World Journal of Fish and Marine Sciences 8 (4): 164-169, 2016
ISSN 2078-4589

DOI: 10.5829/idosi.wjfms.2016.164.169

Integrating Multi-Trophic Species to Improve Nutrient Retention Using Closed Aquaculture System

[1]Zaki Sharawy, [2]Mohamed S. Hassaan and [2]Ashraf M. A.S Goda

[1]Aquaculture Division, Invertebrates Laboratory,
National Institute of Oceanography and Fisheries (NIOF), Suez, Egypt
[2]Aquaculture Division, Fish Nutrition Laboratory,
National Institute of Oceanography and Fisheries (NIOF), Cairo, Egypt

Abstract: In the present study, both whiteleg shrimp and red tilapia (all male cultivar) were maintained separately in monoculture (control groups) and in co-culture under controlled conditions in a recirculating aquaculture system (RAS). Both monoculture fish (T_1) and monoculture shrimp (T_2) received their commercial diets. Shrimp in co-culture either fed only on tilapia waste (T_3) or on tilapia waste with a low supplement of commercial shrimp diet (T_4). At the end of the experiment, weight gains and specific growth rates were significantly higher in tilapia and shrimp of both co-culture treatments (T_3 and T_4). Growth rates results clearly indicated that tilapia bio-waste/faeces can be used as a sole diet for high value shrimp irrespective of the feeding regime. Lower survival rates in the T_3 treatment remain however unexplained. Based on the costs of feed, the highest income was detected in T_3 and T_4 respectively. Overall, current results show that the integration of these species together was highly successful in terms of nutritional provision and shrimp health status especially when companied with RAS. Although the concept is not very new but it is innovative when came to apply and more investigations are needed.

Key words: RAS · IMTA · Monosex · Red tilapia · Whiteleg shrimp

INTRODUCTION

Integrated multi-trophic aquaculture (IMTA) systems combine species with different feeding and habitat requirements to maximize production through efficient occupation of physical space and resource use [1- 4]. Such systems must maximize the use of food and space resources without provoking feed competition between the cultured species [5, 6]. IMTA creates new food sources from system wastes as species often feed on each other's faeces, generates less organic matter as waste and production units subsequently become more profitable through the sale of two or more species that are well accepted in the market [1, 7]. Integrated culture of shrimp and tilapia is an example of a highly promising IMTA production method with an increasing number of proven benefits including markedly reduced diet costs [8]. As with all IMTA, both biotic and abiotic conditions in the co-culture systems must be optimal to ensure better growth and survival rates of cultured species. This includes, but not limited to, ensuring the optimal nutritional provision to all culture species as well as ensuring that conditions do not induce stress on all integrated species. Diet provision, in particular quantity and quality of dietary protein are the primary factors influencing shrimp growth, nitrogen loading of the culture system and feed costs [9]. Hence, the protein content as well as the size and age of animals [10] or the addition of probiotics to the diet [11] affect the activity of midgut gland enzymes. Lee and Lawrence [12], found that the exhibition of several enzymes in *L. vannamei*: trypsin, carboxy-peptidase A and B, leucine aminopeptidase, acid protease, general protease, amylase, chitinase, esterase and lipase. Trypsin and chymotrypsin, for example, are the most abundant proteases present in the midgut gland of decapods [13]. Nevertheless, the expressed efficiency is less important for the assessment of nutrients in diet than the true potential, i.e. ability to hydrolyze a suitable diet [10].

Corresponding Author: Zaki Sharawy, Aquaculture Division, Invertebrates Laboratory, National Institute of Oceanography and Fisheries (NIOF), Suez, Egypt. Tel: +201001845218, E-mail: zaki_sharawy@yahoo.com.

In the current study, whiteleg shrimp (*Litopenaeus vannamei*) and red tilapia (*Oreochromis niloticus*) were maintained separately in monoculture treatments and also in two distinct IMTA treatments. In the first IMTA treatment only the Tilapia were fed and the shrimp fed solely on Tilapia feces and fish food remains. In the second IMTA treatment, Tilapia were fed with fish food and the shrimp were provided with 25% of the recommended commercial diet. Growth, feeding efficiency and nutrition efficiency rates were followed.

