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An Evaluation of Wolffia Meal (*Wolffia arrhiza*) in Replacing Soybean Meal in Some Formulated Rations of Nile Tilapia (*Oreochromis niloticus* L.)

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Abstract: An investigation on the evaluation of Wolffia meal being used as a replacement material for soybean meal in ration of Nile tilapia (*Oreochromis niloticus*) was carried out at Khon Kaen University, Khon Kaen 40002, Thailand. The two experiments were laid in a randomized complete block design with three replications and they were carried out in a laboratory. The results showed that digestibility of fresh Wolffia by Nile tilapia fingerlings (2.5 g) and Nile tilapia adults (40-50 g) were not significantly differed from each other. Growth performance of Nile tilapia adults with respect to percentages of weight, length, feed intake, feed conversion ratio, protein efficiency ratio, net protein utilization and specific growth rate were not statisticaly significant in all levels of Wolffia. However, the highest total production occurred in fish being fed on formulated ration containing 15% Wolffia meal. An increase in Wolffia meal beyond 15 % in the formulated ration decreased the survival rate and total production of the cultured fish. Wolffia meal could be successfully used in place of soybean meal but the amount being used should not exceede 15 %.

Key words: *Wolffia arrhiza*, formulated rations, Nile tilapia, feed replacement.

Introduction

For tropical fish culture, fish production depends mostly on the quality of rations being prepared as pellets or any other forms apart from suitable environmental conditions. It is well advocated that protein contents in feeding ration play its significant value in fish production. Protein sources for fish rations can be chosen either from dried fish materials or plant protein (Stickney, 1979). Up to 25% replacement of animal protein with plant protein is acceptable in freshwater fish feeding rations. Similarly, El Sayed (1992) stated that a partial replacement of animal protein with plant protein in the ration could be possible with the use of some plant sources but not all as a whole. Bowen (1982) has also determined that Nile tilapias could be particularly considered as outstanding adapted fish at assimilating energy from algal chlorophyll and other plant sources. This ability has been evaluated and based on low pH values generated in Nile tilapia stomach (as low as 1-1.25), compared with moderate values in other animals (2-2.2). Coupled with low stomach pH value is a lengthen intestine, which may lengthen from 7 to 10 times the fish's length. Both of these adapted features make Nile tilapia to manifest high competency in extracting energy from plant materials and allow inexpensive supplementary feeds to be used in Nile tilapia culture (Diana, 1997). As a result of food diversity habit of Nile tilapia, some large varieties of supplementary feeds have been used in Nile tilapia production. This includes many materials derived from soybean, corn, peanut or cotton seed meal as well as broken rice, rice bran or other cereals. These materials had been commonly fed to Nile tilapia.

A number of plant varieties have been practically used for fish feeding both as a direct supplementary feeds or raw materials for formulated rations particularly legume crops with high protein contents. Some aquatic plants have been commonly used as supplementary feeds, e.g. high protein *Azolla*, *Lemna* whilst Wolffia, a floating aquatic plant with high protein contents could possibly do the same. This aquatic plant could be considered as a feeding material for fish either as a direct feeding stuff or a source of protein for any formulated rations and it could possibly be substituted in place of soybean meal. The availability of soybean meal in the market seems inadequate due to the small amount of soybean seeds being produced annually. Furthermore, the market prices could be relatively high. Therefore, other sources of high protein contents from aquatic plants should be of imperative value in substituting the amount of soybean meal in the formulated rations. Aquatic plants as that of Wolffia (*Wolffia arrhiza*) could be one of many promising sources of high protein (Okeyo, 1989) for some formulated fish rations since this plant has high amount of protein contents and its availability could be relatively huge in most natural fertile stagnated water, lakes and other constructed reservoirs and it could be technologically cultured for use in most formulated animal rations. The objective of present investigation lies on the search for plant materials being used as a substitute for soybean meal and should have no negative effects on fish's performance. Wolffia meal has been chosen for this work.

