]	3.41[a, b]	3.06[b, c]	2.57[c]	1.92[d]	0.16
Methionine	1.34[a]	1.22[a, b]	1.06[b, c]	0.93[c]	0.66[d]	0.04
Tyrosine	1.36[a]	1.25[a]	1.12[a, b]	0.93[b, c]	0.79[c]	0.06
Threonine	2.42[a]	2.19[a]	1.90[b]	1.69[b]	1.38[c]	0.06
Valine	2.28[a]	2.01[a, b]	1.94[a, b]	1.67[a, b]	1.38[b]	0.14
Nonessential						
Cysteic Acid	0.62[a]	0.52[a, b]	0.40[b, c]	0.46[b, c]	0.31[c]	0.03
Aspartic Acid	5.58[a]	4.93[b]	4.27[c]	3.84[c]	3.15[d]	0.12
Serine	2.43[a]	2.23[a]	1.87[b]	1.67[b]	1.42[c]	0.06
Glutamic Acid	8.08[a]	7.35[a]	6.38[b]	5.66[b, c]	4.73[c]	0.26
Proline	3.58[a]	3.17[a, b]	2.48[b, c]	2.47[b, c]	1.95[c]	0.18
Glycine	5.46[a]	4.76[b]	4.15[b, c]	3.77[c, d]	3.18[d]	0.19

Alanine	4.14[a]	3.62[b]	3.16[c]	2.81[c]	2.32[d]	0.11
Phenylalanine	2.04[a]	1.84[a, b]	1.69[a, b]	1.47[b, c]	1.25[c]	0.08
Cysteine + Cystine	0.44[a]	0.37[a, b]	0.29[b, c]	0.32[b, c]	0.22[c]	0.02

*PSE: Pooled standard error

acids ranging between 4.73-8.08 %, 3.15-5.58 %, and 3.18-5.46 %, respectively. Amongst the EAAs, leucine, lysine, and arginine had the largest proportions whilst methionine and tyrosine had the least proportions with significant differences amongst treatments.

Fatty acid profiles showed no significant differences between the proportion of total saturated fatty acids (SFAs) in CF (27.14 %) and WBF (30.92 %) with palmitic acid (16:0) being the dominant one in both diets (Table 3.3). Mono-unsaturated fatty acid (MUFA) proportions were significantly different between diets with oleic acid (18:1) comprising 23.09 and 37.88 % of total fatty acids in CF and WBF, respectively. Polyunsaturated fatty acids (PUFAs) including all ω-6 and the 2 major ω-3 (EPA and DHA) fatty acids accounted for 44.82 and 30.12 % of total fatty acids in CF and WBF, respectively. The dominant ω-6 PUFA in both diets was linoleic acid (LA; 18:2n-6) but differed significantly in content between the two. The second most abundant ω-6 PUFA was arachidonic acid (AA; 20:4n-6). As for the ω-3 PUFAs, α-linolenic acid (ALA; 18:3n-3) constituted 3.99 % of total fatty acids, DHA (22:6n-3) (1.70 %), and EPA (20:5n-3) (1.59 %) were the major ones present in the CF. However, all these three fatty acids were only present in very small amounts in the WBF (0.02-0.76 %).

Fatty acid composition of Nile tilapia fingerlings offered increasing dietary proportions of waste-based feed showed a significant increase in SFAs and MUFAs with the increase in WBF in the regimen (Table 3.4). On the other hand, ω-3 and ω-6 fatty acids decreased significantly with the increase in dietary WBF. The major SFA was palmitic acid ranging between 18.49-24.07 % and the major MUFA was oleic acid (26.96-42.52%). As for PUFAs, linoleic acid and arachidonic acid were the dominant ω-6 fatty acids in all treatments but decreased

Table 3.3 Fatty acids profile of experimental diets (g/100 g of total FA) (mean ± SE)

Fatty acid	Commercial Feed (CF)	Waste Based Feed (WBF)	*P- value*
Pentadecanoic	0.14 ± 0.003	0.13 ± 0.007	0.0890
C15:1	0.01 ± 0.003	0.007 ± 0.003	0.2302
Palmitic	18.56 ± 0.204	15.64 ± 0.909	0.0351
Palmitoleic	2.08 ± 0.100	1.53 ± 0.080	0.0127
Margaric	0.30 ± 0.003	0.48 ± 0.042	0.0153
C17:1	0.10 ± 0.009	0.23 ± 0.018	0.0026
Stearic	3.98 ± 0.102	5.05 ± 0.25	0.0170
Oleic	23.09 ± 0.303	37.88 ± 0.377	< 0.0001
Linoleic	41.02 ± 0.606	29.88 ± 2.13	0.0075
Linolenic	3.99 ± 0.100	0.76 ± 0.152	< 0.0001
γ-linolenic	0.04 ± 0.009	0.04 ± 0.040	0.8683
Arachidic	0.17 ± 0.009	0.22 ± 0.024	0.1226
C20:1	0.69 ± 0.081	0.24 ± 0.038	0.0078
C20:2ω6	0.13 ± 0.020	0.02 ± 0.007	0.0084
C20:3ω3	0.03 ± 0.009	0.00 ± 0.00	0.0194
C20:3ω6	0.05 ± 0.015	0.04 ± 0.023	0.6575
Arachidonic	0.27 ± 0.023	0.08 ± 0.044	0.0183
EPA	1.59 ± 0.060	0.02 ± 0.02	< 0.0001
Heneicosanoic	0.02 ± 0.000	0.01 ± 0.00	< 0.0001
DHA	1.70 ± 0.065	0.04 ± 0.026	< 0.0001
Σ SFA	27.14	30.92	0.1459

Σ MUFA	23.86	38.14	< 0.0001
Σ ω-3 PUFA (EPA + DHA)	3.30	0.06	< 0.0001
Σ ω-6 PUFA	41.52	30.06	0.0084
ω3/ω6	0.080	0.002	< 0.0001

