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Effect of moringa (*Moringa oleifera* Lam.) leaf meal supplementation on growth and nutrient digestibility of rohu and PACU under different culture practices

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Abstract
An eight-week experiment was conducted to assess the influence of 10% moringa leaf meal as a replacement of fish meal in the supplementary diet and its effect on the growth performance of monoculture and polyculture of pacu (*Piaractus brachypomus*) and rohu (*Labeo rohita*). 120 fingerlings were randomly assigned to four treatments: control (Mono and Polyculture of pacu and rohu), T1 (Monoculture of pacu), T2 (Monoculture of rohu), and T3 (Polyculture of rohu and pacu) in triplicates of circular tanks of 25m^3 for 60 days. The basal and reference diets with crude protein and lipid were supplemented throughout the experiment. The growth performance of the fishes had significantly higher in the reference diets over the basal diet. The results showed that the growth performance of rohu and pacu in T3 had significantly higher than other treatments. Compatibility was observed between rohu and pacu in the polyculture system. It can be concluded that *Moringa oleifera* leaf meal 10% supplemented feed improves the polyculture system's overall growth performance than a monoculture system.

Keywords: Moringa leaf meal, pacu, rohu, monoculture, polyculture, growth parameters

1. Introduction

Fishmeal is an excellent protein source in aqua-feeds due to its high palatability, digestibility, presence of essential fatty acids, minerals, and amino acids (Olsen & Hasan, 2012) [21]. With the limited supply and higher cost, alternative protein sources are required for the sustainable aquafeed industry. Researchers evaluate the deployment of non-conventional ingredients such as plants and plant by-products predominantly due to their high protein contents to attain economically unassailable and environment-friendly feed production (Arsalan *et al*., 2016; Raji *et al*., 2018) [5, 24].

Moringa oleifera is one of the future plant-based protein sources for incorporating aquaculture dietsm (Chiseva, 2006) [7]. It is a highly cultivated plant species containing high crude protein (251 g/kg DM) in the leaf, with low tannins and other anti-nutritional compounds (Nouala *et al*., 2006) [9]. Moringa leaf meals (MOLM) contain high levels of sulfur-containing amino acids like soybean, which is usually considered a high-quality plant protein (Francis, Makkar, & Becker, 2001) [10]. They are also rich in n-3 fatty acid and are considered essential carotenoids, minerals, ascorbic acid, and iron. Moringa oleifera leaf meal as a replacement for soybean meal in the diets of *Cyprinus carpio* juveniles significantly improved the growth performance, enhanced immunity of the fish, and reduced the feed cost (Adeshina, Sani, Adewale, Tiamiyu, & Umma, 2018; Yuangsoi and Masumoto, 2012) [1, 28]. Polyculture of Pacu with Indian Major Carps improves overall growth performance, decreases the manufacturing cost, and increases farmers' income (Kumar *et al*., 2018) [15]. Earlier studies have shown that 10% of *M. oleifera* leaf meal is a promising protein source and improves growth performance, nutrient digestibility, with low cost for use in the diet of Tilapia, Rohu, Asian seabass, African catfish, African mud catfish, and Pangasius (Puycha *et al*., 2017; Richter, Siddhuraju, & Becker, 2003) [2, 23].

Red-bellied pacu (*Piaractus brachypomus*) is a freshwater fish indigenous to the Plate River Basin in South America. It is an omnivorous fish and preferentially feeds on the leaf, fruits, flowers, and superior plants' seeds. *P. brachypomus* is cultured widely due to its easy adaption to cultural conditions, omnivorous characteristics, rapid growth, and good flesh quality (Uchoi, Shyama, Golandaj, Sreenath, & Yadav, 2018) [26].

