1 RECYCLE FOOD WASTES INTO HIGH QUALITY FISH FEEDS FOR SAFE AND QUALITY FISH 2 PRODUCTION

3 Ming-Hung Wong^{1,2,3*}, Wing-Yin Mo³, Wai-Ming Choi³, Zhang Cheng³, Yu-Bon Man³

4 ¹ Key Laboratory for Heavy Metal Pollution Control and Reutilization, School of
5 Environment and Energy, Peking University - Shenzhen Graduate School, Shenzhen,
6 China

7 ² School of Environment, Jinan University, Guangzhou, China

8 ³ Consortium on Health, Environment, Education and Research (CHEER), and
9 Department of Science and Environmental Studies, The Education University of Hong
10 Kong, Tai Po, Hong Kong, China

11 ABSTRACT

12 The amount of food waste generated from modern societies is increasing, which has
13 imposed a tremendous pressure on its treatment and disposal. Food waste should be
14 treated as a valuable resource rather than waste, and turning it into fish feeds would be
15 a viable alternative. This paper attempts to review the feasibility of using food waste to
16 formulate feed pellets to culture a few freshwater fish species, such as grass carp, grey
17 mullet, and tilapia, under polyculture mode (growing different species in the same
18 pond). These species occupy different ecological niches, with different feeding modes
19 (i.e., herbivorous, filter feeding, etc.), and therefore all the nutrients derived from the
20 food waste could be efficiently recycled within the ecosystem. The problems facing
21 environmental pollution and fish contamination; the past and present situation of inland
22 fish culture (focusing on South China); upgrade of food waste based feed pellets by
23 adding enzymes, vitamin-mineral premix, probiotics (yeast), prebiotics, and Chinese
24 medicinal herbs into feeds; and potential health risks of fish cultivated by food waste
25 based pellets are discussed, citing some local examples. It can be concluded that
26 appropriate portions of different types of food waste could satisfy basic nutritional
27 requirements of lower trophic level fish species such as grass carp and tilapia. Upgrading
28 the fish pellets by adding different supplements mentioned above could further
29 elevated the quality of feeds, leading to higher growth rates, and enhanced immunity of
30 fish. Health risk assessments based on the major environmental contaminants (mercury,
31 PAHs and DDTs) in fish flesh showed that fish fed food waste based pellets are safer for
32 consumption, when compared with those fed commercial feed pellets.

33 Capsule Abstract:

34 Potential health risks of fish cultivated by suitable portion and type of food waste based
35 pellets which are safer for consumption when compared with fed commercial feed
36 pellets.

Key Words:

Fish feed pellets, feed conversion ratio, health risk assessments, low trophic level fish, polyculture of fish.

Corresponding author: Ming-Hung Wong; Email address: minghwong@ied.edu.hk

1. INTRODUCTION

Food waste, included raw or cooked, is any food substance which is discarded, or intended or required to be discarded (USEPA, 2012). In view of the fact that there is an increasing risk of a food crisis due to potential crop failure, food waste should be regarded as a valuable resource. Furthermore, it has been estimated that about one third of food (about 1.3 billion tonne [t]) produced world wide is wasted, and at the same time 925 million people are starving (FAO, 2015). The amount of food waste generated daily in Hong Kong has reached 3,600 t, comprising of one third of the municipal solid waste (HKEPD, 2012). This will lead to rapid depletion of the limited landfill space and produce landfill gases and leachates, which are detrimental to the environment.

Therefore, food waste should be recycled as a resource for producing fuel, fertilizer and feed, which would partially ease its disposal pressure. The Organic Waste Treatment Facilities (OWTF) being built in Hong Kong, are intended to stabilize the organic waste by means of aerobic and anaerobic decomposition, to produce compost and biogas. However, the 1st phase establishment can only treat about 200 t food waste/day (HKEPD, 2012). There seems to be an urgent need to explore other alternatives for recycling and reuse of food waste.

It seems logical and feasible to turn food waste into animal feeds. However, due to the mad cow disease happened in Britain, a number of countries (including Australia, New Zealand, Canada, USA, and UK) have set rather straight regulations on feeding restricted animal materials (i.e., tissue or blood taken from an animal, and meal obtained from rendering tissues or blood from animals such as blood meal, meat and bone meal, fish meal, feather meal, and compounded feeds made from these products) to ruminants (Westendorf, 2000). Therefore, turning food wastes into fish feeds seem to be the best alternative.

In fact, aquaculture is gaining its importance in recent years. It produced about 3 million t of fish in the 1970s, but reached 66.63 million t by 2012, with the Asia-Pacific region continued to dominate the aquaculture sector, accounting for 88.5% of global production (FAO, 2012). However, food safety is a major public concern, especially in South China which has been developed rapidly in the past 30 years.

Fish feed accounts for more than 50% of the total cost in fish culture industry, as the costs of major feed ingredients: fish meal and cereal grains (i.e. soybean, rice bran, wheat, corn, etc.) are affected by climate, weather conditions, global economic growth and fuel costs. As a matter of fact, the costs of fishmeal, soybean meal, corn and wheat rose by 55, 67, 124, 130, and 250% respectively from 2000 to 2009, mainly due to the increasing energy cost and demand, and also higher manufacturing and transportation costs (Rana et al., 2009).