Table 3.4 Fatty acid composition (g/100 g of total FA) of *Oreochromis niloticus* fingerlings fed increasing dietary proportions of waste-based feed (WBF). Values in the same row with different superscripts are significantly different from each other based on SNK mean separation test ($P < 0.05$)

Fatty acid	Treatment					
	CF 100% (T1)	WBF 25% (T2)	WBF 50% (T3)	WBF 75% (T4)	WBF 100% (T5)	PSE*
Pentadecanoic	0.24[a]	0.18[b]	0.15[b, c]	0.13[c]	0.16[b, c]	0.009
C15:1	0.03[a]	0.02[a, b]	0.01[b]	0.02[a, b]	0.15[b]	0.002
Palmitic	22.13[a, b]	18.82[b]	18.49[b]	18.96[b]	24.07[a]	0.856
Palmitoleic	4.23[a]	3.83[a]	3.70[a]	3.60[a]	3.90[a]	0.255
Margaric	0.34[a]	0.34[a]	0.30[a]	0.29[a]	0.38[a]	0.020
C17:1	0.19[a]	0.21[a]	0.26[b]	0.25[b]	0.30[c]	0.010
Stearic	4.81[a]	4.90[a]	4.60[a]	4.78[a]	6.71[b]	0.130
Oleic	26.96[a]	33.07[b]	35.76[c]	37.50[c]	42.52[d]	0.542
Linoleic	26.29[a]	25.80[a]	24.71[a]	22.78[b]	11.60[c]	0.433
Linolenic	2.18[a]	1.52[b]	1.13[c]	0.77[d]	0.26[e]	0.039
γ-linolenic	0.93[a]	0.99[a]	0.89[a]	0.88[a]	0.29[b]	0.042
Arachidic	0.17[a]	0.15[a]	0.15[a]	0.15[a]	0.18[a]	0.007
C20:1	1.24[a, b, c]	1.08[a]	1.12[a, b]	1.34[b, c]	1.45[c]	0.048
C20:2ω6	0.93[a]	0.88[a]	0.91[a]	0.93[a]	0.51[b]	0.043
C20:3ω3	0.32[a]	0.20[b]	0.18[c]	0.12[d]	0.03[e]	0.007
C20:3ω6	1.27[a]	1.20[a]	1.21[a]	1.29[a]	0.43[b]	0.062
Arachidonic	1.85[a]	1.53[a, b]	1.28[b]	1.11[b]	0.30[c]	0.113
EPA	0.28[a]	0.13[b]	0.11[b]	0.06[b, c]	0.00[c]	0.021

Heneicosanoic	0.03[a]	0.02[a]	0.02[a]	0.02[a]	0.02[a]	0.004
DHA	3.08[a]	1.80[b]	1.21[b, c]	0.68[c, d]	0.09[d]	0.231
Σ SFA	34.59[a]	31.72[a]	31.43[a]	32.44[a]	42.50[b]	1.000
Σ MUFA	28.27[a]	34.22[b]	36.93[c]	38.91[c]	44.04[d]	0.686
Σ ω-3 PUFA (EPA + DHA)	3.35[a]	1.93[b]	1.32[b, c]	0.74[c, d]	0.09[d]	0.248
Σ ω-6 PUFA	31.31[a]	30.44[a]	29.04[a, b]	27.02[b]	13.10[c]	0.619

*PSE: Pooled standard error

significantly with the increase in WBF in the regimen. Similarly, ω-3 fatty acids EPA and DHA showed a significant decrease with increased WBF in the diet.

D. Discussion

Proximate analysis of restaurant food waste revealed major deficiencies in dietary protein content, essential amino acids and essential fatty acids. Concomitantly, body composition of Nile tilapia offered increasing proportions of waste-based feed in their regimen showed a significant decrease in tissue amino acids and essential fatty acids.

Sufficient amounts of digestible energy were available to all experimental fish. Commercial feed provided the necessary protein requirements for Nile tilapia in addition to all essential amino acids. Waste based feed, however, was significantly deficient in dietary protein, and consequently did not provide the needed amino acids especially the essential ones. Possibly, glutamine, the most abundant amino acid in the diets, was used for the synthesis of other dispensable amino acids, easing the deterioration in intestinal villi and enhancing weight gain in Tilapia fingerlings in all treatments (Wu *et al.* 1997, Da Silva *et al.* 2010). However, as the proportion of WBF offered to the fish increased beyond 25 %, dietary amino acid mix was not suitable for protein manufacture and thus we observed a significant decrease in the whole-body protein proportion in the fish.

Replacement of CF with more than 25 % WBF provided more than normal requirements of lipids to the fish. These excess lipids caused a significant increase in lipid to protein ratio that is probably the main cause of the increase in whole-body lipid proportion in the fingerlings.

Fatty acid profile of fish tissue varies according to species, diet, season, water quality conditions, reproductive stage, age, and general activity (Al Souti and Claereboudt 2014, Turchini *et al.* 2009). In tilapia, as many other fish, when

sufficient amounts of PUFAs are present in the diet or if there are enough stores in the tissue lipids of fish, the conversion of 18-C PUFAs to longer chain PUFA derivatives is typically suppressed (Olsen *et al.* 1990, Turchini *et al.* 2006, Leger *et al.* 1981). However, when the EFAs (ω-3 & ω-6) are deficient in the diet, the fish will start depleting EPA and DHA from tissue stores before starting to convert LA and ALA into PUFAs (Olsen *et al.* 1990). Studies have shown that replacing fish oil in fish feed with other lipid sources such as vegetable oils that are deficient in ω-3 PUFAs could enhance the activity of the enzymes elongase and desaturase and can increase ω-3 PUFAs such as EPA and DHA (Turchini *et al.* 2009, Francis *et al.* 2007, Yones *et al.* 2013). Nile tilapia was shown to successfully bio-convert LA and ALA into EPA and DHA when PUFAs are deficient in their diet and sufficient amounts of LA and ALA are present (Olsen *et al.* 1990, Visentainer *et al.* 2005, Sargent *et al.* 2002).