Materials and Methods

Fresh Wolffia samples collected from different sources in Northeast Thailand were oven-dried at 60 °C for three days and then finely ground into meshes. The oven dried Wolffia samples were analysed for its proximate analysis using the method of AOAC (1980). Two laboratory experiments were carried out, i.e. the first experiment was concerned mainly digestibility and the second experiment was emphasized most on feeding effects on growth of Nile tilapia. With the first experiment, two groups of Nile tilapia were used, i.e. Nile tilapia fingerlings with the average live weight of 2.5 g (80 fish for each replication) and Nile tilapia adults with live weights ranging from 240-250 g (4 fish for each replication). All of the experimental fish were acclimatised to a normal condition for 5 days prior to the experimental commencement. For the experiment I, fresh Wolffia feeding materials were fed as *ad libitum* to both groups of fish by spreading the feeding materials (ration) over the surface water of the experimental aquaria. Fish excrements from both sources were collected at half an hour after feeding and then oven-dried at 60 °C for three days and kept in desiccators for proximate analyses (AOAC, 1980). Hydrolysis-resistant organic matter (HROM) was used as internal indicator for digestibility evaluation.

The second experiment was carried out using Randomized Complete Block Design (RCBD) with three replications. 40-litre glass aquaria were used. The four treatments consisted of four levels of Wolffia meal in replacing the amount of soybean meal in the formulated feeding rations, i.e. 0%, 15%, 30% and 45% (Table 1). Twenty Nile tilapia fingerlings were used for each replication. They were solely fed with experimentaly formulated rations and fed as *ad libitum* twice a day for 8 weeks (the amount being fed at each time was approximately 7 % of the body weight). Body live weights and total length of the experimental fish were recorded at two-week intervals. The results obtained were analyzed statistically using one-way analysis of variance and Duncan's Multiple Range Test (Steele and Torrie, 1980).

Table 1: Composition of the formulated diets and proximate analyses of the four experimental formulated diets.

Materials	Percentage formulated			
	1	2	3	4
Fish meal	20	20	20	20
Rice bran	25	25	25	25
Wolffia meal	0	15	30	45
Soybean meal	20	14	7	0
Broken rice	29	20	12	4
Wheat flour	2	2	2	2
Plant oil	2	2	2	2
Vitamin/mineral premixes*	2	2	2	2
Total (%)	100	100	100	100
Proximate analysis				
Crude protein (%)	25.47	25.56	25.29	25.02
Dry weight (%)	98.48	98.32	98.61	98.56
Gross energy (Kcal/kg)	3,800.87	3,747.59	3,794.31	3,741.03
Protein (%)	24.85	24.84	26.61	24.41
Fat (%)	9.81	10.37	9.71	10.67
Fiber (%)	7.84	7.94	6.83	5.73
Ash (%)	9.44	10.47	11.06	9.02
Calcium (%)	2.28	2.14	1.95	1.64
Phosphorus (%)	1.38	1.35	1.39	1.12

*Vitamins and minerals (g/kg ration): 0.024 g AD3, 0.500 g chlorine chloride, 0.250 g $MnSO_4$, 0.100 g E, 0.024 g panthothenic, 0.010 g KI, 0.004 g K, 0.024 g folic acid, 0.050 g ZnO, 0.005 g B, 3,000 g NaCl, 0.010 g $CuSO_4.H_2$, 0.080 g B2, 1,000 g KCl, 6.000 g dicalcium phosphate, 0.100 g B6, 1,400 g $MgSO_4$, 0.125 g BHT, 0.020 C, 0.320 g $FeSO_4.5H_2O$.