MATERIALS AND METHODS

Aquatic Species Husbandry: Whiteleg shrimp, *Litopenaeus vannamei* post larval (PL_{12}) were bought from SIS commercial hatchery in Miami, Florida (USA). All male red tilapia, *Oreochromis niloticus* were purchased from private farm, TilapiCo (The Netherlands). Shrimp and tilapia were acclimated separately to the experimental conditions (salinity of 12-15% and temperature of 28°C) for three weeks at the Centre for Aquaculture Research (ZAF) within the Alfred Wegener Institute, Helmholtz Centre for Polar and Marine Research (AWI) in Bremerhaven, State of Bremen, Germany in recirculating aquaculture system (RAS) under indoor conditions. During the acclimation period, shrimp and red tilapia were fed with a commercial diet (40% crud protein) to satiation four times daily.

Experimental Design and Culture Technique: Preliminary experiment was conducted to construct a holding system for best possible experimental co-culture of these two species (red tilapia and whiteleg shrimp) at the Alfred-Wegener-Institute (AWI) within the Centre for Aquaculture Research (ZAF) in Bremerhaven city, State of Bremen, Germany in recirculating aquaculture system (RAS) under indoor conditions for 90 days. Four units were conducted in this study, each unit consisted of two plastic box frames, placed one on top of the other, the upper pox was (35×60×24cm) to stock red tilapia (12 fish/box) and the downer was (15×60×24cm) to stock shrimp (50 shrimp/box). Two boxes in each unit were tied together and coated with 1mm nylon nets to avoid loss of shrimps, feeds and feces. Moreover, two boxes were connected together with 40cm tube with diameter of 7cm to provide shrimp diets. Each tube was connected with 65cm rubber hoses connected with small pump to pump faces from upper box to downer one. Four units were completely randomized then transferred and emerged into one tank (600L) with three repetitions, attached to the recirculating aquaculture system. The first (T_1) and second unit (T_2) are monoculture which containing (red tilapia in the upper box and no shrimps in the downer) and (no red tilapia in the upper box and shrimp in the downer box), respectively. Fish in T_1 fed commercial diet at a rate of 5% of its biomass. Shrimp in T_2 fed commercial shrimp diet at a rate of 10% of its biomass. The third (T_3) and four units (T_4) are integrated multi-trophic aquaculture (IMTA) which contained (red tilapia in the upper box and shrimps in the downer) in each unit. Fish in T_3 were fed commercial diet at a rate of 5% of its biomass, but shrimp were fed the waste of fish faces only. Fish in T_3 were fed commercial at a rate of 5% of its biomass, but shrimp were fed the waste of fish faces only. Whereas, fish of T_4 were fed commercial diet) at a rate of 5% of its biomass and shrimp of the same treatment T_4 were fed commercial shrimp diet at a rate of 2.5% (to provide any micronutrients which may needed for shrimp and not existed in fish feces) of its biomass as well as the waste of fish faces only. Fish and shrimp which received commercial diet (370g protein kg $diet^{-1}$, 19.1MJ gross energy kg $diet^{-1}$ and 410g protein kg $diet^{-1}$, 20.1MJ gross energy kg $diet^{-1}$) respectively, were fed three times daily at 7:00, 12:00 and 18:00h. Aeration was continuously provided using compressed air. A photoperiod of 12h light, 12h dark (08.00–20.00h) was used. Fluorescent ceiling lights has supplied the illumination.

Water Quality: Salinity, temperature, dissolved oxygen, pH and ammonia were continuously monitored during the experiment. Water temperature was recorded daily at 13.00h using a mercury thermometer placed at 30cm depth. Dissolved oxygen was measured at 07:00h using a YSI model 56 oxygen meter (YSI Company, Yellow Springs Instrument, Yellow Springs, Ohio, USA) and pH at 09.00h by using a pH meter (Orion pH meter, Abilene, Texas, USA). Ammonia was measured three times a week according to APHA [14]. Water and subsamples of feces were taken twice a week for ammonia and phosphate analysis.

