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The Combination of Aquatic Species in Integrated Multi-Trophic Aquaculture with Paddy in Brackish Water: An Investigation of Feed Utilization Performance

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ABSTRACT This study investigated the performance of artificial feed in the Integrated Multi Trophic Aquaculture (IMTA) system through a combination of milkfish, tiger shrimp, and clam involving paddy in brackish water. The three combination groups (C) and triplicate including milkfish and paddy (C-2), milkfish, tiger shrimp, and paddy (C-3), and milkfish, tiger prawns, clams, and paddy (C-4) were examinated for 80 days on a model sheeting pond designed to have a volume of 280 L and a density of 30 individuals. Meanwhile, 30 clumps of paddy were maintained through the floating method which is constructed from bamboo with an area of 1 m^2. Feeding 3 times a day with 5% of biomass resulted a mean Feed Conversion Ratio (FCR) and Index Profit (IP) of 2.1 and 2.0 for C-2, 2.0 and 2.9 for C-3, and 1.9 and 3.1 for C-4, respectively. The results showed that the best trend pattern were the combination of C-3 species which were statistically found to have a significant effect ($P<0.05$) on FCR and IP performances. The findings of current study have practical significance for the expansion of co-culture of paddy and aquatic animals in brackish water which important by ecologically, biologically, and economically.

Keywords: Brackish water; feed conversion ratio; IMTA-Paddy; index profit; species combination

INTRODUCTION

The trade-off phenomenon associated with artificial feed utilization in aquaculture is currently an interesting focus of study in feed management in line with the ecosystem approaches towards sustainable aquaculture. It is important to note that aquaculture production depends on the survival and growth of organisms which are largely determined by the quality and quantity of artificial feed. Moreover, feed costs take more than 50% of the production cost and this further affects profitability (Braga *et al.*, 2016; Fry *et al.*, 2018; Bharati *et al.*, 2019). The feed usually utilized is not more than 50% with the rest passed as waste in the form of residual feed and excretion such as feces and urine (Bosma & Verdegem, 2011; Altinok & Ozturk, 2017; Azad *et al.*, 2017).

Co-culture is a sustainable aquaculture model developed to increase productivity while simultaneously reducing costs and ecological impact (Zhang *et al.*, 2019; Bashir *et al.*, 2020; He *et al.*, 2020). An example of this system widely used is the Integrated Multi Trophic Aquaculture (IMTA) and paddy-fish/shrimp (P-F/S) which is known as Minapadi in Indonesia. The IMTA system integrates different levels of tropical species to create several advantages such as nutrient waste utilization, productivity as well as social and economic benefits (Chang *et al.*, 2019; Knowler *et al.*, 2020). Moreover, the P-F/S system which involves the integration of paddy and aquatic animals species also provides ecological, social, and economic advantages (Kunda *et al.*, 2014; Nurhayati *et al.*, 2016; Li *et al.*, 2019; Bashir *et al.*, 2020; He *et al.*, 2020). These two sustainable aquaculture systems, in principle, integrate synergistically different species in order to utilize all the organic and inorganic nutrients in the water column including the waste from the artificial feed.

The development of the IMTA systems in seawater, brackish water, and freshwater through several combinations of species has been widely reported and the same was observed from the implementation of the P-F/S system in freshwater but its application in brackish water is limited. Meanwhile, paddy and tiger shrimp (P-S) co-culture has been applied in brackish water by the Research Institute for Coastal Aquaculture and Fisheries Extension, Maros, Indonesia (Sahabuddin *et al.*, 2019). There is, however, the need for more research on different models, species, locations, and previous studies on the application of the IMTA and P-F/S systems. They are, therefore, integrated into a dual co-culture system in this study and this is relevant to the principles of the Ecosystem Approach to Aquaculture which has been described to be sustainable, prosperous, and multisectoral (FAO, 2018).