Rohu (*Labeo rohita*) is the most important species among Indian major carps. It lives in the column of the pond and feeds on zooplankton. Due to its broader feeding niche, which extends from column to bottom, rohu is usually stocked at relatively higher levels and has rapid growth (Gandotra, Kumari, Sharma, & Singh, 2017) [11]. This study involves a comparative study on the growth performance of *Labeo rohita* and *Piaractus brachypomus* using moringa leaf as a feed supplement in monoculture and polyculture systems.

2. Materials and Methods
2.1 Experimental design
The experiment was conducted at the Advanced Research Farm Facility, Madhavaram campus, Tamil Nadu, (13.1488 N, 80.2306 E), India. Rohu and Pacu fingerlings obtained from a local private farm were acclimatized to the experimental conditions for two weeks before the start of the experiment. Animals (initial weight ± 3.2 g) were distributed into twelve tanks with 100 fingerlings per tank. The stocked fingerlings were healthy and disease-free. The experimental species such as Pacu and Rohu were stocked at different combinations. Control had Monoculture of pacu and rohu, Polyculture with pacu and rohu. Treatment -1 with pacu alone (Monoculture), rohu was stocked alone in Treatment -2 (Monoculture), whereas pacu and rohu in Treatment -3 (Polyculture). Fish were cultured in a circular tank of 25 m^3 capacity, and experiments were carried out in triplicates for 60 days.

2.2 Preparation of reference diets
Fresh *Moringa oleifera* leaf was collected from Advanced Research Farm Facility, Madhavaram campus, air-dried for 24 h, powdered, packed, and stored in airtight bags at 4-degree Celsius.
Fish meal in the reference diets was replaced with 10% Moringa leaf meal as suggested by (Raji *et al*., 2018) [24]. Ingredients of the basal diet and reference diet composition were shown in Table 1. Fishes were fed two times a day at 09:00 AM and 2:00 PM at a rate of 6% of their body weight (Gandotra *et al*., 2017) [11].

2.3 Water quality analysis
Water samples were collected between 09.00 AM and 10.00 AM every day to analyse important physicochemical parameters. Temperature (Pro 20, YSI-USA), pH (Pro 20, YSI-USA), and Dissolved Oxygen (Pro 20, YSI-USA), were monitored daily. Total Alkalinity, Total Hardness, Ammonia (NH_3-N) were tested every week (American Public Health Association, 1995) [4].

2.4 Estimation of growth indices
Growth in terms of length and weight was further assessed through periodic samplings at 15-days intervals.

2.5 Growth parameters
According to (Olvera-Novoa, Campos, Sabido, & Palacios, 1990), the following parameters were estimated.
Body Weight Gain (BWG) (%) = 100 x ((Final body mass (g) - Initial body mass (g))/ Initial body mass (g))
Average Daily Gain (ADG) = Final Weight – Initial Weight/ Rearing Period (g/day)
Specific Growth Rate (SGR) (%) = (((ln Final body mass in g) – (ln Initial body mass in g))/ Number of trial days) x 100
Feed Conversion Ratio (FCR) = Feed taken / weight gain
Survival rate (%) = 100 x (Number of fishes recovered / Number of fishes stocked)

2.6 Proximate composition analysis of dietary ingredients and experimental feeds
Dietary ingredients and experimental feeds were subjected to proximate composition analysis. Crude protein, ether extract, crude fibre, total ash, moisture, and gross energy were analysed according to (AOAC, 1990). The results were shown in Table 2 and 3.
The biochemical analysis of moringa leaf powder was carried out for moisture, total ash, crude protein, crude fat (ether extract), gross energy contents following standard methods (AOAC, 1990), and results are presented in Table 1.

2.7 Nutrient digestibility
The chromium oxide was added for the reference diet, and faecal samples were collected to determine the nutrient digestibility, according to (Bolin, King, & Klosterman, 1952) [6]. The apparent digestibility coefficients (ADC) of dietary nutrients and gross energy were calculated using (Ngugi, Oyoo-Okoth, Manyala, Fitzsimmons, & Kimotho, 2017) [18].