Growth Performance and Nutrient Utilization Indices: Fish and shrimp PL weight (g) and length (cm) was determined at the initial of the experiment and, thereafter, every two weeks for 90 days. Shrimp weight gain (WG), specific growth rate (SGR), survival % (SR), feed

conversion ratio (FCR), Protein efficiency ratio (PER), protein productive value (PPV), energy retention (ER) and fat retention (FR) were calculated using the given formula as:

SR=(Final number of shrimp/Initial number of shrimp)×100.

WG = Final body weight (g) - Initial body weight (g); SGR = [(ln FBW - ln IBW)/t] ×100; where: FBW is final body weight (g); IBW is initial body weight (g); ln= natural logarithmic; t = time in days; FCR= Total weight of feed consumed / Wet biomass gain; PER = Weight gain (g)/Protein fed (g); PPV = (Protein gain (g) / Protein intake (g)) x 100.

Chemical Composition of Fish and Shrimp: At the experimental onset, a random pooled sample of five red tilapia and twelve shrimps were collected and stored at -20°C for the determination of the initial whole-body proximate composition. At termination of the feeding trial, three red tilapia and three shrimp were randomly selected from each tank, to determine the proximate whole body composition. The animals were blended and homogenized, oven-dried, ground and stored at -20°C for subsequent analysis. The proximate composition of red tilapia and shrimp samples were determined according to AOAC [15]. Dry matter was determined after oven drying (105°C) for 3h (MEMMERT Drying Oven, GE-174, Memmert GmbH, Germany) [15] method number 930.15. Ash was measured by incineration at 550°C for 12 h (Thermo Scientific Heraeus M 110 Muffle Furnace, Thermo Fisher Scientific Inc., Waltham, MA 02454, Germany) [15] method number 942.05. Crude protein was determined by the micro-Kjeldhal method, N% × 6.25 (using Kjeltech autoanalyzer, Model 1030, Tecator, Höganäs, Sweden) [15] method number 984.13 and crude fat by Soxhlet extraction with diethyl ether (40-60°C) (Soxtec System HT6, Tecator, Höganäs, Sweden) [15] method number 920.39.

Statistical Analysis: Statistical analysis was conducted using a statistical package (SPSS 13.0 for Windows; SPSS Inc., Chicago, IL, USA). Before analysis, data in the form of percentages were transformed (arcsine of the square root) to obtain normality and homogeneity, but were presented as non-transformed percentages. One-way analysis of variance (ANOVA) was used to test for significant difference ($P<0.05$) between treatment and control groups for measured parameters. Duncan's multiple range test was used to compare differences between treatment means when significant F values were observed [16]. The effects of the co-culture on the antioxidant-related gene expression were assessed by one-way ANOVA followed by Tukey's multiple comparisons test using GraphPad Prism version 6.00 for Windows, GraphPad Software, La Jolla California USA, www.graphpad.com. P value < 0.05 was considered to be statistically significant. Data are reported as means± standard error of mean (SEM) unless otherwise stated.

RESULTS

Growth Performance and Nutrient Utilization: Growth performances and feed utilization expressed by the body weight, specific growth rate and survival rate of Red tilapia and shrimp rearing in mono and integrated culture system are summarized in Table 1&2. Growth performance of Red tilapia in monoculture (T_1) was significantly ($P<0.05$) higher in final body weight (FBW) and specific growth rate (SGR) compared with red tilapia in integrated multi-trophic aquaculture (IMTA) system. However, the shrimp in IMTA system (T_4) which was received fish feces only recorded the higher value of FBW, WG, SGR and survival rate compared with shrimp in mono (T_1) and IMTA (T_3). Feed conversion ratio for Red tilapia was significantly ($P<0.05$) lower (best) in monoculture than IMTA. Also, the same trend was observed in PER. With regard to shrimp, Feed conversion ratio was significantly ($P<0.05$) lower (best) in the IMTA system (T_4) compared with the shrimp in monoculture and shrimp in IMTA system (T_3). Protein efficiency ratio of shrimp in IMTA system (T_4) was significantly ($P<0.05$) than other culture.