This present study involved the integrated cultivation of milkfish (*Chanos chanos*), tiger shrimp (*Penaeus monodon*), mussels *Glauconome virens* as well as paddy (*Oryza sativa*) which is an important foodstuff for the world's population. Each species in integrated aquaculture is required to be at different trophic levels including the fed, inorganic extractive, and organic extractive species in order to provide different functions in the ecosystem (Buck *et al.*, 2018; Chang *et al.*, 2019; Zhang *et al.*, 2019; Knowler *et al.*, 2020). Paddy is specifically realized due to the tolerance of different varieties to brackish water (Jamil *et al.*, 2016). The Paddy in this study were reared by this floating method which has been widely applied (Kang *et al.*, 2016; Irianto *et al.*, 2018) to demonstrate a more adaptive capacity and sustainable production (Nguyen & Howie, 2018) as well as the ability to remediate ecological waters and assimilate nutrients effectively to ensure efficient growth (Srivastava *et al.*, 2017).

A key factor observed to be affecting the success of co-culture operations is the combination of species influencing the pond ecosystem's ability to utilize the nutrient waste (Wahab *et al*., 2011). The right combination was reported to have minimized the antagonistic and maximize the synergistic of multispecies (Milstein, 1992) and this means the selection of the appropriate combination with synergistic ecological functions is very relevant to the cultivation of multispecies. This research is, however, the first to simulate a combination of species in a dual co-culture system (IMTA-Paddy) in brackish water with the focus on the performance of feed utilization due to its significance to production cost and ecology. The results are expected to provide basic knowledge on the cultivation of the IMTA-Rice system in brackish water with the potential for development along coastal areas. It is also hoped that it can be applied by cultivators, especially the small-scalers which are numerous in Indonesia, to increase productivity.

MATERIALS AND METHODS

Materials

Experimental tanks were constructed using wood and sheets measuring 200 cm × 200 cm × 100 cm placed on the surface of the pond with a volume of 280 L as indicated in Figure 1. For paddy clumps, it was planted using palm fiber and soil in a medium measuring 100 × 100 cm which was floated in an experimental tank as shown in Figure 2. Meanwhile, milkfish and tiger shrimp were used as fed species, mussels as organic extractive species, and paddy

Figure 1. Experimental unit.

Figure 2. Media floating of paddy.

as the inorganic extractive species. Water quality measuring instruments used in this study included a digital CE thermometer, an ATC handrefractometer, a Lutron DO-5509 DO meter, a digital Lutron PH-201, and a 752 Uv-Vis spectrophotometer. Other tools used are Camry EHA401 digital scales and 500 mL sampling bottles.

Methods

The study was conducted from May to August 2020 in the traditional ponds of Lerang Village, Lanrisang District, Pinrang Regency, South Sulawesi Province, Indonesia at coordinates 3°54'19.0" South Latitude and 119°33'59.8" East Longitude. It is important to note that milkfish and tiger shrimp seeds were obtained from the nursery unit around the research site with an initial weight of 12.3±0.1 g and 0.4±0.1 g, respectively while mud clam were collected from the estuary of Lanrisang District with an initial weight of 30.9±0.2 g. The organisms were, however, acclimatized to the experimental conditions for 3 days before the experimentation was initiated. Meanwhile, the paddy seeds obtained from the Research Institute for Cereal Plants of Maros Indonesia were first sowed in the pond until they reach a plant height of 17.8±0.10 cm.

The research created three combination groups in triplicate and these include the combination of milkfish and paddy as C-2 species followed by milkfish, tiger shrimp, and paddy as C-3 species and milkfish, tiger shrimp, mud clam, and paddy as C-4 species as indicated in Figure 3. The density of the milkfish, tiger shrimp, and mud clam is 30 individuals per tank, respectively. Meanwhile, the milkfish and tiger shrimp received a commercial diet thrice a day at 06.00 am, 12.00 am, and 06.00 pm at the rate of 5% biomass during the experiment. The commercial feed used based on the label packaging contains protein (min) 21%, fat (min) 8%, fibre (max) 9%, ash (max) 9%, and water content (max) 12%. The milkfish and tiger shrimp were weighed every 10 days to determine the increase in weight. An aeration system was designed using blowers connected to plastic pipes in each experimental unit to provide continuous air.