It has been estimated that by 2020, the global demand of aquaculture feed would reach 71 million t, which is doubled the figure (29.2 million t) in 2008, and almost ten-fold of 1995 production (7.6 million t). Furthermore, the aquaculture feed is mainly catered for rearing carps, accounting for 31.3% of the total feed production (FAO, 2012). The use of food wastes for fish culture would be a more sustainable strategy to reactive local fish culture industry which once prosperous during 1960s – 1990s in Hong Kong.

The major aim of this presentation is to demonstrate that food wastes could be recycled into high quality fish feeds for culturing freshwater fish for producing safe and quality products, and at the same time partially ease its disposal pressure.

2. CURRENT PROBLEMS ON FOOD SAFETY AND FISH CONTAMINATION

2.1 Environmental pollution and food safety

Food safety is any action and policy which ensures food is safe, in the entire food chain, i.e. from production to consumption (WHO, 2013). In fact, “chemical food contaminants” is one of the 3 key global food safety concerns. The other two are “spread of microbiological hazards”, and “assessments of new food technologies (e.g., genetically modified food). As a matter of fact, food safety is currently one of the major public health issues in the world, especially in rapidly developing countries, due to rapid population growth, urbanization and industrialization. A wide range of persistent toxic substances (PTS) has been emitted into different ecological compartments (i.e., air, water, and soil/sediment), threatening their ability to meet safety standards, and jeopardize food production. These PTS included heavy metals and metalloids (such as cadmium [Cd], mercury [Hg], lead [Pb] and arsenic [As, a metalloid]; and persistent organic pollutants (POPs) (such as organochlorine pesticides, dioxins and flame retardants); which are widespread in the environment.

Most of these PTS share the common characteristics of being persistent (resisting biological and photo-degradation), toxic (causing adverse environmental and health problems), bioaccumulative (lipid seeking, leading to biomagnification in food chains) and capable of long-range transport through different media (reaching to remote areas, due

to their semi-volatile nature, known as grasshopping effect). Some of the chemicals management issues in developing countries and countries with economy in transition have been recently reviewed (Bouwman et al., 2013). Some emerging chemicals of concern (such as bisphenol A and phthalates) recently found in contaminated fish have also become public health concerns (Wong et al., 2012). There seems to be rather concrete evidence showing the potential associations of PTS found in contaminated fish, their body loadings and health hazards of consumers.

One typical example is Hg, where the subfertile males (with abnormal semen parameters, including inferior quantity and quality of sperms), with higher hair Hg levels significantly correlated with their frequency of fish consumption (Dickmen et al., 1988). Liang et al. (2013) indicated the relationships between Hg levels in Hong Kong residents and their seafood consumption. A study revealed that human hair Hg levels collected from fish farming areas scattered around the PRD, were significantly correlated with frequency of fish consumption (Shao et al., 2013). Being neurotoxins, the high hair Hg (and very often Pb) levels are linked with higher fish consumption rates of autistic children (Ko et al., 2012).

Uncontrolled recycling of electronic waste in Guiyu (South China), has generated a wide range of toxic chemicals, including Pb, Hg, dioxins, and flame retardants (Leung et al., 2007; Wong et al., 2007), which linked with food contamination, body loadings in residents and workers, and the morbidity of major diseases (cancer, respiratory diseases, etc.) in the village (Chan et al., 2013). Our early study showed that human milk samples collected from Hong Kong and Guangzhou showed that higher concentrations of DDT, HCH and PCB detected in samples are significantly correlated to the frequency of fish consumption of the donors (Wong et al., 2002). Recently, it has been revealed that higher concentrations of heavy metals (Hg, Pb), as well as POPs (DDTs, HCHs and PCBs) detected in adipose tissues of patients with uterine leiomyomas, are also associated with their preference for seafood diets, when compared with their healthy counterparts (Qin et al., 2010). Therefore, there seems to be an urgent need to reduce human body loadings of these PTS, by lowering their concentrations in our food items, in particular fish.

2.2 Reasons of fish contamination

It is commonly observed that environmental contaminants such as Hg and DDTs can be efficiently taken up by fish, especially larger carnivorous fish (such as tuna, swordfish and shark). Fortunately, fish cultivated in inland fish ponds tend to contain lower levels of contaminants, due to less complicated trophic levels within fish ponds, and the relatively short culture periods (several months to a year) (Cheng et al., 2011; 2013). Nevertheless, consumption of contaminated fish in certain populations has become a growing public health concern in different parts of the world (Hinck et al., 2006).

Furthermore, there is also a risk of contamination of aquatic products due to the use of contaminated trash fish, fishmeal and oil as feed ingredients (Dorea, 2006).

The use of trash fish for feeding carnivorous species in Asian region is a traditional practice of local fish farmers, making use of the high protein content of small fish, without much commercial value. Fish meal, largely made from trash fish, is an important component of commercial fish feed pellets. Both trash fish and fish meal provide an important source of protein and oil for most carnivorous fish and even animals, due to the unique amino acid profile, high digestibility and oil content (Huntington & Hasan, 2009). Unfortunately, it has been noted that both trash fish and fish meal available in South China (including Hong Kong) are contaminated with total and methyl Hg (Liang et al., 2011).