Results and Discussion

The results on proximate analysis revealed that crude protein content on dry weight basis of Wolffia meal was approximately 20.4 % (Table 2). This crude protein level had been attained from natural sources in Northeast Thailand where nutrient contents of the water sources, were relatively poor as a result of low fertility of soil types due to high percentages of sand particles and poor amount of organic matter as reported by Suksri (1996). If the growth of Wolffia had been looking after with high level of nutrients then it could be possible that, higher level of crude protein may be achieved. Okeyo (1989) showed that Wolffia attained crude protein content up to 21.5 %. Therefore, protein content of Wolffia could possibly be manipulated by the use of nutrient levels in the growth media. The protein content of this plant could be comparable to some protein levels in some other plants, e.g. Alma and Gastanares (1990) reported that crude protein contents of *Azolla* approximately ranged from 19-31% whilst, crude protein content of soybean meal could reach 44 % as reported by Fritz (1973). Therefore, Wolffia meal could have high potential in replacing some amounts of soybean meal in most formulated animal rations particularly fish diets if being cultured with high level of nutrient solutions. Furthermore, this plant has a high tendency to multiply themselves most rapidly with time in most tropical countries. It is not desirable to have a fiber content exceeding 12 % in fish feeding stuff, as an increase in fiber content could consequently lead to the decrease in the quantity of some usable nutrients in the feeding ration as stated by De Silva and Anderson (1995). The common problems encountered with the utilization of aquatic macrophytes could be the low level of essential nutrients and high level of crude fiber and finally poor digestibility. These could possibly reflect poor feed conversion ratio as stated by Rifai (1979). When the fiber content is excessively available in the diets then it could decrease the total dry matter and nutrient digestibility and eventually resulted in poor growth performance as reported by De Silva and Anderson (1995). The rations being prepared for this experiment contain ash within the range of 9.02-11.06%, which could presumably be accepted for the range of allowable ash percentages as reported by De Silva and Anderson (1995).

Table 2: Mean values of proximate analyses on dry weight basis of Wolffia from the different collected sources in Northeast Thailand.

Item analysed	Means and SE values
Moisture	94.7 ± 0.07
Dry weight	5.28 ± 0.07
Gross energy (GE)	4,74 ± 46.2
Crude protein (CP)	20.4 ± 0.36
Crude fat	4.63 ± 0.13
Crude fiber	11.6 ± 0.17
Ash	17.6 ± 1.52
Calcium (Ca)	0.16 ± 0.03
Phosphorus (P)	0.41 ± 0.05
Ca:P ratio	0.41 ± 0.08
GE:CP ratio (kcal/Kg)	23.3 ± 0.25
HROM	15.8 ± 1.28

SE = Standard errors;
HROM = Hydrolysis-resistant organic matter.

Table 3: Digestibility percentage determinations of Wolffia meal by Nile tilapia fingerlings and adults.

Indicators	Fish sizes	
	Fingerlings	Adults
Dry weight	58.0	58.5
Gross energy	68.6	68.2
Protein	69.4	68.6
Fat	86.4	88.6
Calcium	67.2	79.3
Phosphorus	86.0	87.7

Table 4: Growth performance of Nile tilapia for 8 weeks being fed with the four experimental formulated diets.

Parameters	Feed No.			
	1	2	3	4
Weight gain (%)	716.9	931.6	910.6	1012.8
Length gain (%)	126.4	116.3	127.6	131.1
Total production (g)	55.0 ^{b}	79.3 ^{a}	34.3 ^{c}	30.4 ^{c}
Specific growth rate (%/day)	3.83	4.02	4.16	4.25
Survival rate (%)	58.8 ^{a}	72.5 ^{a}	35.0 ^{b}	27.5 ^{b}
Feed intake (g/fish)	10.5	11.4	10.4	9.94
Feed conversion ratio	2.6	2.3	2.2	2.0
Protein efficiency ratio	1.6	1.8	1.9	2.14
Net protein utilization	46.9	48.9	55.13	61.1

Letters indicate Duncan's Multiple Range Test (P=0.05).