2.8 Statistical analysis
Statistical analysis for all the parameters in the study was performed using Analysis of Variance followed by Tukey's multiple range test at 5% ($P < 0.05$) level to compare the mean between the treatments with SAS® 9.4 (SAS, 2020).

3. Result and Discussion
During the experimental period, the water temperature (28 to 33°C), pH (7.6 to 9.4), dissolved oxygen (4.2 to 5.2 mg/L), total alkalinity (65 to 100 mg/L), total hardness (60 to 85 mg/L), ammonia (0.01 to 0.03 mg/L) and nitrite (<0.003 mg/L) were within the optimal ranges of rohu and pacu cultures (Jena, Ayyappan, & Aravindakshan, 2002; Kumar *et al*., 2018) [15] with no drastic variation (Table 4). No significant differences were found in measured water quality parameters for the monoculture and polyculture tanks. Such values of the parameters indicated that the addition of moringa leaf meal does as a feed supplement does not affect the culture tanks' water quality.
The growth performances of rohu and pacu in monoculture and polyculture systems over 60 days fed on *Moringa oleifera* leaf meal as a feed supplement and basal diet are presented in Table 5.
The percentage weight gains of pacu and rohu of this experiment ranged between 320- 446% and 500-845%, respectively. The highest percentage weight gain of pacu and rohu was observed in T3, compared to T1 and T2. Such results reveal that there is no overlapping of the feeding niche of the two species and compatibility of these two species in polyculture. These results agree with (Kumar *et al*., 2018) [15], who reported the percent weight gains between 230-300%, 541.9-657.4% in IMC and pacu polyculture. Hussain *et al*. (2018) [12] studied 10% of moringa leaf meal as a reference diet had a significant increase in percentage weight gain of rohu was higher (349.59%) than basal diet 263.33%. The highest mean specific growth rate and average daily weight gain of pacu and rohu were higher in T-3 than in other treatments.
The feed conversion ratio (FCR), calculated based on the supplemented feed, significantly differed between the treatments, and it was lower in Treatment 3 (Table 5). As the SGR and ADG were higher in T3 due to the higher growth of rohu and pacu, the increased feed intake by the animals leads

to the decreased FCR. The lower FCR value in T3 indicates the better and more efficient utilization of the diet used for growth and meets the nutritional requirements of rohu and pacu species without compromising the intended effects. The present results agree with the reports of (Algrient, Romeo, Peguy, Thomas, & Salifou, 2019; Cruz-Velásquez, Kijora, Vergara-Hernández, & Schulz, 2014; Ibrahim & Naggar, 2010) [3, 9, 13]. (Uchoi *et al.*, 2018) [26] who reported that monoculture of pacu with artificial feeding had higher growth performance, followed by polyculture with IMC with artificial feeding than other treatments. It is mainly due to different types of culture strategies like feed type, animal combination, and culture type followed for each treatment.

In all the treatments, the survival of rohu and pacu was not significantly different ($p > 0.05$). The average survival of rohu and pacu in this study was above (monoculture) and within (polyculture) the range reported by other studies and concluded that pacu is a compatible species for culturing along with Indian major carps (Kumar *et al.*, 2018; Uchoi *et al.*, 2018) [26].

The present study admittedly defined that significant differences ($P< 0.05$) were found in the final weight (FW), weight gain (WG), average daily growth (ADG), specific growth rate (SGR), feed conversion ratio (FCR) between the treatments (Table 5). The growth parameters (FW, WG, ADG, SGR) of the fishes were significantly ($P< 0.05$) higher in the reference diet and polyculture system. The lower FCR obtained from this study indicated the positive effect on the species combination in polyculture system with MOLM incorporated diet. This experiment indicates that the highest growth performance was observed in polyculture systems compared to monoculture.

Chromic oxide used as an inert marker was also added to the diets. At the end of the study, faeces from each tank were collected to estimate the nutrient digestibility parameters.