Maule et al. (2007) analyzed fish feeds used in US Fish and Wildlife Service's National Fish Hatcheries and noted that out of the 55 samples collected, 39 contained polychlorinated dibenzo-p-dioxin (PCDDs), 24 contained polychlorinated dibenzofuran (PCDFs) and 24 contained dichlorodiphenyltrichloroethane (DDT) or its metabolites. In general, it is recognized that fish feeds are major sources of heavy metals and POPs in farmed fish (SCAN, 2000), and the concentrations of several POPs (e.g. dioxins and BFRs) and heavy metals (notably methyl-mercury [MeHg], the most toxic form of Hg) in farmed fish are correlated with their concentrations in feeds (Karl et al., 2003; Lundebye et al., 2004). As such, the European Community set the Maximum Level and Action Level of aquafeeds, in terms of MeHg: fishmeal (1.25, 1.00 ng/kg), fish oil (6.00, 4.50 ng/kg) and commercial fish feed (2.25, 1.50 ng/kg) (SCAN, 2000), in addition to reducing POP inputs into the environment.

3. USE OF FOOD WASTES FOR FISH CULTURE

3.1 Freshwater fish culture- past and present in South China

In view of the above, there seems to be a need to ensure that the feeding materials are relatively free of PTS. Presumably, the leftover of food items, fit for human consumption should be safe to be recycled as fish feeds. This has been a long term practice in China, where kitchen wastes, and even pig manure (serves as pond fertilizer) are applied for polyculture of freshwater fish. Different fish species with different ecological niches and feeding modes are reared in the same pond and all the residual energy derived from the wastes could be fully utilized.

Pig manure serves as pond fertilizer to promote growth of plankton, which would be utilized by bighead carp (*Aristichthys nobilis*), which mainly feeds on zooplankton, and silver carp (*Hypophthalmichthys nobilis*), on plankton. Being an herbivore, grass carp (*Ctenopharyngodon idella*) feeds on macrophytes (Napier grass is grown as feeds for grass carp) (Cheng et al., 2015a). Bottom dwellers included common carp (*Cyprinus*

carpio) and mud carp (*Cirrhinus molitorella*) feed on benthos and detritus, while black carp (*Mylopharyngodon piceus*) prefers benthos only. Polyculture of mixed fish species (mainly members of the carp family) has been a long-term tradition in China, with different types of stocking composition. One of the most popular stocking composition adopted in Hong Kong included: grey mullet (60-70%), grass carp (10%), and bighead (15%) together with small numbers of other species including silver carp, common carp and tilapia (Lau et al., 2003). These species occupy different niches and feed on different organisms, which can fully utilize all the materials derived from the wastes. However, due to the recent outbreaks of Avian influenza (the disease caused by infection with avian [bird] influenza [flu] Type A viruses), the traditional practice of integrated agricultural and aquacultural systems is questionable, and should be carried out with extreme caution.

Carnivorous fish such as freshwater bass or largemouth bass (*Micropterus salmoides*) and Mandarin fish (*Synchiropus splendidus*) are often mono-cultured, under high density, using trash fish or compound feeds with higher protein contents (Wong et al., 2004). However, there has been a shift from the use of animal protein to plant based protein in fish feed production industry, with the fish meal gradually and partially replaced by other protein sources, such as soybean meal and rapeseed meal. Salmon fed with a diet containing 20% soybean protein achieved a similar growth rate, when compared to those fed with fish meal solely (Olli et al., 1995). Table 1 shows the proportions of fish meal included into feeds of different species (e.g., trout, salmon and carps) have been declined in 2008, compared with the proportions included in 1995. It is predicted that the proportions of fish meal included in feeds would be further declined in 2020 (Tacon et al., 2011).

Table 1. Reduced fish meal inclusions (%) in aqua feed of different aquaculture species (FAO, 2012)

Species/species group	1995	2008	2020*
Carps	10	3	1
Tilapias	10	5	1
Catfishes	5	7	2
Milkfish	15	5	2
Miscellaneous freshwater fishes	55	30	8
Salmons	45	25	12
Trouts	40	25	12
Eels	65	48	30
Marine fishes	50	29	12
Marine shrimps	28	20	8
Freshwater crustaceans	25	18	8

*Predicted values (Tacon et al., 2011)

A study observed that poultry –by product could replace fish meal completely for culturing hybrid striped bass (Rawles et al., 2009). Attempts have been made to incorporate different types of food and industrial wastes (from poultry, soy sauce, rice wine, beer and papaya producing/processing industries) into fish feed pellets (Kang et al., 2010; Vechklang et al., 2011).

In fact, some of the food wastes, including meat waste, fruit waste, vegetable waste and fish waste, are rich in nutrients, and could be considered as substitutes for original raw materials for feeding animals including fish (Garcia et al., 2005). Therefore, it will be a good idea to explore ways of turning food waste into fish feeds, in view of (1) the increasing amount of food waste generated in cities such as Hong Kong; (2) contamination of PTS in feeding materials and associated health risks; and (3) the importance of aquaculture in terms of food security and fish as a key protein source.

