Utilization of Methanol Extracted Of Moringa And Mulberry Leaves To Evaluate Energy and Protein Balance Of Nile Tilapia

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Abstract

Fish ration should have high protein content. Source of feed protein usually comes from animal such as fish meal and waste of fishery industries. The price of animal protein like fish meal is quite expensive and that ingredient of feed has competitive problem with human food. Plant protein like legume leaves or other forage can be used for covering protein requirement of herbivore fish, but they contain high secondary compounds. These compounds may be removed by methanol extraction of the plant material, e.g. for moringa. The present study was carried out to evaluate energy balance of diet containing extracted moringa leaves and mulberry leaf meal each as 30 % protein replacement for fish meal in diets for Nile Tilapia. Three diets were designated as control diet prepared with fishmeal (C), diet 1 contain methanol extracted moringa (D-1) and diet 2 contained mulberry leaves (D-2). Fifteen Nile Tilapia were randomly kept in a 5 L capacity individual respiration chamber in which the oxygen consumption of each fish could be measured continuously (Focken et al., 1994). Prior to the experiment fish were fasted for two days in order to measure standard metabolic rate (SMR), routine metabolic rate (RMR) and spontaneous activity (SSA). After those measurements, fish were divided into three groups and fed with the test diets C, D-1 and D-2 at around 10-g feed per MBW (kg $^{0.8}$) using automatic feeders. Fish were weighed individually every week and the oxygen consumption continuously measured for gain information on the energy expenditure (EE). At the end of the eight week, fish were sacrificed and analyzed for energy retention (ER). Feed analyses were conducted to evaluate gross energy intake (GEI), while energy metabolism (ME) was calculated from EE plus ER. The data were subjected to ANOVA and statistical comparisons between the feeding groups were made using the Duncan's Multiple Range Test. Results showed that the average values of SMR, RMR and SSA were 49, 67 and 105 mg.kg$^{-0.8}$.h^{-1}, respectively. Energy intake for the control group was lower than for the other groups, while final body weight in group D-1 was the highest. The ratio EE and ME from GEI (%) were similar for groups, while ER (g) for group D-2 is the highest. It was concluded that methanol extracted moringa leaves and mulberry leaves are quite palatable and could replace 30 % of protein fish meal in diets for Nile Tilapia.

Keywords: Moringa, Mulberry, legume tree, SMR, RMR, SSA

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Introduction

Fish ration should have high protein content. Source of feed protein usually comes from animal such as fish meal and waste of fishery industries. The price of animal protein like fish meal is quite expensive and that ingredient has competitive problem with human food. Plant protein like legume leaves can be used for covering protein requirement of herbivore fish, but in tropical legumes they contain high secondary compounds, which have a side effect to the user. The other alternative forage which also content high protein is *Moringa oleifera* Lam and mulberry (*Morus* sp.) leaves.

Moringa oleifera is tree which grows throughout most of the tropics and has several industrial and medicinal uses (Becker and Makkar, 1999). They are not legumes and also not a gramineae; some people call it "The Miracle Tree". They has multifunction such as human food, water purification, medicinal products and animal and fish feed (Becker and Makkar, 1999: Foidl *et al.*, 2001). In Indonesia, in such area like Bali, Madura, Nort Sumatra and South Sulawesi island, people eat those leaves and especially for lactating mother. While in India, Nicaragua and Niger there are a lot of *Moringa oleifera* plantation and uses for multi purposes. It was reported that replacement of 20 and 30 % of the total dietary protein with freeze-dried *Moringa oleifera* leaf meal for Nile tilapia had decreasing of growth performance caused of the relatively high secondary compounds like total phenolics, saponin and phytic acid, as well as NDF and ADF (Richter *et al.*, 2003). Afuang *et al.* (2003) reported that methanol-extracted residues and methanol extracts of moringa leaf meal had no significant effect on the growth performance compared with control diet in Nile tilapia and so far it was concluded that those diets reduced the plasma and muscle cholesterol. The nutritional and energy content of extracted and unextracted moringa leaves are 43.50 and 25.10; 1.40 and 5.40 ; 47,40 and 21.90; 16.30 and 14.10 %; 17.70 and 18.70 MJ/kg for CP, CL, NDF, ADF and GE, respectively (Gupta *et al.*, 1989). Makkar and Becker (1996) reported that anti nutritional components of whole and extracted moringa leaves which is important information for animal feed are glucosinolates, saponin, total phenols, tannins and cyanogenic glycosides in pars of moringa plant.