In the present study, nutrient digestibility of crude protein, crude lipid, and gross energy of rohu and pacu faeces at 10% inclusion level of moringa leaf meal as a reference diet and basal diets were analysed (Table 6).

Crude protein in moringa leaf was well digested by rohu and pacu (81% and 75%), which fell within limits regarded as high, i.e., 75%-95% (Cho & Kaushik, 1990) [8]. It may be due to a high proportion of pepsin soluble nitrogen (82-91%) and a low proportion (1-2%) of acid detergent insoluble protein (Makkar & Becker, 1996) [16].

The ADC of crude lipid (80% and 75%) was low compared to the routinely reported range of 85-95% (National Research Council, 1993). It is likely because the lipid fraction of MOLM contains significant amounts of indigestible waxes (Afuang, Siddhuraju, & Becker, 2003) [2, 25].

Our results demonstrated that gross energy digestibility was highest at experimental diets, and it was significantly different from control diets. According to Kumar *et al.* (2018) [15], the highest gross energy digestibility value (77%) was observed when Nile tilapia fed on MOLM based diet and significantly different from the values of the reference diet.

The nutrient digestibility activity depends on digestive capability, feeding habits, and environment (Uchoi *et al.*, 2018) [26]. Hussain *et al.* (2018) [12] reported that ADC of crude protein, crude fat, and gross energy was highest at 10% level of MOLM than basal diet in *Labeo rohita*, and the values are similar to our results. The result showed that the reference diet had a significantly higher nutrient digestibility than the basal diet, and it could be due to the fish meal replacement for the basal diet of fish.

The 10% moringa leaf meal supplementation in fish feed gives better growth performance in polyculture system and higher nutrient digestibility over the basal diet.

Table 1: Ingredient composition of the reference diet (g per 100 g)

Ingredient	Basal diet (g)	Reference diet (g)
Fish Meal	25	20
Rice bran	33.5	28.5
Groundnut oil cake	40	40
Moringa leaf meal	-	10
Vitamin Premix	0.5	0.5
Mineral Premix	0.5	0.5
Chromium oxide	0.5	0.5

Table 2: Proximate composition of experimental feed ingredients

Ingredients	Proximate composition (%)				
	Crude Protein	Crude Lipid	Moisture	Crude Fibre	Total Ash
Fish meal	58.82	12.56	10.32	<1	13.93
Rice bran	10.52	11.41	12.11	6.89	7.45
Groundnut oil cake	39.21	10.11	17.65	4.35	3.77
Moringa leaf meal	27.22	8.65	10.84	4.89	7.97

Table 3: Proximate composition of the experimental diet (g per 100g^{-1}, as fed)

Parameters	Percentage composition (%)	
	Basal diet	Reference diet
Crude protein	32.38 ± 0.29	32.66 ± 0.16
Ether extract	9.78 ± 0.28	9.60 ± 0.34
Crude fibre	3.64 ± 0.19	3.72 ± 0.21
Total ash	4.26 ± 0.16	4.34 ± 0.13
Moisture	8.26 ± 0.32	8.05 ± 0.21
Gross Energy (Kcal per g)	3.36 ± 0.12	3.46 ± 0.09

Table 4: Water quality parameters observed during the experiment period

Treatment / Parameters	Control	Treatment 1	Treatment 2	Treatment 3
Dissolved Oxygen (mg L^{-1})	4.51 ± 0.28^a	4.53 ± 0.39^a	4.64 ± 0.32^a	4.57 ± 0.30^a
pH	7.44 ± 0.36^a	7.42 ± 0.45^a	7.35 ± 0.38^a	7.35 ± 0.38^a
Temperature (°C)	29.32 ± 1.33^a	29.30 ± 1.30^a	29.50 ± 1.51^a	28.91 ± 1.69^a
Total alkalinity (ppm)	86.48 ± 5.37^a	85.25 ± 6.80^a	89.50 ± 5.50^a	87.00 ± 8.50^a
Total Hardness (ppm)	74.39 ± 8.24^a	75.00 ± 10.00^a	69.50 ± 7.50^a	73.25 ± 11.50^a
Total Ammonia (NH_3-N) (mg L^{-1})	0.02 ± 0.00^a	0.03 ± 0.00^a	0.02 ± 0.01^a	0.01 ±0.00^a
Nitrite (NO_2-N) (ppm)	0.001 ± 0.00^a	0.002 ± 0.00^a	0.003 ± 0.00^a	0.001 ± 0.00^a