3.2 Use of food wastes to formulate fish feed pellets

Wheat bran, soybean and peanut cake are often used as feed ingredients, but corn meal, bread, noodles, flour and biscuits also applied to ponds for feeding freshwater fish in South China including Hong Kong (Lau et al., 2003). Only a small portion of farmers uses commercial feed pellets as regular feeds, due to their higher costs.

Table 2 shows the categories of different food wastes, including cooked and non-cooked items produced from hotels in Hong Kong (Choi et al., 2015a). It is envisaged that the major problem of utilizing food wastes to formulate feed pellets would be the diverse range of food wastes generated from different sources, e.g. domestic homes. Without proper separation at source, it would be very difficult to produce fish feed with desirable composition and nutritional contents. For handling food waste that cannot be separated into categories, using other treatment would be essential. For example, making use of solid state fermentation (SSF) to process food waste from heterogeneous sources could enhance the content of crude protein content and digestible carbohydrate while decreasing the content of non-digestible fiber (Lateef et al. 2008; Wang et al., 2010)

Table 2 Food waste categories collected from hotels in Hong Kong (Choi et al., 2015a).

Categories	Food	Amount (%)	Non-cooked or cooked
Fruit peel and vegetables	-Pineapple	20	Non-cooked
	-Watermelon	20	
	-Cantaloupe	15	
	-Strawberry, banana, apple	30	
	-Leaf vegetables (such as lettuce and spinach)	15	

Meat	-Beef, pork and chicken -Salmon -Grouper	60-70 35 5	Cooked Non-cooked Cooked
Cereals	-Rice bran -Soybean meal -Rice grain -Spaghetti	50 20 20 10	Non-cooked Cooked Cooked Non-cooked
Bone	-Cow, pig and chicken -Salmon -Grouper	60-70 30 10	Cooked Non-cooked Cooked

3.3 Use of food wastes based pellets for fish culture

Although food wastes (or kitchen wastes including noodle, biscuit, rice bran, etc.) have been applied to inland fish ponds for feeding fish for years in South China, but there is a severe lack of reliable data, in terms of the types, combinations and amounts of food wastes used, the growth performance and feed conversion ratio in culturing different fish species. The nutritional contents (essential amino acids) of some common feeds (Napier grass, rice bran, soybean dreg, noodles, breads and Hamburger buns) used by local farmers, were compared with the three food waste based pellets (Table 3), and a commercial pellet (Jinfeng, 613 formulated feed, with about 30% protein). It was observed that the crude protein and lysine contents (except soybean dreg, the two food waste based pellets and the commercial pellet) of those common feed stuff are insufficient (Mo, 2014). Therefore, the nutritional contents of these two food waste based pellets seemed to be suitable for culturing freshwater fish.

Table 3 Food waste based feed pellets (containing 75% food wastes) for rearing grey mullet and grass carp (Mo et al., 2014; Choi et al., 2016).

	Fruit/ vegetable	Meat	Cereals	Bone	other	Fishmeal	Corn starch	TOTAL %
FWA	10	0	53	8	5	10	15	100
FWB	10	25	28	8	5	10	15	100
FWC	10	10	43	8	5	10	15	100

FW: food waste

Our early laboratory experiment showed that the food waste based pellets resulted in lower growth rates of grass carp and grey mullet, when compared with the control, which fed a commercial diet: Jinfeng 613 formulated feed (with about 30% protein), containing wheat middling, flour, bean pulp, rapeseed meal, fish meal, bean oil and fish oil (Choi et al., 2015a). It was also observed that Food Waste B and C (FWB and FWC) containing meat would be less suitable than FWA or Jinfeng 613 for rearing grey mullet (omnivore) and grass carp (herbivore), as they prefer plant protein rather than animal

protein. Furthermore, the higher level of lipid detected in feeds containing meat products would also be a major reason for hindering growth of these two species.

A field trial using FWA and FWB to feed several freshwater fish species under polyculture (stocking different fish species in the same ponds): grass carp (*Ctenopharyngodon idella*), bighead carp (*Hypophthalmichthys nobilis*) and mud carp (*Cirrhinus molitorella*), at the ratio of 3:1:1 favored fish growth, in general. FWA contained a higher level of phosphorus enhanced plankton growth, leading to better growth of bighead carp (a filter feeder). FWB promoted the growth of grass carp, possibly due to the relatively lower amount of carbohydrates (24.2%) and carbohydrates: lipid (CHO: L) ratio (1.83), than control (fed the commercial diet) and FWA (Mo et al., 2014). Results indicated various formulations of food waste based fish could be beneficial to fish with their unique mode of nutrition. Further investigations focusing on other fish species cultured in polyculture ponds as well as other food waste fish feed formulations should be conducted in order to optimize the formulations.

4. UPGRADING FOOD WASTES INTO HIGH QUALITY FISH FEEDS

Adding supplements to fish feeds to enhance growth and health of the cultivated fish is common practice. Table 4 shows the general benefits of incorporating enzymes, vitamin-mineral premix, probiotic (yeast), prebiotic fibres and Chinese medicinal herbs into fish feeds.