There are a lot of Mulberry species. Ekastuti *et al.* (1996) reported the nutrient content of five kinds of mulberry leaves such as Morus cathayana, M. nigra, M. canva, M. multicaulis and M. alba from Indonesia in different cutting stage which have 15.71 – 22.59; 3.70 –

615 ; 8 – 16.8 % and 3.5 – 4.6 Kal/ kg for protein, lipid, fiber and gross energy respectively.. Those leaves also content vitamin A, where M. cathayana has the highest one compared to the other (5671 and 5736 mg%, in young and old leaves respectively. The proximate analysis of sun-dried mulberry leaves also reported by Phiny *et al.*, (2003) which were contained (%DM) 20 and 22 % of CP and CL respectively, and around 75 % of DM digestibility.

There is no information regarding the utilisation of methanol extracted of moringa and raw mulberry leaf meal to report the digestibility and energy balance of Nile tilapia. Therefore, the present study was carried out to evaluate of digestibility and energy balance of diet containing extraets moringa and mulbery leaf meal as 30 percent protein replacement for fish meal in diets for Nile tilapia.

Material and methods

Diet formulation

Moringa *oleifera* and mulberry leaves were obtained from Indonesia with treated oven-dried 40 ° C before transportation to Germany. On receipt at Hohenheim University, they were finaly ground in a laboratory mill and from the moringa stock; sample was extracted with 90 % methanol using a Soxhlet apparatus for 48 h. The leaf extracts were separeted from the residues through filltered using a filter paper and the residues were freeze-dried soon after air-drying and all material above stored at freezer until analysis and feed formulation. Prior to feed formulation, the proximate composition and amino acid analysis of methanol extracted moringa and morus leaves were determined, while wheat meal and fish meal were analyzed by previous reseacher (Riehter *et al*, 2003).

Three diets, were designated as control diet ©, diet 1 (D-1) and diet 2 (D-2), were used in this experiment. The control diet was prepared with fishmeal as the primary source of protein by mixing with various ingredients as shown in Table 1. Diet 1 and diet 2 were designated to replace 30 % of fishmeal-derived dietary protein in diet using methanol extracted residues of moringa leaf and raw mulberry leaf meal respectively.

Table 1. Chemical composition and anti-nutrient content of ingredients (g % DM)

	Wheat meal	Fish meal	Me-OH moringa	Morus
DM	87.70	90.90	91.30	90.30
CP	14.00	69.90	37.40	29.58
CL	1.50	8.10	3.80	1.40
CF	1.70	-	7.60	20.12
Ash	1.63	19.47	12.52	9.64
NDF	-	-	42.28	20.34
ADF	-	-	22.01	15.59
Met + Cys	0.80	3.19	1.42	0.65
Val	0.91	4.40	1.97	1.47
Isoleu	0.68	3.63	1.75	1.22
Leu	1.14	6.16	3.61	2.46
Phe +Tyr	1.60	5.39	4.05	1.53
His	0.46	1.65	1.31	0.92
Lys	0.57	5.61	2.08	1.84
Thre	0.91	5.50	1.97	1.39
Arg	0.57	3.41	2.85	1.86
Tryp	0.23	0.88	0.77	0.54
Saponin	-	-	3.04	1.72
Tannin	-	-	trace	0.46
Phytic acid	-	-	trace	2.91
Total phenolic	-	-	trace	1.04

Experimental Design

A group of nile tilapia (11 – 24 g) fingerlings were used in fish laboratoriun of Departement of Aquaculture System and Animal Nutrition, Institute for Animal Production in the Tropics and Subtropics, Hohenheim Universty. There were two batches of experiment where experiment 1. was for evaluation of energy balance using respiratory boxes while experiment 2. was for evaluation of digestibility of diets containing methanol extracted moringa and raw mulberry leaf meal. At the beginning of the experiment, three fish of the same population were killed and frozen for the determination of initial body composition and the rest of fish were fed at level of maintenance requirement according to body weight.