Means bearing the same superscript are not significantly different from one another ($P\leq 0.05$)

Table 5: Growth performance of Pacu and Rohu in monoculture and polyculture fed with basal and reference diets

Treatment		Weight		Weight gain (%)	ADG (g/day)	SGR (%)	Survival rate (%)	FCR
		Initial WT (g kg^{-1})	Final WT (g kg^{-1})					
Control Pacu	Mono Culture	3.25 ± 0.21^a	13.26 ± 1.54^a	306.67 ± 20.84^a	0.16 ± 0.02^a	2.33 ± 0.08^a	96.00 ± 0.00^a	2.78 ± 0.12^a
	Poly Culture	3.25 ± 0.25^a	15.61 ± 2.20^b	378.12 ± 30.51^b	0.21 ± 0.03^b	2.60 ± 0.10^b	98.00 ± 0.00^a	2.67 ± 0.21^b
Treatment Pacu	Mono Culture (T1)	3.28 ± 0.54^a	15.61 ± 2.18^a	371.50 ± 113.98^a	0.24 ± 0.05^a	2.53 ± 0.43^a	98.00 ± 0.00^a	2.43 ± 0.32^a
	Poly Culture (T3)	3.24 ± 0.46^a	19.19 ± 2.72^b	446.21 ± 81.99^b	0.30 ± 0.04^b	2.81 ± 0.25^b	94.00 ± 0.00^a	2.25 ± 0.22^b
Control Rohu	Mono Culture	3.25 ± 0.27^a	19.87 ± 0.87^a	420.08 ± 22.96^a	0.27 ± 0.01^a	3.01 ± 0.07^a	94.00 ± 0.00^a	2.51 ± 0.14^a
	Poly Culture	3.25 ± 0.31^a	22.32 ± 1.89^b	497.47 ± 17.54^b	0.31 ± 0.03^b	3.21 ± 0.02^b	96.00 ± 0.00^a	2.43 ± 0.19^b
Treatment Rohu	Mono Culture (T2)	3.24 ± 0.49^a	21.63 ± 3.18^a	661.87 ± 400.32^a	0.34 ± 0.05^a	3.22 ± 0.69^a	96.00 ± 0.00^a	2.33 ± 0.23^a
	Poly Culture (T3)	3.26 ± 0.37^a	27.82 ± 3.12^b	845.25 ± 424.35^a	0.45 ± 0.05^b	3.60 ± 0.69^a	96.00 ± 0.00^a	2.18 ± 0.21^b

Means bearing the same superscript within a subgroup are not significantly different from one another ($P \leq 0.05$)

Table 6: Fecal matter apparent digestibility of Pacu and Rohu fed with reference diets

	Control		T-1	T-2	T-3	T-3
	Pacu	Rohu			Pacu	Rohu
Dry Matter	75.70 ± 1.56^a	78.02 ± 3.76^a	77.85 ± 1.01^b	81.91 ± 2.28^b	79.14 ± 1.75^b	81.09 ± 1.92^b
Crude Protein	75.69 ± 1.60^a	80.86 ± 0.98^a	76.11 ± 1.57^b	80.98 ± 1.59^b	74.41 ± 1.84^b	80.66 ± 2.30^b
Crude Lipid	71.89 ± 1.02^a	76.79 ± 1.21^a	72.05 ± 1.09^b	80.26 ± 2.09^b	71.01 ± 1.27^b	80.34 ± 1.23^b
Gross Energy	69.27 ± 1.35^a	71.02 ± 0.79^a	72.77 ± 1.38^b	75.68 ± 1.68^b	73.03 ± 1.63^b	75.77 ± 1.24^b