Table 4: Food waste based pellets added with supplements: Effects on fish growth

Food Waste Based Pellets with Supplements	Enzymes	Vitamin-mineral premix	Probiotic (yeast)	Prebiotic fibres (such as inulin)	Chinese medicinal herbs (e.g. huanqi and goji)
Benefits	Hydrolyzed feed proteins into smaller protein in peptides with higher digestibility. Enhanced digestibility, feed conversion ratio (FCR).	Raised activities of various enzymes. Increased weight gain, FCR, and Protein Efficiency Ratio (PER).	Improved intestinal balance of fish. Enhanced digestibility of feeds, stimulated innate immune responses, protected fish against	Improved fish health. Enhanced growth, and non-specific immune systems, or innate immune systems.	Improved growth and specific immunity, against infection by pathogens, in general.

			infections.		
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293 **4.1 Use of enzymes for enhancing feed conversion of fish feeds**

294 After adding enzyme mixtures (Bromelain and papain, 1% and 2%, weight by weight)
 295 into the food waste based pellets, the feed conversion ratio and the total
 296 immunoglobulin of the cultured fish (grass carp and grey mullet) were enhanced, when
 297 compared with those fed the commercial diet.

298

299 In fact, enzyme supplementation in animal feeds in poultry and swine industries has
 300 been proved to be effective in enhancing feed conversion and utilization (Kitchen, 1997).
 301 This is the same with fish feeds. Our early study also demonstrated that pretreatment of
 302 soybean residue by papain could enhance the feed digestibility and conversion of
 303 common carp, in addition to cleaner water of the culture tanks (Wong et al., 1996).
 304 Proteases such as papain (from papaya) and bromelain (from pineapple) are able to
 305 hydrolyze the feed proteins into smaller protein in peptides with higher digestibility
 306 (Nilsang et al., 2005).

307 It has also been noted that a commercial enzyme, Ronozyme™ VP is able to enhance
 308 growth performance and net protein utilization of tilapia fed palm kernel meal based
 309 feed (Boonyaratpalin et al., 2000). Fish processing by-products (wastes containing heads,
 310 viscera, skin and skeleton) treated with enzyme in the form of fish protein hydrolysate is
 311 a potential ingredient producing aquaculture feeds (Bhaskar & Mahendrakar, 2008).
 312 Inclusion of fruit processing residues involving papaya or pineapple, into making food
 313 waste pellets, may be a good idea to cut down the cost.

314 **4.2 Use of vitamin-mineral premix for enhancing nutritional values of food wastes**

315 In order to increase the nutritional qualities of animal feeds especially vegetable-based
 316 diets, adding minerals and vitamins is a common practice in animal husbandry (Deyhim
 317 et al., 1995). For fish culture, it has been shown that inclusion of vitamin-mineral premix
 318 in the feed for broadhead catfish (*Clarias microcephalus*) fry enhanced the survival rate
 319 and weight gain, compared to those without inclusion (Taechajanta & Sitasit, 1981).
 320 Another study noted that phosphorus in mineral premix could raise the weight gain,
 321 Feed Conversion Ratio (PCR) and Protein Efficiency Ratio (PER) of tilapia (Datocajegas &
 322 Yakupitiyage, 1996).

323 It is expected that inclusion of the full range vitamins and minerals would also be
 324 important for fish growth, e.g. Li et al. (2007) revealed that hybrid tilapia (*Oreochromis*
 325 *niloticus* x *O. aureus*) fed with diets supplemented with copper, iron and zinc
 326 significantly raised the activities of amylase in hepatopancreas homogenates, amylase in
 327 intestine and lipase in intestine; while Huang et al. (2011) showed thiamin included in
 328 the diet significantly raised the activities of various enzymes (such as lipase, trypsin,

alkaline phosphatase, etc.) in the intestine of juvenile Jian carp (*Cyprinus carpio* var. *Jian*).

Adding vitamin-mineral premix in food waste based pellet B (FWB) for feeding grass carp and tilapia, resulted in improved FCR (grass carp=1.29, tilapia=1.04). In fact, addition of the premix enhanced the protein digestibility (from 65-80%) of food waste based pellets, leading to better growth performance of both fish (Mo et al., 2015). Therefore, adding vitamin-mineral premix in food waste based pellets will be beneficial for fish growth.

4.3 Use of “probiotics” for enhancing fish health

“Probiotics” referred to “a live microbial feed supplement which beneficially affects the host animal by improving its intestinal balance” (Fuller, 1989). Our study attempted to upgrade food waste based diets was made by adding baker’s yeast (2% yeast, *Saccharomyces cerevisiae*) into the pellets. Results showed that fish (grass carp) fed with these diets led to better growth rates, in terms of FCR and PER, leading to superior Specific Growth Rate (SGR) and Relative Weight Gain (RGW) (Mo et al., 2015).