Food waste from Lebanese restaurants is usually rich in oils and fats especially olive oil, sunflower oil and butter. These lipids are known to be quite high in MUFAs especially olive oil (55-83 % MUFAs). The excess MUFAs provided by the WBF could have been used to spare the minor amounts of ω-3 PUFAs from catabolism (Stubhaug *et al.* 2006) but ultimately the EFAs became limiting in the growing fish, especially those offered regimens with high replacement of CF with WBF. Also, the decrease in LA and ALA in the diets as WBF proportion increased could have contributed to the decrease in tissue levels of ω-3 PUFAs noted in Nile tilapia fingerlings because of the failure of the metabolic effort to compensate for the deficiency in ω-3 PUFAs (Turchini and Francis 2009, Turchini *et al.* 2009). Ultimately, the use of high proportions of WBF affected the nutritional characteristics of the cultured fish tissue, as the fish could not maintain the same amounts of flesh EPA and DHA as those offered the fishmeal based commercial diet.

CHAPTER IV
EXPERIMENT THREE

Effect of using post-consumer food waste as feed on growth parameters and hematological indices of marbled spinefoot, *Siganus rivulatus*

A. Introduction

Food spoilage and food waste have always been among the most challenging predicaments of humankind. In recent years, governmental and non-governmental organizations have drawn attention to lost or wasted foods and their implications on food security, sustainability and the environment.

In fact, food loss and food waste are major ethical dilemmas as demand for food increases worldwide (FAO 2014) and yet more than 925 million people continue to suffer from undernourishment (Gustavsson *et al.* 2011). Various waste management strategies and guidelines have emerged with the "food waste hierarchy" provided by the EU (European Union) being the most prominent. This food waste hierarchy prioritizes the efforts to reduce the amount of waste generated by households, industries and governmental sectors; reuse of surplus food resources for human consumption; recycle food waste into useful material such as animal feed or compost; and recover energy by means of anaerobic digestion (EC 1975).

Food waste generated at various levels of the food supply chain is attracting a lot of attention because of its environmental, social and economic impacts (Mourad 2016). Food waste management faces many challenges because of the negative impacts such as despoliation of natural resources, i.e., *soil nutrients, water and energy* (Rockström *et al.* 2009); cost for waste management (Gustavsson *et al.* 2011); impact on greenhouse gases from the early stages of food production through spoilage (Padfield *et al.* 2012); dumping in landfills (FAO 2014); and social implications in relation to food security issues (Parfitt *et*

al. 2010). Accordingly, several food waste management processes are being considered while maintaining the notion that food waste can be a valuable resource (Bringezu and Bleischwitz 2017).

Most food waste mitigation programs recommend the use of a food recovery hierarchy which stipulates:(1) food waste prevention by decreasing excess at the source, improving processing technologies and adjusting production to needs; (2) reallocation of excess food to people in need (e.g., charity and social accountability); and finally (3) recycling food waste to produce animal feed, energy or fertilizers (EC 1975, FAO 2013). Some experts suggest that recovery and recycling of food waste are not as efficient as waste prevention in diminishing such wastes (Lorek and Spangenberg 2014, Tukker *et al.* 2017). Nevertheless, most work on food loss prevention focuses on the bottom of the hierarchy (i.e., recycling), because prevention and recovery are typically difficult to measure and more indefinite in relation to environmental impacts (Eriksson *et al.* 2015). Additionally, social and economic criteria should be taken into consideration when deciding on a waste management plan, especially since patterns of global food waste suggest a direct relationship between the economic status of a region/ country, the quantities of food lost/wasted, and the stage of the food supply chain at which they occur. Developing countries or low-income nations experience more food loss in the early stages of the food supply chain (production and post-harvesting), while in industrialized or high-income countries higher waste occurs during processing, retail and consumption (Papargyropoulou *et al.* 2014). Accordingly, the waste hierarchy is used as a flexible guideline for setting waste management plans and not a prescription to end all problems (Porter 2010, Price and Joseph 2000, Rasmussen *et al.* 2005). The choice of which food waste management strategy to implement depends on the desired outcomes whether to protect the environment, enhance trade and profit (economic), and/or improve

food availability (social). Under the environmental hierarchy, the most promoted solutions are ranked within recycling and include among many suggestions the reuse of food waste as animal feed (Mourad 2016, Salemdeeb *et al.* 2017). A major advantage of such recycling is keeping food waste away from landfills while using it productively.

Post-consumer food waste is mainly comprised of food plate leftovers or food purchased but not consumed. It often includes non-consumable components such as eggshells, pineapple skin, apple cores, bones and coffee grounds in addition to a substantial quantity of protein, lipids and carbohydrates. In other words, food waste includes quality food along the value chain suitable for human consumption but ultimately unconsumed and/or discarded (Gustavsson *et al.* 2011). Other factors such as cooking practices, demand anticipation, food quality, expiry dates and menu choices also contribute to food waste (Monier *et al.* 2010, Priefer *et al.* 2013).

Aquaculture is the fastest growing sector of animal production which requires *circa* 75 million tons of prepared feed annually amounting to approximately 250–300 million tons of fresh ingredients. Commercial feeds tend to be quite expensive partly because of a necessary uniformity of feeds in terms of nutritional content, consistency in form, and practicality of use. Feed accounts for more than 50% of production costs in aquaculture (Chong 1993), often reaching 80% of operation costs of a fish farm. Thus, any scheme that reduces feed cost without affecting fish production is welcome.

A major concern with residential and restaurant trash as well as expired foods at supermarkets and agricultural processing wastes is that they are often not a complete nutrient source for eukaryotic organisms. Frequently, wastes are mixed with other sources of nutrition such as fishmeal, vitamins and minerals and processed into fish feed. This is difficult in developing nations where most fish

farming takes place. Moreover, fish tend to conserve essential amino acids and other nutrients for necessary body building blocks and use non-essential nutrients for metabolism and energy. Accordingly, we postulate that farmers can offer their fish a feed containing complete dietary necessities in order to supply essential nutrients and then offer processed wastes to supplement caloric needs. Thus, farmers could use recycled waste to supplant expensive feed without deleterious effects. The processed wastes can be produced locally, thus saving on shipping and storage as well as reducing fish production costs. To the best of our knowledge, most existing studies have only focused on utilizing vegetable waste from farms and food processing industry to produce animal feed for swine (San Martin *et al.* 2016), but limited operations are those focusing on fish feed and fish production (Cheng *et al.* 2016, Choi *et al.* 2016).