Means bearing the same superscript within a subgroup are not significantly different from one another ($P \leq 0.05$)

4. Conclusion

The results concluded that the comparative studies on monoculture and polyculture of pacu and rohu, Polyculture had a higher growth rate, survival, and low FCR than monoculture. Thus, the use of costly feed ingredients like fish meal and soybean meal can be replaced with cost-effective plant-derived by-products, i.e., 10% Moringa leaf meal without any adverse effects on growth performance and nutrient digestibility of pacu and rohu. Hence, moringa leaf meal could be used as an alternative plant protein source in the rohu and pacu diet for cost-effective feed production. The present study encourages the use of moringa leaf meal as a viable feed ingredient in the polyculture of rohu and pacu to enhance farmers' production and income and suggests a way forward for field validation of the results to achieve sustainable aquaculture practices.

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6. References

1. Adeshina I, Sani RA, Adewale YA, Tiamiyu LO, Umma SB. Effects of dietary Moringa oleifera leaf meal as a replacement for soybean meal on growth, body composition and health status in Cyprinus carpio juveniles. Croatian Journal of Fisheries: Ribarstvo 2018;76(4):174-182.
2. Afuang W, Siddhuraju P, Becker K. Comparative nutritional evaluation of raw, methanol extracted residues and methanol extracts of moringa (*Moringa oleifera* Lam.) leaves on growth performance and feed utilization in Nile tilapia (*Oreochromis niloticus* L.). Aquaculture Research 2003;34(13)1147-1159.
3. Algrient NT, Romeo NR, Peguy T, Thomas EE, Salifou J. Comparative Effect of Monoculture and Polyculture in Two Species of Clariidae: Heterobranchus longifilis and Clarias gariepinus in Post Fingerlings Growth. International Journal Fisheries Science and Research 2019;3(1):1010.
4. APHA (American Public Health Association), Standard methods for the examination of water and waste water, 19th edition. American Public Health Association, Washington, DC, 1995
5. Arsalan MZH, Hussain SM, Asrar M, Anwar H, Rehan MMH, Shahzad MM *et al*. Effects of Moringa oleifera leaf meal (MOLM) based diets on carcass composition and hematology of Labeo rohita fingerlings. Journal of Biodiversity and environmental. Science 2016;9:214-223.
6. Bolin DW, King RP, Klosterman EW. A simplified method for the determination of chromic oxide (Cr2O3) when used as an index substance. Science (Washington) 1952;116:634-635.
7. Chiseva S. The growth rates and feed conversion ratios of fry fed conventional fry diets and Moringa oleifera supplemented diets. Unpublished B. Sc. Dissertation, Bindura University of Science Education, Bindura, Zimbabwe 2006.
8. Cho CY, Kaushik SJ. Nutritional energetics in fish: energy and protein utilization in rainbow trout (*Salmo gairdneri*). Aspects of food production, consumption and energy values 1990;61:132-172.
9. Cruz-Velásquez Y, Kijora C, Vergara-Hernández W, Schulz C. On-farm evaluation of Cachama blanca and Nile tilapia fed fermented aquatic plants in a polyculture. Orinoquia 2014;18(2):269-277.
10. Francis G, Makkar HP, Becker K. Antinutritional factors present in plant-derived alternate fish feed ingredients and their effects in fish. Aquaculture 2001;199(3-4):197-227.
11. Gandotra R, Kumari R, Sharma M, Singh D. Growth Response of Juveniles of Rohu, Labeo Rohita to Different Levels of Lipid in the Diet. Journal of Fisheries and Aquatic Science 2017;8(210):2.
12. Hussain SM, Javid A, Hussain AI, Aslam N, Ali Q, Hussain M *et al*. Replacement of Fish Meal with Moringa oleifera Leaf Meal (MOLM) and its Effect on Growth Performance and Nutrient Digestibility in Labeo rohita Fingerlings. Pakistan Journal of Zoology, 2018, 50(5).
13. Ibrahim N, Naggar GE. Water quality, fish production and economics of Nile tilapia, Oreochromis niloticus, and African catfish, Clarias gariepinus, monoculture and polycultures. Journal of the World Aquaculture Society 2010;41(4):574-582.