Chemical Composition: The influence of monoculture and IMTA system on body composition of Red tilapia and shrimp is present in Table (3). No significant differences ($P<0.05$) of dry matter, protein, lipid contents in red tilapia and shrimp which was stocked monoculture or IMTA system. However, Ash content was significantly higher in red tilapia and shrimp of IMTA system (T_3).

Economical Evaluation: The calculation of the economic efficiency of monoculture and IMTA system was based on the costs of feed. As described in Table (4) the highest income was detected in T_3 followed by T_4.

Table 1: Growth performance for red tilapia and shrimp culture either monoculture or IMTA systems.

	Monoculture				IMTA system			
	T_1		T_2		T_3		T_4	
Items	Tilapia	Shrimp	Tilapia	Shrimp	Tilapia	Shrimp	Tilapia	Shrimp
IBW (g)	10.92±2.59	-	-	0.024±0.061	10.92±2.59	0.024±0.061	10.92±2.59	0.024±0.061
F BW (g)	173.65±5.46^a	-	-	9.62±1.06c	139.16±1.80^b	11.32±1.48^b	130.33±4.47^c	13.02±2.38^a
SR (%)	79.17±6.24^b	-	-	81.5±2.50^a	91.67±4.16^a	78.50±4.50^b	81.25±2.08^b	46.00±7.00^c
WG (g)	162.73±5.22^a	-	-	9.60±1.21^c	128.24±3.94^b	11.30±3.25^b	119.41±3.11^c	13.00±5.27^a
SGR	2.38±0.19^a	-	-	6.60±0.06^b	2.16±0.20^a	6.76±0.17^b	2.09±0.08^b	6.90±0.38^a

Values are mean ± SEM. Values in the same row with same superscripts are not significantly different (P > 0.05).

Table 2: Feed utilization for red tilapia and shrimp culture either monoculture or IMTA systems.

	Monoculture				IMTA system			
	T1		T2		T3		T4	
Items	Tilapia	Shrimp	Tilapia	Shrimp	Tilapia	Shrimp	Tilapia	Shrimp
FCR	1.43	-	-	1.31	1.56	1.20*	1.67	0.81**
PER	2.24	-	-	1.83	1.42	0.16*	1.41	3.14**
PPV	34.20			26.97	23.26	2.55*	26.97	47.88**

*Estimated based on body gain

**Calculated based on shrimp data only (with regardless to leftover feed and feces effect)

Table 3: Chemical composition for red tilapia and shrimp culture either monoculture or IMTA systems.

	Monoculture				IMTA system			
	T1		T2		T3		T4	
Items (%)	Tilapia	Shrimp	Tilapia	Shrimp	Tilapia	Shrimp	Tilapia	Shrimp
Dry mater	25.91±1.2	-	-	26.51±1.3	25.43±1.2	25.63±1.1	2.62±0.21	25.44±1.2
Protein content	49.34±3.6	-	-	61.63±2.3	58.37±3.5	61.41±2.3	56.39±2.3	60.14±2.4
Lipid content	19.50±0.78	-	-	20.82±0.89	19.60±1.02	20.66±1.5	18.30±1.2	20.35±1.2
Ash content	13.23±0.51	-	-	15.4±0.6	14.33±1.2	17.61±1.2	15.37±0.98	18.23±1.3

Values are mean ± SEM. Values in the same row with same superscripts are not significantly different (P > 0.05).

Table 4: Economic evaluation for red tilapia and shrimp culture either monoculture or IMTA systems.