The present work assesses whether post-consumer food waste could be used to supplant diets of marbled rabbitfish (*Siganus rivulatus*), an algaevorous marine fish native to the West Indian Ocean region and the Red Sea (Forsskal 1775). Marbled rabbitfish is an economically valuable species with growing interest for aquaculture in Eastern Mediterranean countries (El-Dakar *et al.* 2011, Saoud *et al.* 2008b). Several qualities make *S. rivulatus* good candidates for aquaculture such as being eurythermal and euryhaline (Saoud *et al.* 2008a, Saoud *et al.* 2007) in addition to their tolerance to high stocking densities (Saoud *et al.* 2008b). Although *S. rivulatus* are algaevorous in nature, they readily accept artificial feed when raised in culture systems (Abou-Daoud *et al.* 2014, El-Dakar *et al.* 2011, Barakat *et al.* 2011). The present study evaluates the use of restaurant food discards as partial replacement of commercial fish feed on survival, growth, feed conversion, hematology and proximate composition of marbled rabbitfish. The work was performed at the aquaculture research laboratory of the American University of Beirut (AUB), Lebanon, between October and December 2016.

B. Materials and Methods

1. Waste collection and feed processing

Post-consumer restaurant food waste was collected in the period extending between June and July 2016 from local restaurants serving traditional Lebanese–Mediterranean food. The collected waste was sorted, ground and then dried at 70 ± 5 °C in a forced-air oven to a moisture content of 6 ± 1%. Following drying, the samples were further pulverized to 50 μm using a burr grinder and stored at room temperature in vacuum-sealed bags.

To determine the proper mix that will provide the desired water stability of the fish feed, the samples were extruded and tested after mixing with a variety of gelatinizing ingredients such as wheat flour or cellulose (Vitacel R200 ® J. Rettenmaier & Söhne GmbH & Co. KG, Rosenberg, Germany). The diet with a ratio of 4:1—food waste to cellulose—was then selected as it provided the longest water stability for at least 10 min. Accordingly, the fish feed was prepared and extruded using a Model BD-GP50 (Henan Bedo Machinery Equipment Co., Ltd, China) extruder. The resulting pellets were dried at 40 °C to a moisture content of 8% and stored at − 20 °C. A commercial fish feed (Rangen 35; Rangen Inc., Buhl, Idaho, USA) was ground to match the size of the locally prepared feed pellets and used as the control diet.

Proximate composition of feeds and fish was performed as described in Nasser *et al.* (2018). This includes the measurement of moisture, total nitrogen, crude protein, fiber, ash, carbohydrate, lipid, carbohydrate-to-lipid ratio, energy, protein-to-energy ratio and protein solubility. Following the proximate analysis, the digestible energy in the feeds was calculated as 16.72, 17.62 and 37.62 kJ/g for protein, carbohydrate and lipid, respectively (Garling and Wilson 1976).

2. Fish experiment

The university animal care approval was obtained prior to fish handling. Juvenile marbled rabbitfish were caught from the wild and quarantined for 1 month in a recirculating saltwater system connected to a biological filter and a pump. The fish were offered a 36% protein and 6% crude lipid commercial feed (Rangen Inc., Buhl, Idaho, USA) twice daily.

The experiment was performed in fifteen 180-L fiberglass tanks (60 × 60 × 50 cm) connected to a biological filter and settling tank. Water was aerated using a regenerative blower with submerged air diffusers. The temperature in the tanks was maintained at 26–28 °C using submerged heating elements. Salinity, temperature and dissolved oxygen were monitored daily using a handheld YSI Model 85 dissolved oxygen (DO) meter (YSI Inc., Yellow Springs, OH, USA), whereas the total ammonia nitrogen (TAN), nitrite nitrogen and pH were measured weekly using a HACH saltwater aquaculture test kit, Model FF-3. The photoperiod during the duration of the experiment was maintained at 14:10 h (light/dark), and 30% of the water in each system was exchanged weekly or as necessary to maintain the optimal water quality.

The fish used in the experiment were size-sorted by hand, and 15 fish 7.5 ± 1.46 g per fish (mean ± SD) were randomly stocked into each of the 15 tanks. The fish were offered one of five feed regimens with three replicate tanks per treatment where the treatments are as follows:

(i) T1: 100% commercial feed (CF);

(ii) T2: 75% CF and 25% waste-based feed (WBF);

(iii) T3: 50% CF, 50% WBF;

(iv) T4: 25% CF, 75% WBF; and

(v) T5: 100% WBF.

The feed offered to the fish was measured as 5% of total body weight divided into four daily portions over 12 h (7:00 h, 11:00 h, 15:00 h and 19:00 h)

over a period of 6 days per week. The commercial feed was always offered in the morning followed by WBF whenever applicable. For example, in treatment T2 (75% CF, 25% WBF) the first three portions were CF, whereas the fourth was WBF. The feed ratio was increased by 10% on weeks when biomass was not measured. The fish were group-weighed biweekly following a day of fasting and the feed ration was adjusted accordingly (5% of the average total biomass of the largest treatment). The experiment was terminated after 8 weeks.