The digestibility and growth performance of Wolffia by Nile tilapia fingerlings and Nile tilapia adults did not significantly differ from each other (Tables 3 and 4). This may be attributed to Nile tilapia's high digestibility as previously discussed. Percentages of weight, length, feed intake, feed conversion ratio, protein efficiency ratio, and net protein utilization and specific growth rate of Nile tilapia adults were not statisticaly significant in all levels of Wolffia being used. However, total production of Nile tilapia being fed with the feed number 2 (15 % Wolffia meal) was significantly higher than the rest. This includes soybean meal formulated ration (feed number 1, being used as a control treatment). The highest live fish production was found with that of the formulated feed number 2 (15 % Wolffia meal) followed by the feed numbers 1, 3 and 4, respectively (Table 3). The results suggested that 15 % Wolffia in the ration should be adequate for use in the ration for Nile tilapia production. The survival rates of Nile tilapia had a trend as that of the total live fish production. The results indicated the appropriate level of Wolffia meal for growth and production of the experimental fish. 15 % of Wolffia meal should be the highest rate for use. An increase in the level of

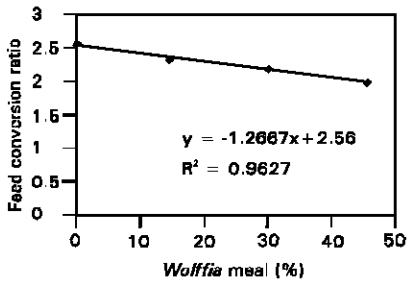

Fig. 1: Feed conversion ratio of Nile tilapia being fed with diets containing *wolffia* meal, 0%-45% for 8-week duration.

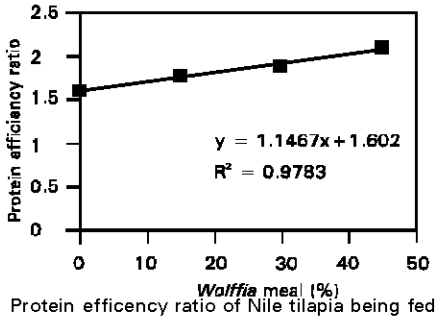

Fig. 2: Protein efficency ratio of Nile tilapia being fed with diets containing Wolffia meal, 0%-45% for 8- week duration.

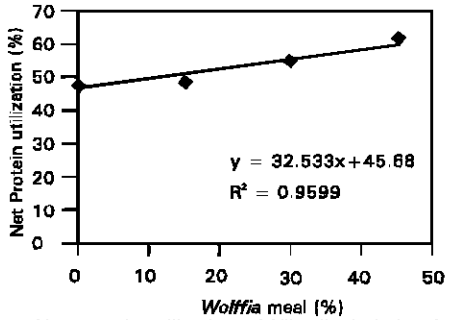

Fig. 3: Net protein utilization of Nile tilapia being fed with diets containing Wolffia meal 0%-45% for 8-week duration

Wolffia meal in the formulated ration decreased the survival rate of the fish tremendously (r^2 = 0.96, Fig. 1). Feed conversion ratio was similar for all treated fish. De Silva and Anderson (1995) reported that feed conversion ratio should be at the range between 1.2-1.5. Nevertheless, the results of the present work were much higher than these figures even at the highest rate of Wolffia (feed number 4). The results suggest some outstanding features in growth of the fish due to Wolffia meal. An increase in Wolffia meal in the ration increased protein efficiency ratio of the experimental fish, (r^2 = 0.97, Fig. 2) and also did with the net protein utilization (r^2 = 0.96, Fig.3). However, no statistical differences were recorded. Jauncey and Ross (1982) reported that crude protein and crude lipid requirements of Nile tilapia during growth period could be 25-30% and 6-10%, respectively. However, there were no significant differences among the formulated feeds being used. An increase in the amount of Wolffia meal in the formulated rations failed to increase the fish production. The results implied that greater percentages of Wolffia in the ration (beyond 15 %) could have produced less degree of palatability for Nile tilapia. Furthermore, protein contents and quality of feeding rations may have decreased due to the additional amount of Wolffia meal hence the survival of fish was tremendously affected.

In conclusion, the replacement of Wolffia meal in place of soybean meal could be possible only up to 15 % in the ration and an increase beyond 15 % would decrease total fish production, survival rate, feed intake, and feed conversion ratio of the Nile talipia, whilst protein efficiency ratio, specific growth rate, and net protein utilization were increased with an increase in the levels of Wolffia meal.

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