The temperature, salinity, dissolved oxygen (DO), and pH were measured every day in the morning and evening. Meanwhile, the ammonia was measured at the beginning, middle, and end of the experiment at the Water Quality Laboratory, State Agricultural Polytechnic, Pangkep. The water samples were analyzed in the laboratory according to the American Public Health Association 2005 standard method (Greenberg, 1984) and all the water quality parameters were measured in each tank.

The feed performance was expressed as the Feed Conversion Ratio (FCR) and the value was calculated at the end of the experiment using Lalramchhani *et al*. (2020) method as follows:

$$\text{FCR} = \frac{\text{feed applied (g)}}{\text{live weight gain (g)}}$$

It is important to note that the total feed applied obtained from the total feed given based on the biomass of milkfish and tiger shrimp while the live weight gain were obtained from the total weight of milkfish and tiger shrimp at the end, respectively during the rearing period. It was assumed that milkfish and tiger shrimp consumed the feed provided. The weight of the organisms and the feed was measured using

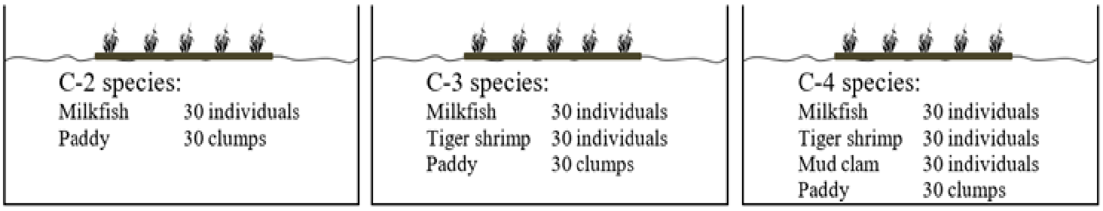

Figure 3. Treatments of experiment

a digital scale with an accuracy of 0.1 g.

A simple economic analysis was later conducted using the Index of Profit (IP) to determine the cost-efficiency of feed and this involved using Obirikorang *et al*. (2016) method as follows:

$$IP = \frac{\text{values of fish}}{\text{cost of fish}}$$

The value of fish and the cost of feed was assumed based on local market prices at the time of the research while the weight of the fish products was the absolute growth produced during the rearing and not the final weight.

Data FCR and IP were expressed as mean ± standard deviation (SD). Based on the Shapiro-Wilk Test of Normality and the Test of Homogeneity of Variances, all data were normally distributed and has homogeneity of variance (P>0.05). Furthermore, FCR and IP among different combination species were analyzed throught one-way ANOVA and Tukey's HSD test at a significant rate of 95% (p<0.05). All of the statistical analyses were performed using the IBM SPSS Statistics Version 25 (SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

Water Quality

The result measuring of water quality parameters for each species combination during in 80 days cultivation are summarized in Table 1. The five general water quality parameters recorded during the study did not show wide variations in each biota combination treatment. Temperature and salinity are relatively stable, and their range is not a problem for milkfish and tiger prawns, which are eurithermal and euryhaline, although optimal at temperatures of 25-32 °C and 15-35 ppt salinity for milkfish (Yap *et al*., 2007), and temperatures of 26-33 °C (Supono, 2017) and 10-20 ppt salinity (Rahi *et al*., 2021) for tiger prawns. Similar to mussels, their tolerance to temperature and salinity is also wide, between 10-50 °C and 0-25 ppt, respectively (Verween *et al*., 2007). Dissolved oxygen is always above 5 mg L^{-1} due to continuous aeration during the experiment, which is conducive to brackish water organisms (Pantjara *et al*., 2015). The pH parameter is also conducive all organisms (Yap *et al*., 2007; Aftabuddin *et al*., 2018).