3. Fish performance

At termination, the fish were fasted for 24 h prior to data collection. Accordingly, the fish were anesthetized using a solution of MS 222 (Western Chemical Inc., Washington, USA) and group-weighed. The individual weight and length of each fish were also measured. Fulton's condition index (K), survival rate reported in % and feed conversion ratio were calculated according to Eqs. (1), (2) and (3), respectively.

$$K = \frac{M}{L^3} \times 10^5 \quad (1)$$

where M is mass (g) and L is length (mm)

$$S = \left(\frac{No.\ of\ fish\ at\ end\ of\ experiment}{No.\ of\ fish\ at\ beginning\ of\ experiment} \right) \times 100 \quad (2)$$

$$FCR = \frac{total\ mass\ of\ feed\ provided}{final\ biomass - initial\ biomass} \quad (3)$$

4. Fish hematology and proximate composition

Blood samples were collected by cardiac puncture using heparinized 1-ml syringes with 20-gauge 4 cm needles. At least four samples were taken from each tank. All hematology tests were performed as described in Nasser *et al.* (2018) The parameters tested include hematocrit (Hct), total plasma protein (TPP) (g/dL),

and hemoglobin (Hb) (g/ dL). Differential white blood cells (WBCs) counts were also performed on blood smears that were fixed with absolute ethanol and stained with Wright-Giemsa stains (Vázquez and Guerrero 2007). Eight hundred cells were counted on each slide in areas with no extensive overlap of cells.

Following blood extraction, the fish were weighed and subsequently the liver and viscera were removed and weighed. The hepatosomatic (HSI) and viscerosomatic (VSI) indices, reported as a percentage, were calculated as per Eqs. (4) and (5), respectively.

$$HSI = \frac{Liver\ Mass\ (g)}{Body\ Mass\ (g)} \times 100 \quad (4)$$

$$VSI = \frac{Viscera\ Mass\ (g)}{Body\ Mass\ (g)} \times 100 \quad (5)$$

In addition, four fish from each tank were also macerated and dried in an oven at 95 °C to constant mass. These samples were stored for proximate analysis which was performed following the procedures described in Nasser *et al.* (2018).

5. Statistical analysis

Results were analyzed using SAS (V.9.2, SAS Institute Inc., Cary, NC, USA). A simple analysis of variance (ANOVA) was used to compare means of all parameters tested followed when necessary by a Student–Newman–Keuls (SNK) means separation test to determine significant differences among treatments means. Significance was at $P < 0.05$.

C. Results and Discussion

Water quality parameters remained within narrow ranges throughout the experiment with reported temperature of 25.4 ± 1.1 °C; salinity 35.7 ± 1.5 ppt; pH

7.9 ± 0.12; and dissolved oxygen (DO) of 6.65 ± 0.49 ppm (mean ± SD). These parameters are all within optimal ranges for marbled rabbitfish.

Proximate compositions of the commercial and waste-based feeds used in the present work are summarized in Table 2.1. The commercial feed had almost twice the protein content of the waste-based feed: 40.7 versus 18.9%, respectively. Conversely, the waste-based feed contained around four times more lipids than the commercial feed: 22.2 versus 5.2%, respectively. Although both feeds were iso-energetic (20 kJ per g of diet), they had different amounts of digestible energy, ash and fiber.

The fish in all treatments gained weight and length during the first 4 weeks. At that point, the fish in treatment T5 which were offered 100% WBF started exhibiting a significant decrease in growth and started dying (Table 4.1; Fig. 4.1). A breakpoint analysis of growth (Fig. 4.2) reveals a breakpoint of 73.2% feed replacement. The equations of the two-best fit models are given by Eqs. (6) and (7):

$$y = -0.038 \cdot x + 22.34 \text{ with } R^2 = 0.709 \quad (6)$$

$$y = -0.728 \cdot x + 72.87 \text{ with } R^2 = 0.997 \quad (7)$$

where y is average weight (g) and x is the percent replacement of WBF.

Mean values of the final body weight (FBW), final length (FL), FCR and HSI showed significant differences among treatments (*P value < 0.05*). However, K and VSI were not significantly different (*P value > 0.05*) among the treatments. FBW and FL of treatment T4, 18.2 ± 4.22 g (mean ± SD) and 11.25 ± 0.79 cm, respectively, were different from all other treatments. Furthermore, treatment T4 had the greatest FCR (2.43 ± 0.15). Protein and energy retention or efficiency are not reported because in most treatments except treatment T1, fish were offered

Table 4.1 Survival (S; %), final body weight (FBW; g), final length (FL; cm), Fulton's condition index at harvest (K), feed conversion ratio (FCR), and hepatosomatic (HSI; %) and viscerosomatic (VSI; %) indices of juvenile *Siganus rivulatus*. Values in the same column with different superscripts are significantly different from each other based on SNK mean separation test ($P < 0.05$)

Treatment	S	FBW	FL	K	FCR	HSI	VSI
CF 100% (T1)	97.8 [a]	21.7 [a]	11.87 [a]	1.27 [a]	1.93 [a]	2.21 [a]	11.80[a]
WBF 25% (T2)	97.8 [a]	21.4 [a]	11.83 [a]	1.26 [a]	1.97 [a]	2.42 [a,b]	12.30[a]
WBF 50% (T3)	97.8 [a]	20.5 [a]	11.73 [a]	1.26 [a]	2.03 [a]	2.44 [a,b]	12.29[a]
WBF 75% (T4)	100 [a]	18.2[b]	11.25[b]	1.25 [a]	2.43[b]	2.91[b]	13.36[a]
WBF100% (T5)	N/A[**]	N/A[**]	N/A[**]	N/A[**]	N/A[**]	N/A[**]	N/A[**]
PSE[*]	2.10	0.80	0.15	0.01	0.07	0.001	0.004

[*]PSE: Pooled standard error

[**] Mortality in treatment 5 was 100 %. Accordingly, no data were available for the studied parameters.