The positive effects provided by addition of yeasts is mainly due to the adhesion of yeast cells to the intestinal wall, and their amylase enzymes would increase the digestibility (Scholz et al., 1999) of the food waste based pellets. Furthermore, yeast cells attached on the intestinal wall could also stimulate innate immune responses, protecting fish against infections (Esteban et al., 2001). This is due to B-glucans, mannan oligosaccharides and nucleic acid contained in the yeast, serving as immune-stimulants (White et al., 2002). It has been noted in feeding trials that addition of these active ingredients in feeds enhanced disease resistances of fish to *Aeromonas hydrophila* in different fish: Asian catfish (*Clarias batrachus*) (Kumari & Sahoo, 2006), common carp (*Cyprinus carpio*) (Selvaraji et al., 2005).

Other studies also yielded positive effects using whole yeast, on the immune response of seabream (*Sparus aurata*) (Rodriguez et al., 2003) and tilapia (*Oreochromis niloticus*) (He et al., 2009). A study also revealed that the whole baker’s yeast is a potential alternative to replace fish meal protein for rearing sea bass (*Dicentrarchus labrax*) juveniles, with a higher nutritional requirement (Oliva-Teles & Goncalves, 2001). Therefore, addition of whole yeast into food waste based pellets would be more convenient, than extracted and purified constituents, with associated lower cost and lessened technique. By-products generated from brewing industry containing yeasts could be collected easily, and used for this purpose, at low cost.

4.4 Use of “prebiotics” to enhance feed digestibility, growth and non-specific immunity of fish

“Prebiotics” such as inulin, an oligosaccharide; mannanoligosaccharides (MOS), a glucomannoprotein complex derived from yeast cell wall; fructooligosaccharides (FOS); and galactooligosaccharides (GOS) could be used to improve health of aquatic animals, replacing antibiotics (Ringo et al., 2010). Nile tilapia fed with 5 g inulin/kg diet had significantly higher body weight gain, specific growth rate, condition factor, survival rate and nitroblue tetrazolium (NBT) value, which indicated enhanced immunity (Ibrahim et al 2010). Salmon treated with FOS resulted in improved feed efficiency (1.38 compared with the control) (Grisdale-Helland et al., 2008). Rainbow trout fed with MOS (2/kg) had improved growth performance, survival rate, and also antibody titer and lysozyme activity (Staykov et al., 2007).

An attempt has been made to add inulin and MOS into food waste based pellets for rearing grass carp and Nile tilapia. In general, 0.2% inulin and 0.2% MOS could enhance growth (reflected by RWG, SGR, PER, and FCR) of grass carp, and the non-specific immune systems, or innate immune systems (with better total serum immunoglobulin, bactericidal activity and anti-protease activity) of both species (Mo et al., 2015).

4.5 Use of Chinese medicinal herbs to enhance fish immunity

Bacterial infection is one of the major reasons causing fish mortality in aquaculture (Grisez & Ollevier, 1995). *Aeromonas hydrophila*, *Streptococcus* spp. and *Vibrio* spp. are the most common fish pathogens associated with great economic loss. Abuse and overuse of antibiotics in aquaculture has caused tremendous problems, with more and more antibiotics resistant fish pathogens detected around aquaculture sites (Cabello, 2006). There is also a danger of terrestrial veterinary pathogens and even human pathogens gaining antibiotic resistance genes in the aquaculture environment, through horizontal gene transmissions from bacteria (Heuer et al., 2009).

Despite the fact that the World Health Organization (WHO), Food and Agriculture Organization (FAO) of the United Nations and the World Organization for Animal Health (OIE) jointly issued guidelines for responsible use of antimicrobial agents for veterinary uses (FAO/OIE/WHO, 2006), application of antibiotics (e.g. quinolones) in aquaculture in some countries such as China and Chile is still common (Jacoby, 2005).

Traditional Chinese Medicines (TCMs) have been used for human therapeutic purposes as immune boosters or medicines. They have also been used in aquaculture for a long period of time, and some of these could serve as antimicrobial agents and immune-stimulants, to replace certain antibiotics used in aquaculture. To cite a few: By adding 1-2% of anthraquinone extract from rhubarb (*Rheum rhabarbarum*) as a feed supplement, it was effective in preventing pathogenic infection, mitigating overcrowding stress and promoting growth of common carp (Xie et al., 2008). Zakes et al. (2008) noted that juvenile pikeperch (*Sander lucioperca*) fed diet with 0.1% supplement of huanqi (*Astragalus radix*) improved feed conversion ratio and growth. In addition to enhanced

growth, Yin et al. (2006) showed 0.1 and 0.5% extracts of the same herb added in feeds enhanced lysozyme activity and phagocytosis by phagocytic white cells.

Mo (2014) revealed that the use of 0.2% (weight/volume) goji (*Lycium barbarum*) extract significantly improved the RWG, FCR, SGR, PER and digestibility of diets, leading to significantly higher total immunoglobulin bactericidal activity and anti-protease activity of Nile tilapia and grass carp, when compared with the control groups (without any addition of this herb extract). Our early study using the mixtures of four TCMs: *Radix scutellaria*, *Rhizoma coptidis*, *Herba andrographis* and *Radix sophorae flavescentis* at the ratio of 1:1:2:3 (2% weight/weight) added to feed pellets significantly improved the bactericidal activity and total immunoglobulin in the plasma of grass carp, after 21 days. Subsequent laboratory and field trials confirmed that 1% and 2% TCM feeding groups had a significant reduction of mortality against *Aeromonas hydrophila* (pathogen causing enteritis) challenge towards the end of both trials. It was shown that the cost of using TCM formulation in grass carp culture was insignificant and could be compensated by improved yield (Choi et al., 2014a).