	Monoculture				IMTA system			
	T_1		T_2		T_3		T_4	
Items	Tilapia	Shrimp	Tilapia	Shrimp	Tilapia	Shrimp	Tilapia	Shrimp
Totalproduction(kg)/treatment	7.08	-	-	1.64	6.40	2.13	5.26	1.40
Total cost of feed (EURO)	8.01	-	-	2.15	7.94	2.56	7.03	1.13
Total income (EURO)	49.56	-	-	21.32	44.8	27.69	36.82	18.20
Net income (EURO)	41.46	-	-	19.17	36.86	25.13	29.79	17.09

DISCUSSIONS

The integrated multi-trophic aquaculture (IMTA) technique is characterized by cultivating two or more species of aquatic organisms of different trophic levels in a single production unit, this system could, therefore, be a viable alternative to make aquaculture more profitable through efficient occupation of physical space and different ecological niches and sustainable [6, 17]. Both tilapia, *Oreochromis niloticus* and whiteleg shrimp *Litopenaeus vannamei* are two extremely commercially important tropical aquaculture species suitable for tank and pond culture systems and they are considered as a highly promising production within IMTA [8]. In the present study final body weight of the shrimp in IMTA system which did not offer any extra diets, but is fed only with the waste of red tilapia was higher than shrimp in IMTA system was fed shrimp diet at 2.5% of biomass as well as feces of red tilapia may be budget nutrient in this system which assume to a cause of more stress on both fish shrimp consequently. Dietary protein requirements of Penaeidea range between 27 and 50% depending on species [18]. Interestingly, in this study, the level of protein and lipid of waste of fish on dry matter base was 33.5 and 0.4% respectively, survival rate in shrimp was higher when it was fed with this waste only than that it was fed by this waste and supplementary diet. Biowaste of fish could provide certain nutrients or digestion regulators to shrimp, which were vital to the metabolism of shrimp. Besides, beneficial bacteria in wet fish waste also benefit for growth [19]. The present study, indicated that shrimp has the potential of reducing organic pollution of red tilapia waste. As a measure to reduce this organic loading it has been suggested to cultivate inorganic and organic extractive species at lower trophic levels in close vicinity or together to the fish farms in IMTA system. In conclusion, our combined systems (IMTA and RAS) have two non-conflicting overall objectives: 1) Increased biomass production and added value based on the feed investments and 2) mitigating potentially negative environmental impacts of waste nutrients. In this way, IMTA may contribute to a more sustainable aquaculture production [20, 21]. Such co-culture can optimize the use of food, maximize the production of both species, decrease the generation of organic matter and and finally, make aquaculture facilities more profitable. However, biotic and abiotic conditions in co-culture systems must be optimal to ensure growth, health and survival. As with monoculture, this includes ensuring the optimal nutritional provision to all culture species as well as ensuring that conditions do not induce stress. However, more investigations are needed to apply such system commercially.

ACKNOWLEDGEMENT

The authors wish to acknowledge the support of Ms. Christina Hörterer, Mr. Kai Lorkowski and Mr. Jan Köbel in assisting during system design and sampling. The project was funded by the Science and Technology Development Fund (STDF), Capacity Building Grant no. 12326, Ministry of Scientific Research, EGYPT. The authors are grateful for all the support.

REFERENCES

1. Lutz, C.G., 2003. Polyculture: principles, practices, problems and promise. Aquaculture Magazine, 5: 34-39.
2. Milstein, A., A.F. Ahmed, O.A. Masud, M.A. Kadir and M.A. Wahab, 2006. Effects of the filter feeder silver carp and the bottom feeders mrigal and common carp on small indigenous fish species (SIS) and pond ecology. Aquaculture, 258: 439-451.
3. Goda, A.M.A-S., M.A. Essa, M.S. Hassaan and Z. Sharawy, 2014. Bio–Economic Study of Freshwater Integrated Multi-Trophic Aquaculture (IMTA) Systems in Egypt. World Applied Sciences Journal, 32: 2367-2374.
4. Goda, A.M.A-S, M.A. Essa, M. Abou-Taleb, M.S. Hassaan, M.E. Goher and Z. Sharawy, 2015. Utilization of Fish Feed Waste by Freshwater Mussels of *Aspatharia chaiziana* and *Aspatharia marnoi* (Family: Iridinidae) in Integrated Multi-Trophic Aquaculture (IMTA) System. World Journal of Fish Marine Sciences, 7: 185-194.
5. Chopin, T., A.H. Buschmann, C. Halling, M. Troell, N. Kautsky, A. Neori, G. Kraemer, J. Zertuche-Gonzalez, C. Yarish and C. Neefus, 2001. Integrating seaweeds into aquaculture systems: a key towards sustainability. Journal of Phycology, 37: 975-986.
6. Muangkeow, B., K. Ikejima, S. Powtongsook and Y. Yi, 2007. Effects of white shrimp, *Litopenaeus vannamei* (Boone) and Nile tilapia, *Oreochromis niloticus* L., stocking density on growth, nutrient conversion rate and economic return in integrated closed circulation system. Aquaculture, 269: 363-476.
7. McGinty, A.S. and D.E. Alston, 1987. Polyculture of all male tilapia hybrids with low densities of *Macrobrachium rosenbergii*. Journal of Agriculture of the University of Puerto Rico, 71: 225-229.