The quantification of organic and inorganic nutrient waste from milkfish and tiger shrimp and the uptake of these wastes by *G. virens* and paddy was not measured, thus becoming the limitation of this study. However, there was an indication of the waste load from the NH_3 measurement range during the study, namely 0.0013-0.0429 mg L^{-1} in C-2 species, 0.0015-0.0598 mg L^{-1} in C-3 species and 0.0014-0.0320 mg L^{-1} in C-4 species. The lower NH_3 concentration in the C-4 species may be due to the presence of mussel as an extractive species of nutrient organic. Although shrimp are benthic feeders (Eldani & Primavera, 1981), it seems is not sufficient to compensate for the load of organic waste (feces) from milkfish and shrimp themselves. Overall NH_3 in this study <1 mg L^{-1}, according to the quality standards of the culture medium (Lawson, 1995). The lower NH_3 concentration creates more conducive water conditions for feeding activities as well as metabolic and growth processes.

Feed Conversion Ratio (FCR)

Table 2. Feed conversion ratio (FCR) during rearing of IMTA-Paddy system.

Combination of species	FCR (Mean ± SD)
C-2 species (milkfish and paddy)	2.1±0.17[a]
C-3 species (milkfish, tiger shrimp and paddy)	2.0±0.03[b]
C-4 species (milkfish, tiger shrimp, mussel, and paddy)	1.9±0.04[c]

FCR = Feed Convertion Ratio; SD = Standard of Deviation; [a, b, c] = The different superscript letters indicate significant differences (P<0.05) by Tukey's HSD

The result of calculating FCR means for each species combination of IMTA-Paddy system during on 80 days cultivation are summarized in Table 1. The best FCR in C-4 species followed by C-3 and the worst was obtained by C-2 species. Statistically, the combination of species has a significant effect to increased FCR and there was a

Table 1. Water quality during rearing of IMTA-Paddy system.

Water quality parameters	Combination of species		
	C-2 (milkfish and paddy)	C-3 (milkfish, tiger shrimp and paddy)	C-4 (milkfish, tiger shrimp, mussel, and paddy)
Temperatures (°C)	27.6-28.9	27.6-28.7	27.7-28.7
Salinity (ppt)	9-15	9-15	9-15
Dissolved oxygen (mg L^{-1})	5.5-6.9	5.6-6.8	5.5-7.0
pH	6.7-7.0	6.6-7.0	6.7-7.1
Ammonia (mg L^{-1})	0.0013-0.0429	0.0015-0.0598	0.0014-0.0320

significant difference for each combination of species ($P<0.05$). In C-4 species, FCR increased significantly by 5.7-9.3%. From this results, it is clear that the FCR performance was directly related with increasing of the number of species combinations. The more species combination, the better values of FCR obtained.

FCR is defined as the ratio between the weight of feed used and the weight of the biomass produced, where the lower FCR (close to 1) means the better the utilization of feed (Fry *et al*., 2018). When comparing with the IMTA studies, our results are consistent with the findings of several previous studies. Lalramchhani *et al*. (2020) tested three species combinations (crab, mullet, shrimp, oyster and periphyton) in brackish water ponds, the better the FCR obtained for the combination of more species, namely 3.7, 3.4 and 3.31 for each a combination of 3, 4 and 5 species. Biswas et al. (2019) obtained an FCR of 1.1 in culturing of mullets and tiger shrimp as fed-species and estuarine oyster and seaweed as extractive species, was better than only mullets and tiger shrimp combination, with FCR 1.34. When compared with polyculture and monoculture systems for milkfish and tiger shrimp, there are variations in consistency with several previous studies. Tsutsui *et al*. (2015) reported the FCR value of tiger shrimp of 2.39 for monoculture systems and 1.46 with co-cultur green algae. FCR in polyculture milkfish with crab 1.92 (Mondal *et al*., 2020), with *P. monodon* 1.99 (Biswas *et al*., 2012) with *P. indicus* 1.64 (Lalramchhani *et al*., 2019). The most likely reasons for this difference in FCR are water quality and species co-culture.