Grass carp is the second important (in terms of production, more than 3 million t) freshwater fish species, with China contributed to about 95.7% of the global production in 2002 (FAO, 2009). Enteritis, a kind of intestinal infection is the most common problem during its juvenile stage, with a high mortality rate (Yang, 2008). The use of this TCM formulation seemed to be effective in stimulating immunity of grass carp, and preventing its *Aeromonas* infection. A patent related to this has been successfully filed (Wong et al., 2011). Choi et al. (2004b) also revealed that the modified Huanglian Jiedu decoction was effective on the disease resistance in grey mullet (*Mugil cephalus*) to *Lactococcus garvieae* (bacterium which caused infection).

It is hoped that other TCMs could be explored for combating other pathogens commonly encountered in aquaculture. There seems to be a large amount of potential herbs and their combinations, for dealing with different microbes commonly encountered in different fish species. Possible usage of TCM residues in freshwater (herbivorous) fish culture could also be trialed, as it is envisaged that the residues may still contain active ingredients.

5. HEALTH RISK ASSESSMENTS OF FISH CULTIVATED BY FOOD WASTES

As indicated above, environmental pollutants such as Hg and DDTs can be effectively bioaccumulated and biomagnified in the aquatic food chains, and they are commonly found in trash fish and fishmeal used for fish culture. It is therefore essential to conduct health risk assessments to ensure aqua-products cultivated by food waste based pellets are safe for human consumption.

Methyl mercury (MeHg), PAHs and DDTs are the most common contaminants found in different fish species, which are of public health concerns in our region. Health risk assessments were therefore conducted, based on the contaminant concentrations detected in fish (bighead carp, grass carp and mud carp) fed food waste based pellets, based on body weight of 60 kg for adult, and 21.8 kg for a child (Lee et al., 1994). The average fish consumption rate stipulated by the Centre for Food Safety in Hong Kong is 57.48 g/day (CFS, 2010), but we adopted the daily consumption rates of fish of 93 and 50 g/day for adults and children respectively (Leung et al., 2000), in order to more suited the local conditions, and to protect children.

5.2 Results of health risk assessments

The fish meal used in producing food waste based pellets was the major sources of MeHg, and DDTs, while bone meal the major source of PAHs. Results on health risk assessments obtained did not show any Life-time Cancer Risk for the three common contaminants detected in flesh of the 3 species fed the food waste based pellets, as well as the commercial pellets.

In terms of non-cancer risk, for both PAHs and DDTs, there were unlikely non-cancer risk exerted on both adults and children via consumption of all the 3 species fed with both food waste based pellets and the commercial diets (Cheng et al., 2015b; 2015c). However, for MeHg, there were potential non-cancer risks for MeHg via consumption of bighead carp, and common carp fed the commercial pellets in both adults and children, but not those fed food waste based pellets; and also in children via consumption of bighead carp fed FWB pellets. Non-cancer risks based on bioaccessible MeHg via consumption of mud carp fed the commercial pellets, were observed in children, but not those fed the food waste based pellets (Cheng et al., 2015d).

Results showed that consumption of grass carp (herbivorous species) will be safer than bighead carp (a filter feeder) and mud carp. Mud carp is an omnivorous fish which thrives at the bottom of fish ponds. It will be easily contaminated by sediment, which serves as sinks for environmental contaminants, notably Hg. Hopefully, replacing fish meal in the commercial feed pellets by food waste will be able to lower the MeHg concentrations in fish, in particular mud carp.

6. CONCLUSION

Using food wastes to formulate fish feed pellets seems to be feasible. Different combinations of food wastes should be chosen to suit the feeding modes of different fish species, notably freshwater fish associated with low-trophic levels, e.g. herbivores (such as grass carp) and omnivores (such as grey mullet). This is due to the fact that the protein and nutritional requirements of these low- trophic level fish are lower, when compared with carnivorous fish (such as freshwater bass). Food waste based pellets could be upgraded by adding enzymes and baker's yeast, leading to better growth rates

and enhanced immunity of the cultured fish. Inclusion of Chinese medical herbs would also help to replace certain antibiotics used in aquaculture.

In general, fish fed with food waste based diets are safer for human consumption, when compared with those fed the commercial diets, due to the higher contaminant concentrations in fishmeal contained in the commercial diets. Further studies should focus on the feasibility of adding different Chinese medicinal herbs, enzymes or other additives in order to enhance the quality and efficacy of fish feeds.

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822 **International Conference on Green Technology for Sustainable Ecosystems ICGTSE**
 823 **2016, at St. Joseph's College (Autonomous), Bengaluru, India, 26-27 Feb, 2016.**

824 **PREFACE**

825 The major purpose of this international conference is to provide a platform for
 826 researchers, professionals and academicians from different parts of the world, to
 827 present their ideas and research findings relevant to the area of Green Technologies for
 828 Sustainable Ecosystems.