8. Henry-Silva, G.G., C.S.P. Maia, R.S.T. Moura, J.A.P. Bessa and W.C. Valenti, 2015. Integrated multi-trophic culture of Nile tilapia (*Oreochromis niloticus*) and Amazon river prawn (*Macrobrachium amazonicum*) in brackish water. The Arquivo Brasileiro de Medicina Veterinária e Zootecnia, 67: 265-273.
9. Sharawy, Z., A.M.A-S. Goda and M.S. Hassaan, 2016. Partial or total replacement of fish meal by solid state fermented soybean meal with *Saccharomyces cerevisiae* in diets for Indian prawn shrimp, *Fenneropenaeus indicus*, Postlarvae. Animal Feed Science and Technology, 212: 90-99.
10. Lee, P.G., L.L. Smith and A.L. Lawrence, 1984. Digestive proteases of *Panaeus vannamei* Boone: relationship between enzyme activity, size and diet. Aquaculture, 42: 225-239.
11. Wang, Y.B., 2007. Effect of probiotics on growth performance and digestive enzyme activity of the shrimp *Penaeus vannamei*. Aquaculture, 269(1-4): 259-264.
12. Lee, P.G. and A.L. Lawrence, 1982. A quantitative analysis of digestive enzymes in penaeid shrimp; influence of diet, age and species. Physiologist, 25: 241.
13. Lemos, D., J.M. Ezquerra and F.L. García-Carreño, 2000. Protein digestion in penaeid shrimp: digestive proteinases, proteinase inhibitors and feeding digestibility. Aquaculture, 186: 89-105.
14. APHA 1995. WPCF, Standard methods for the examination of water and wastewater. American Public Health Association/American Water Works Association/Water Environment Federation, Washington DC, USA.
15. AOAC, 1995. Official Methods of Analysis of the Association of Official Analytical Chemistry (16th ed.). AOAC International, Washington.
16. Duncan, D.B., 1955. Multiple range and multiple *F* tests. Biometrics, 11: 1–42.
17. Neori, A., T. Chopin, M. Troell, A.H. Buschmann, G.P. Kraemer and C. Halling, 2004. Integrated aquaculture: rationale, evolution and state of the art emphasizing seaweed bio?ltration in modern mariculture. Aquaculture, 231: 361-391.
18. Allan, G.L., B.M. Smith, 1998. Recent nutrition research with Australian penaeids. Reviews in Fisheries Science, pp: 113-127.
19. Yuan, X.T., H.S. Yang, Y. Zhou, Y.Z. Mao, T. Zhang and Y. Liu, 2006. The influence of diets containing dried bivalve feces and/or powdered algae on growth and energy distribution in sea cucumber *Apostichopus japonicus* (Selenka) (Echinodermata: Holothuroidea). Aquaculture, 256(1-4): 457-467.
20. Chopin, T., S.M.C. Robinson, M. Troell, A. Neori, A.H. Buschmann and J. Fang, 2008. Multitrophic integration for sustainable marine aquaculture. In: Jørgensen SE, Fath BD (eds) The Encyclopedia of Ecology. Elsevier, pp: 2463-2475.
21. Troell, M., A. Joyce, T. Chopin, A. Neori, A.H. Buschmann and J.G. Fang, 2009. Ecological engineering in aquaculture – Potential for integrated multi-trophic aquaculture (IMTA) in marine offshore systems. Aquaculture, 297: 1-9.