Synergy and antagonism (competition and predation) which are the main problems in co-culture (Martínez-Porchas *et al*., 2010) seem to be no problem for every species in this study. Culturing of milkfish, tiger shrimp, mussel and paddy together (co-culture) are feasible in brackish water ecosystem. Milkfish, tiger shrimp and mussel make use of different spaces so that spatial use will be optimal. Milkfish occupies a habitat in the water column and tiger shirmp on the bottom of the water (Aubin *et al*., 2015). Although *G. virens* also occupies the bottom of the waters, they are sessile and tiger shrimp are motile so that space competition for these two species is relatively low. Thus, regardless of its position, either on the surface or column or bottom of the water, the feed is used by species based on its space. This synergy of space leads to reduction in competition for feed, optimized use of feed and improvements in feed efficiency.

In the IMTA-Padi system, milkfish and tiger prawns are fed artificial feed which in the process to produces waste of organic N and P particles (feed residue and feces) and inorganic N and P (excretion) (Knowler *et al*., 2020). Fish or shrimp only assimilate 23% -31% N and 10-13% P from the feed, some of which is wasted in the water column or in the sediment (Azad *et al*., 2017). Furthermore, mussel utilize organic waste (Zhang *et al*., 2019) and paddy absorbs inorganic waste (Li *et al*., 2019) as their energy or nutrients. Artificial feed seems to be dominated by milkfish because they are fast swimmers so tiger shrimp may use more natural feed, especially detritus and other small organisms (Kent *et al*., 2011; Viau *et al*., 2013). Residual feed, feces, and excretion results can stimulate plankton growth (Djumanto *et al*., 2018). For the use of natural food, milkfish prefer plankton and tiger shrimp prefer detritus even though these two species are opportunistic omnivores (Aubin *et al*., 2015). The excretion of milkfish that is decomposed at the bottom of the waters can also be used by tiger shrimp, which are benthic feeders (Eldani & Primavera, 1981). Paddy can directly absorb inorganic N in water (Li *et al*., 2019), transfer O_2 from the atmosphere to the root zone (Colmer *et al*., 2006) and increase O_2 diffusion (Foster-Martinez & Variano, 2016). Li *et al*. (2019) reported that N_2O and NH_3 were significantly reduced by 115.9% and 25.4%, respectively, in the shrimp and paddy co-culture. This trophic niche synergy can optimize nutrient utilization and improvements in feed efficiency.

The presence of mussel as extractive species significantly resulted in a better FCR value in this study. This is an important finding in developing the IMTA-Paddy system in brackish water ecosystems. *G. virens* mussels in this experiment functioned as a deposit feeder and filter feeder (Suwignyo, 2005; Hastuti *et al*., 2012) and suspension feeder (Brewer & Willan, 2018), namely organisms that absorb organic nutrients deposited and suspended in water (Cubillo *et al*., 2016) . Mussel is the main extractive component used in the IMTA system to utilize organic particles from cultivation waste as feed (Sarà *et al*., 2009; Cranford *et al*., 2013). To our knowledge, no studies has reported the potential of *G. virens* in absorbing organic nutrients. As an illustration, Srisunont & Babel (2016) reported absorption efficiency of green shellfish nutrients was 65.1% C, 62.1% N and 79.2% P and was able to remove particles of C, N and P was 08.1, 13.5 and 4.6 mg day^{-1} individual^{-1} respectively.