829
 830 Theoretically, all the elements are recycled within normal healthy ecosystems.
 831 Unfortunately, emissions of persistent toxic substances (PTS) from different industries
 832 since industrial evolution have made recycling of different elements within different
 833 ecosystems rather difficult, and very often impossible. For example, mercury (Hg)
 834 emitted from coal-power plants is the major source of anthropogenic source of Hg in
 835 the world. The elemental Hg entered into the atmosphere could travel long distance,
 836 and deposit onto water and soil/sediment surface. Through the action of sulfide
 837 reducing bacteria under anaerobic condition, inorganic Hg is transformed to organic Hg,
 838 with methyl Hg (MeHg) the most toxic one, which could be bioaccumulated in aquatic
 839 organisms and biomagnified in food webs. Minamata disease happened in Minamata
 840 Bay (a fishing village), Japan was in fact acute poisoning of villagers who consumed
 841 seafood contaminated with extremely high concentrations of MeHg, due to the
 842 discharge of inorganic Hg and its subsequent methylation. Nowadays, the long-term
 843 exposure of lower concentrations of MeHg in seafood, especially predatory fish (such as
 844 tuna and swordfish) seems to be a worldwide public health concern.

845
 846 In fact, a wide range of PTS: persistent organic pollutants (POPs), such as DDTs and
 847 dioxins, and heavy metals, such as Hg, lead and cadmium, is also emitted to the
 848 environment, finding their ways to various food production systems, with products
 849 imposing human health risks. Furthermore, some chemicals such as DDT and PCB,
 850 widely used in the past, had been banned, due to their long-term environmental and
 851 health effects. Emerging chemicals of concern are chemicals that have not been tested
 852 thoroughly when they first entered the market, and detrimental effects on human
 853 health have been noted, after being used for a number of years, e.g. flame retardant,
 854 bisphenol A, phthalates, etc. All these PTS will impose environmental and health hazards
 855 like Hg, although under different exposure routes, and different extents on health
 856 impacts.

857
 858 In order to achieve a more sustainable ecosystem, various green technologies, such as
 859 green production, zero discharge, etc. should be adopted in various industries, to avoid
 860 generation/emission of wastes and PTS. In terms of food production, green agriculture
 861 and aquaculture should be practiced, to ensure food products are safe for consumption,
 862 in addition to a cleaner environment. Waste materials should be treated as resources,
 863 as much as possible, rather than wastes. This is especially true for biological waste,
 864 which should be recycled as food, feed and fuel. The large amount of food waste

generated daily has become a growing concern in densely populated cities, especially those cities which have become more affluent recently. Proper treatment and disposal of food waste is an urgent matter.

Turning food waste to animal feeds, especially fish feeds would be a viable alternative. Laboratory and field trials have been confirmed that appropriate portions of different food waste (e.g., fruit, vegetables, rice, meat, etc.) could be incorporated into fish feed pellets to cultivate lower-trophic level fish, such as grass carp, grey mullet and tilapia, which have a lower nutritional requirement. Further upgrades of food wastes by adding different supplements (e.g., prebiotics, probiotics, Chinese medicinal herbs, etc.) could further improve fish growth and fish health. In addition, fish fed food waste based pellets are safer for consumption as they contained lower concentrations of contaminants, such as MeHg, DDT and PAH, which are often found in trash fish and commercial fish feed pellets (which contained a high proportion of fish meal, made from trash fish).

These fish species are cultivated in the same pond, under “polyculture” mode, which was commonly adopted in the past, throughout Asian and some Eastern European countries. These fish species possess different ecological niches and modes of nutrition, and they are able to utilize all the residues derived from the waste efficiently, within the same pond. Grass carp occupies the upper water column and consumes macrophytes (herbivore), bighead carp stays in middle of the water column and filters planktons (filter feeder), while tilapia eats all sorts of materials (omnivore). There are other popular species also used in polyculture: mud carp, silver carp, grey mullet, etc. Pig manure was the major waste material (as pond fertilizer to enrich nutrients in pond water) added into fish ponds in the past, but its usage has been gradually phased out. It could be replaced by food waste. The practices of some of these green technologies which were popular in the past, should be revisited, and should be adopted or modified, to suit the changing environment.

There are other green technologies to be discussed in this important and timely meeting, for achieving sustainable ecosystems. Hopefully, information included in this book will generate interests, for further meetings and even collaborations, in the near future.

Ming-Hung Wong, BSc (CUHK), MSc, PhD, DSc (Durham), MBA, DSc (Strathclyde)
 Research Chair Professor of Environmental Science, Hong Kong Institute of Education
 Chang Jiang Chair Professor of Environmental Science, Ministry of Environment, China
 Emeritus Chair Professor of Biology, Hong Kong Baptist University
 Editor-in-Chief, Environmental Geochemistry and Health (Springer)
 Book Series Editor, Urbanization, Industrialization, and Environment (CRC/Taylor & Francis)