14. Jena JK, Ayyappan S, Aravindakshan PK. Comparative evaluation of production performance in varied cropping patterns of carp polyculture systems. Aquaculture 2002;207(1-2):49-64.
15. Kumar A, Pradhan PK, Das PC, Srivastava SM, Lal KK, Jena JK. Growth performance and compatibility of pacu, *Piaractus brachypomus* with Indian major carps in polyculture system. Aquaculture 2018;490:236-239.
16. Makkar HA, Becker K. Nutrional value and antinutritional components of whole and ethanol extracted *Moringa oleifera* leaves. Animal feed science and technology 1996;63(1-4):211-228.
17. National Research Council. Nutrient requirements of fish. National Academies Press 1993.
18. Ngugi CC, Oyoo-Okoth E, Manyala JO, Fitzsimmons K, Kimotho A. Characterization of the nutritional quality of amaranth leaf protein concentrates and suitability of fish meal replacement in Nile tilapia feeds. Aquaculture Reports 2017, 562-69.
19. Nouala FS, Akinbamijo OO, Adewumi A, Hoffman E, Muetzel S, Becker K. The influence of Moringa oleifera leaves as substitute to conventional concentrate on the *in vitro* gas production and digestibility of groundnut hay. Livestock Research for Rural Development 2006;18(9):1-4.
20. Official AO. Methods of analysis of AOAC International. AOAC international, Maryland, USA 2003-2005.
21. Olsen RL, Hasan MR. A limited supply of fishmeal: Impact on future increases in global aquaculture production. Trends in Food Science & Technology 2012;27(2):120-128.
22. Olvera-Novoa MA, Campos SG, Sabido MG, Palacios CAM. The use of alfalfa leaf protein concentrates as a protein source in diets for tilapia (*Oreochromis mossambicus*). Aquaculture 1990;90(3-4):291-302.
23. Puycha K, Yuangsoi B, Charoenwattanasak S, Wongmaneeprateep S, Niamphithak P, Wiriyapattanasub P. Effect of moringa (*Moringa oleifera*) leaf supplementation on growth performance and feed Utilization of Bocourti's catfish (*Pangasius bocourti*). Agriculture and natural resources 2017;51(4):286-291.
24. Raji AA, Alaba PA, Yusuf H, Bakar NHA, Taufek NM, Muin H *et al*. Fishmeal replacement with Spirulina Platensis and Chlorella vulgaris in African catfish (*Clarias gariepinus*) diet: Effect on antioxidant enzyme activities and haematological parameters. Research in veterinary science 2018;119:67-75.
25. Richter N, Siddhuraju P, Becker K. Evaluation of nutritional quality of moringa (*Moringa oleifera* Lam.) leaves as an alternative protein source for Nile tilapia (*Oreochromis niloticus* L.). Aquaculture 2003;217(1-4):599-611.
26. Uchoi R, Shyama S, Golandaj A, Sreenath VR, Yadav RP. Growth potential of Pacu, Piaractus brachypomus in different Culture Approach. Research. Journal of Animal, Veterinary and Fishery Science 2018;3(6):8-13.
27. Generalized linear and nonlinear models for correlated data: theory and applications using SAS (Version 9.4). SAS Institute, North Carolina.
28. Yuangsoi B, Masumoto T. Replacing moringa leaf (*Moringa oleifera*) partially by protein replacement in soybean meal of fancy carp (*Cyprinus carpio*). Songklanakarin Journal of Science & Technology, 2012, 34(5).