Figure 4.1 Growth in average individual body weight (g) of juvenile *Siganus rivulatus* fed with increasing % of waste-based feed (WBF) over 8 weeks; T1: 100% commercial feed (CF); T2: 75% CF and 25% waste-based feed (WBF); T3: 50% CF 50% WBF; T4: 25% CF, 75% WBF; and T5: 100% WBF

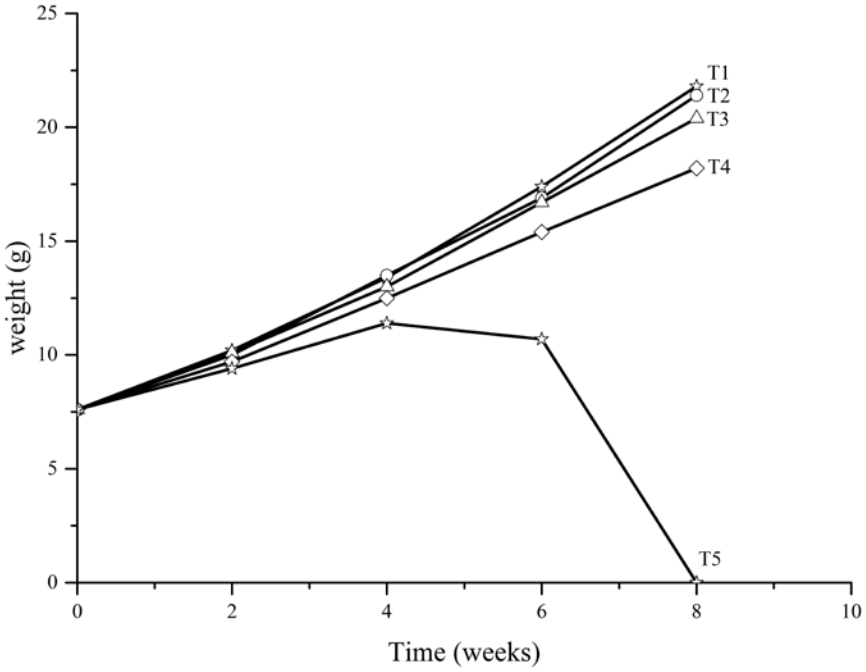
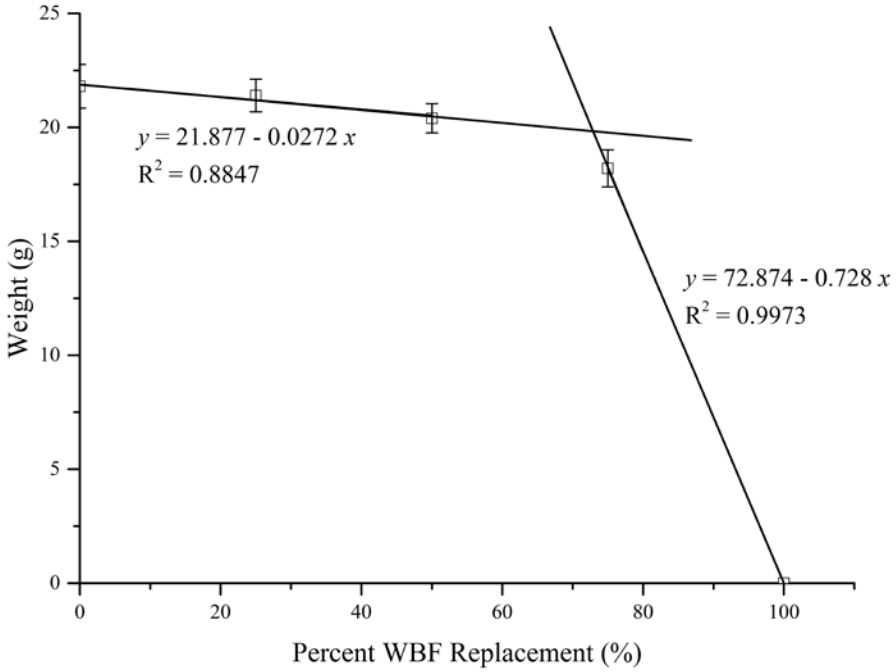


Figure 4.2 Breakpoint analysis of average growth (g) of juvenile *Siganus rivulatus* as a function of % replacement of commercial feed by waste-based feed (WBF) over 8 weeks



excess feed to eliminate the possibility that growth differences would be the result of food limitation.

There were no significant differences in hemoglobin and total plasma protein among the treatments (Table 4.2). Hemoglobin mean values ranged between 11.47 and 13.14 g/dL, while TPP varied between 4.06 and 4.27 g/dL. Hematocrit values were significantly different among treatments with treatment 4 having the smallest mean of 24.06 ± 2.66%. No differences were noted in the differential white blood cell counts among treatments (Table 4.3). Eosinophils and basophils were totally absent from all slides.

Food waste can be used to partially replace fish feed in aquaculture. The present work suggests that under controlled experimental conditions possibly up to 73% replacement of commercial fish feed with waste-based feed does not significantly affect survival, growth, hematology and proximate composition of juvenile *S. rivulatus*. However, significant effects on survival were noted with 100% replacement (100% mortality). This is probably because the food waste diet was deficient in essential amino acids, vitamins, or minerals necessary for fish metabolism. The commercial feed must have contained enough of these nutrients to keep the fish alive. Whole-body proximate compositions reported as a proportion of dry weight of fish are summarized in Table 4.4.

Although significant differences were observed in moisture content, lipid and total protein proportions among treatments, no differences were noted in the ash content. Means of total proteins and lipids for treatment 4 were 50.80 ± 4.09 and 40.42 ± 3.72%, respectively. As total protein offered to the fish decreased and total food lipids increased, the fish started storing adipose tissue at the expense of

Table 4.2 Hemoglobin (Hb; g/dL), hematocrit (Hct; %), and total plasma protein (TPP; g/dL) of juvenile *Siganus rivulatus* fed with increasing %s of waste-based feed (WBF). Values in the same column with different superscripts are significantly different from each other based on SNK mean separation test ($P < 0.05$)

Treatment	Hb	Hct	TPP
CF 100% (T1)	13.14[a]	28.83[a]	4.27[a]
WBF 25% (T2)	12.84[a]	28.07[a]	4.06[a]
WBF 50% (T3)	11.86[a]	27.59[a]	4.24[a]
WBF 75% (T4)	11.47[a]	24.06[b]	4.19[a]
WBF 100% (T5)	N/A[**]	N/A[**]	N/A[**]
PSE[*]	0.50	0.97	1.43

[*]PSE: Pooled standard error

[**] Mortality in treatment 5 was 100 %. Accordingly, no data were available for the studied parameters.