The above discussion provides important information that the IMTA-Paddy system with *C. chanos*, *P. monodon*, *G. virens* and *O. sativa* species has a high synergistic and low antagonistic relationship. These four species are possible in co-culture in brackish water. With a feed amount of 5% of the biomass, the FCR trend obtained better with increasing species combination. This indicates an increase in feed utilization efficiency. Increasing feed efficiency means increasing the production of organisms from the same amount of feed (de Verdal *et al*., 2018). The results of this study also further strengthen the concept of IMTA, which is not enough to consider the number of species, but each species must have a different function based on its trophic level (Buck *et al*., 2018; Chang *et al*., 2019; Knowler *et al*., 2020). Efficiency of feed utilization in the IMTA-Paddy system still needs to be studied more deeply, especially the release and absorption of nutrients of each species which is the limitation of this study.

Index of Profit (IP)

The IP in this study reflecting a comparison of the total cost of feed with the production values of milkfish and tiger shrimp. During the study period, total Cost of feed was IDR 50552, IDR 54404 and IDR 57112 for each a combination of 2, 3 and 4 species. The price of milkfish and tiger shrimp at the site study by September 2020 was IDR 37000 and IDR 135000, respectively. The result calculating of IP means for each species combination during in 80 days cultivation are summarized in Table 2.

Table 3. Index of Profit (IP) during rearing of IMTA-Paddy system.

Combination of species	IP (Mean ± SD)
C-2 species (milkfish and paddy)	2.0±0.15[a]
C-3 species (milkfish, tiger shrimp and paddy)	2.9±0.30[b]
C-4 species (milkfish, tiger shrimp, mussel, and paddy)	3.1±0.10[c]

IP = Index of Profit; SD = Standard of Deviation; [a, b, c] = The different superscript letters indicate significant differences (P<0.05) by Tuke'y HSD

In present study, the efficiency of using feed is determined by calculating IP. This index indicates the benefits of a given feed, where an IP above 1 indicates that feeding is profitable (Jimoh *et al*., 2013). IP performance (Table 2) shows the same trend pattern as FCR performance (Table 1). The best IP in C-4 species followed by C-3 and the worst was obtained by C-2 species. Overall, all species combinations had an IP value was >1 which indicates profitable (Jimoh *et al*., 2013). This means that the co-culture of *C. chanos, P. monodon, G. virens* and *O. sativa* in brackish water is profitable. In line with Ouma (2019), PI polyculture of *Clarias gariepinus* and *Oreochromis niloticus* is significantly profitable than monoculture. Comparatively, IP of C-4 species was significantly higher 21.0% and 34.4% than IP C-3 and C-2 species (P<0.05). These implies that the IP performance was directly related with increasing of the number of species combinations. The more species combination, the better values of IP obtained. From this result, its clear that co-culture IMTA-Paddy with C-4 species may not only be more effectively but also more economical.

The PI values clearly indicate that more profit will result from combining four species at the same feeding rate. Feed costs can be reduced by up to 34.4% if the co-culture uses four species. The present findings can be helpful for farmers in reducing feed costs which have a proportion of around 50-80% production cost in aquaculture (Bharati *et al*., 2019). In this experiment, the higher PI performance in C-4 species could be explained in relation to the fact that a complete co-culture, namely fed species, inorganic extractive species and organic extractive species will increase the efficiency of nutrient utilization (Chang *et al*., 2019) which is represented by maximum growth and production value.

CONCLUSION AND RECOMMENDATION

The study indicates that co-culture of IMTA-Paddy co-culture with the combination of fish, shrimp, mussels and paddy (C-4-species) increased FCR and IP compared to the combination of C-2 and C-3 species. The effectiveness and efficiency of feed utilization increased which lead to increased productivity and diversification of four commercially species from brackish water ponds. Thus, for the implementation of IMTA-Paddy, fish, shrimp, mussel and paddy rearing together is recommended. More further research is needed to optimize this IMTA-Paddy system, especially to investigate the release and absorption of nutrients for each species.

AUTHOR'S CONTRIBUTIONS

HH find ideas and drafting the manuscript; HH, IC and HMS developed the idea, concept, and designed experiments; IC conceived and planned the experiments; HMS collected the data and worked out almost all of the technical details. All authors contributed extensively to the work presented in this manuscript, including data generation, data analysis, and manuscript preparation.

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