Table 4.3 Differential white blood cells count (in %) for juvenile *Siganus rivulatus* fed with increasing %s of waste-based feed (WBF). Values in the same column with different superscripts are significantly different from each other based on SNK mean separation test ($P < 0.05$)

Treatment	Neutrophils	Thrombocytes	Lymphocytes	Monocytes
CF 100% (T1)	2.67[a]	77.67[a]	16.67[a]	3.33[a]
WBF 25% (T2)	2.33[a]	79.33[a]	15.00[a]	3.67[a]
WBF 50% (T3)	3.67[a]	81.00[a]	9.67[a]	5.67[a]
WBF 75% (T4)	3.12[a]	82.20[a]	9.30[a]	3.20[a]
WBF 100% (T5)	N/A[**]	N/A[**]	N/A[**]	N/A[**]
PSE[*]	0.56	2.21	1.66	0.50

[*]PSE: Pooled standard error

[**] Mortality in treatment 5 was 100 %. Accordingly, no data were available for the studied parameters.

Table 4.4 Moisture, ash content, protein, and lipid as percent of dry matter of juvenile *Siganus rivulatus* fed with increasing percentage of waste-based feed (WBF). Values in the same column with different superscripts are significantly different from each other based on SNK mean separation test ($P < 0.05$)

Treatment	Moisture	Ash	Protein	Lipid
CF 100% (T1)	66.86[a]	10.17[a]	57.98[a]	34.13[a]
WBF 25% (T2)	65.50[b]	10.05[a]	56.23[a]	36.07[a,b]
WBF 50% (T3)	65.20[b]	10.27[a]	55.46[a]	37.02[a,b]
WBF 75% (T4)	64.76[b]	10.67[a]	50.80[b]	40.42[b]
WBF 100% (T5)	N/A[**]	N/A[**]	N/A[**]	N/A[**]
PSE[*]	0.30	0.30	1.14	1.41

[*]PSE: Pooled standard error

[**] Mortality in treatment 5 was 100 %. Accordingly, no data were available for the studied parameters.

protein. Consequently, although the fish grew by the same weight, those offered 75% WBF were fattier than those offered 50% or less WBF.

Food wastes include various categories that could provide nutrients and energy for fish. Meat, poultry and fish wastes could be used as protein and lipid sources for fish nutrition, while wastes of fruits and vegetables could provide carbohydrates and fibers (Garcia *et al.* 2005). A mixture of these wastes appears not to affect digestion capacity of rabbitfish, probably because herbivorous fish tend to utilize plant proteins and because of the enzymatic apparatus in the long digestive system of these species (Smith 1989). Partial replacement of fishmeal-based commercial feeds with WBF did not negatively affect growth and feed conversion of *S. rivulatus* juveniles probably because the fish got their necessities of essential amino acids and fatty acids from the commercial diet and used the waste-based diet for energy and non-essential amino acids and fatty acids.

Usually, fish have specific nutrient and energy requirements for survival and healthy growth and metabolism. According to the National Research Council (1993), the diet should provide enough non-protein energy for survival and maintenance; otherwise, the energy needed will be supplied using proteins at the expense of growth. In addition, fish have optimum dietary protein and lipid requirements. In the present work, both diets had similar gross energy content but differed in the amount of digestible energy which was 14.99 MJ/kg and 17.11 MJ/kg for CF and WBF, respectively. Abou-Daoud *et al.* (2014) reported that diets containing around 14.2 MJ of digestible energy with sufficient amounts of crude protein provide adequate growth of juvenile marbled rabbitfish when the essential nutrient requirements are met. Accordingly, both CF and WBF diets used in the present study provided enough digestible energy to the experimental fish although the sources of this energy were different. The fact that up to 73.2% of CF could be replaced with WBF without significant effects on survival and

growth suggests that nutritional energy is provided by both diets. Additional replacement of low-lipid commercial feed with lipid-rich WBF limited growth probably because the ratio of protein to digestible energy dropped below the 17–26 g/MJ range recommended by National Research Council (1993). Alternatively, growth could have been reduced by a lack of sufficient essential nutrients or vitamins and minerals. For example, deficiency in dietary lysine can reduce protein deposition and enhance lipogenesis (Brown *et al.* 2008, Peres and Oliva-Teles 1999, Phumee *et al.* 2011), thus limiting growth. As reported by Ghanawi *et al.* (2011), the increase in lipid content can have an effect on survival, growth, hematological parameters and fish proximate analysis. Although many researchers suggest that fish limit feed intake according to energy content of the diet, Du *et al.* (2005) and Ayisi *et al.* (2017) suggest that protein content is more important. This might explain the reduction in fish growth revealed in the present work when CF was replaced by more than 73% with low-protein WBF. Alternatively, fish growth may have been limited by the increase in lipid-to-protein ratio. Ghanawi *et al.* (2011) established optimal dietary lipid requirements for *S. rivulatus* juveniles as 98 g/kg of fish feed. If the amount of lipid provided exceeds the optimum quantity required, a decrease in growth will be observed (Du *et al.* 2005) as this increase in lipid content tends to affect the ability of the fish to digest food and absorb nutrients (Luo *et al.* 2005, Wang *et al.* 2005). Although break point analysis suggests that fish grow well at 75% WBF dietary replacement, proximate composition of the fish suggests that a 50% WBF replacement is a better value because then fish growth is in protein deposition not fat.

In general, the increase in dietary lipid content of fish is associated with the increased whole-body lipid proportion in various body parts such as the visceral cavity, liver and muscle tissues (Wang *et al.* 2005, López *et al.* 2006, Song *et al.* 2009). HSI was significantly different in the fish of treatment T4 as

compared to the other treatments. This may be attributed to the fact that the fish in treatment T4 grew at a lower rate than the fish in the other treatments and not because of the size of the liver. Ghanawi *et al.* (2011) showed that HSI of *S. rivulatus* was not significantly affected by lipid content in the diet since *S. rivulatus* seem to store lipids in their viscera and muscles and not in their liver. They also observed that fish size had a significant effect on HSI.

Although VSI appeared to be slightly greater in fish of treatment T4, there were no significant differences in VSI among all treatments. Since treatment T4 fish were being offered a lipid intensive regimen, the only explanation for not having a higher VSI is that the fish were consuming less of the diet offered which is very plausible given that the WBF was rich in lipids.

Hematological and biochemical indices are sensitive indicators of the physiological status of fish and are influenced by endogenous and exogenous factors (Wang *et al.* 2011). These indices include hemoglobin (Hb), hematocrit (Hct) and total plasma proteins (TPP), parameters usually tested for an indirect evaluation of the nutritional status of fish (Hoar *et al.* 1992, Riche 2007). Low protein content in the WBF compared to the CF could have affected Hct because it was deficient in certain essential amino acids or other nutrients (Lunger *et al.* 2006). Hemoglobin content tended to decrease as WBF replacement of commercial feed This suggests the possibility of deficiency in some micronutrients such as iron (Clauss *et al.* 2008). Hemoglobin differences among treatments were not statistically different from each other, which suggests that the experiment was not long enough for the deficiency to start affecting blood pigment. It is possible that for the long-term growth of the fish, a lesser proportion of WBF should be used or may be blood meal from local butcheries added to the diet mix. Total plasma proteins were not affected by the replacement of CF by WBF in *S. rivulatus* juveniles. Finally, there were no differences in the white

blood cells profiles among treatments, suggesting that the WBF did not cause intestinal inflammatory responses which are sometimes associated with nondigestible feeds.

Food waste (households or restaurants) is usually characterized by excessive moisture content but requires minimal industrial pre-treatment. Negligible expenses on energy requirement and transportation are needed for local processing of food waste into other useful substances. However, if wastes are to be used as nutritive substances, then protein, lipid and carbohydrate content need to be known to understand amounts to be offered to animals. The moisture in the raw ingredients need not be an adverse property. Although the waste in the present experiment was dried, in commercial settings a feed pellet could be made using the wet raw material. However, the ingredients and processing protocols used should provide a form of pasteurization while still maintaining feed integrity at least for animal-derived proteins in order to avoid biological contamination of the aqua feed and harm the aquatic organism (Wood 1995).

Nutrients present in collected food waste would be expected to vary based on the menu and plates offered and season of collection. However, with a relatively consistent menu in restaurants with similar offerings (Lebanese food in the present work), the end product can be assumed to have a relatively consistent composition in terms of proteins (amino acids), lipids, carbohydrates and fibers. Accordingly, variations over a grow-out period of a fish would not be consequential to growth and other production variables.

Aquaculture, similar to all food production industries, has environmental consequences mainly related to fish feed production and use. Production of aqua feed from food waste decreases the environmental impact of using original raw materials. However, incomplete diets tend to cause incomplete digestion with consequent pollution by unused nutrients (Chong 1993). Moreover, shipping

material back and forth usually has a high carbon footprint because of increased CO_2 emissions (fuel/energy consumed). A feed mix composed of nutritionally complete feeds to offer essential nutrients and waste-based feed that offer energy compounds and non-essential amino acids to complement the diet would be doubly beneficial. It would reduce the environmental impact of producing commercial feeds from new raw ingredients and would reduce the environmental impact of disposing of food wastes. In areas where large feed manufacturing industries exist, the food waste can be incorporated into the feed manufacture process. In isolated rural settings, commercial feed can be safely and productively complemented with WBF as proven in the present work.

D. Conclusion

In a world with more than 800 million people suffering from chronic undernourishment (FAO and IFAD 2015), it is imperative that new ways to produce more food sustainably while reducing food waste be explored. Results of the present study suggest that post-consumer food waste can be productively used to substitute for at least 50% of commercial fish feed for growing marine herbivorous fish without apparent detrimental effects. This could induce huge savings on the cost of feed production which translates into less expensive fish on the market. Globally, 332,000 tons of rabbitfish were produced in 2015 (FAO 2017) which corresponds to *circa* 530,000 tons of commercial feed utilized. If 50% of commercial feed is substituted with food waste-based feed without any significant effects on survival, growth rate and physiological conditions, then 265,000 tons of non-sustainable fresh ingredients could be replaced by post-consumer food waste annually for rabbitfish aquaculture alone. Our results need to be confirmed by on-farm long-term studies but are very promising.

CHAPTER V
CONCLUSION

Partial substitution of traditional commercial fish feed with restaurant waste based feed results in economic benefits for farmers, increased employment in collection, processing and distribution of waste-based fish feed in addition to a reduction in pollution associated with landfill dumping. As the aquaculture industry continues to grow, a sustainable and environmentally friendly source of feed ingredients such as restaurant food waste will become highly attractive. Redirecting food waste away from landfills into production of nutritious protein would increase food security in vulnerable regions of the world while reducing adverse environmental impacts of food waste disposal.

In the present work, it was feasible to collect waste from two restaurants and process the waste immediately after collection thus reducing fermentation, spoilage and contamination. If the present work is to be adapted to commercial situations in regions where the technology is not yet present, logistics need to be worked out for waste collection, storage, processing, and spoilage reduction. Moreover, the present work was performed to assess the feasibility of using restaurant food waste in aquaculture in regions without access to feed manufacturing. In more industrialized regions, the restaurant waste could possibly be incorporated into a commercial diet as a replacement for soy bean meal or possibly even for fish meal. Moreover, if an alternative to commercial fish feed can be produced locally, expenses of importing commercial aquafeed to developing countries where most aquaculture takes place would decrease by saving on shipping and storage as well as reducing feed costs.

A drawback of the present work was that typical waste food from a Lebanese restaurant tends to be rich in lipids which makes feed formulation and

preparation difficult. Moreover, although WBF had significantly larger lipid content compared to CF, the EFAs LA and ALA were relatively lower and the ω-3 PUFAs (EPA and DHA) were much less significant. We suggest that when considering the use of WBF with such a lipid profile to manufacture feed, we could start by extracting lipids from the dried waste to improve and if necessary add some crystalline amino acids and some fish oil or marine algae to the feed as required by the various nutritional requirements of the aquacultured species.

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