Experiment 1: In Respiration chamber

Fifteen nile tilapia were randomly kept in the 5 L capacity individual respiration boxes in which the oxygen consumption of each fish could be measured continously (Focken *et al.*, 1994). The boxes were

illuminated 12 h on and off and water temperature was kept at 28,2 °C. During acclimatisation (3 days) in the experimental set up, the fish received maintenance level of feed. In the prior of experiment fish were fasted for two days according to measure standard metabolic rate. The standard rate of O2 consumption (SMR) was determined when the fish had grown used to the respiration box approximately after 24 h, then the VO2 values collected at the start of the experiments were tested for SMR which was attained when low metabolic rates had been measured constanly over 60 min. Routine VO2 (RMR) is the O2 consumption of a fasting fish over 24 hours including the VO2 resulting from spontaneous activity, the highest VO2 values recorded in the first 48 hours of experiment is the spontaneous activity (SSA) (Becker *et al.*, 1986).

Experiment 2: In Aquaria

Another fifteen nile tilapia fingerlings (11 – 22 g) were kept in the 40-l of individual aquaria for acclimatisation. The aquaria were integrated into a recirculatory system at 26.6 °C. They were divided into three groups of 5 fish each randomly and fed at around maintenance level (3 g/ kg $^{0.8}$ /day) with three kind of diet containing approximately 35 % protein, 7 % lipid, 11 % ash and a gross energy content of 19 kJ g-1 dry matter. After adaptation three fish of the same average body weight were killed and analysed for initial body composition. During the experimental period, the fish were fed at 10 g feed per metabolic body weight (kg $^{0.8}$) per day in four equal installments using an automatic feeder. Fish were weighed individually every week. At the end of eight week of the experiment, fish were weighed, sacrificed and analyzed for whole body composition. Prior to the chemical analyses, both the initial and experimental groups of fish were autoclaved at 120 °C for 30 min, homogenised, refrozen and freeze dried.

Table 2. Formulation of experimental diets (g %DM)

	C	D-1	D-2
Wheat meal	45	31	25
Fish meal	41	29	29
Me-OH moringa	0	28	0
Morus	0	0	37
Mineral mix	2	2	2
Vitamin mix	2	2	2
Sunflower oil	4	4	4
Alpha cellulose	5	3	0
Ti O2	1	1	1
TOTAL	100	100	100

Table 3. Proximate and amino acid composition, of experimental diets and amino acid requirements of Nile tilapia (% DM)

	C	D-1	D-2	tilapia's requirement
Dry matter	94.30	97.5	93.02	-
Crude protein	36.72	35.97	35.95	-
Crude lipid	7.90	6.98	6.37	-
Ash	10.95	11.89	11.74	-
Gross energy (kJ/g)	19.16	18.80	19.80	-
NDF	20.38	31.32	24.38	-
ADF	8.0	10.74	6.99	-
Met + Cys	1.67	1.57	1.36	0.90
Val	2.21	2.11	2.05	0.80
Isoleu	1.79	1.75	1.67	0.90
Leu	3.04	3.15	2.98	1.0
Phe +Tyr	2.93	3.19	2.53	1.60
His	0.88	0.99	0.93	0.50
Lys	2.55	2.39	2.45	1.40
Thr	2.66	2.43	2.34	1.20
Arg	1.65	1.96	1.82	1.10
Tryp	0.46	0.54	0.51	0.30

Biochemical analysis

The proximate analysis of diet ingredients, diet and whole bodies of fish (CP, CL, and ash) were based on the procedures of the AOAC

(1990) standard methods. Dry matter was measured by drying to a constant weight at 105 ° C and gross energy by bomb calorimetry (IKA C 7000) with benzoic acid standard. Fiber constituents such as neutral detergent (NDF) and acid detergent (ADF) fibers of methanol extracted moringa and raw morus leaves were determined according to the procedure described by Van Soest *et al.* (1991). An automated amino acid analyser was used to determine the amino acid composition of feed ingredient. The total phenolics and tannins were determined by the spectrophotometric methods described by Makkar *et al.* (1993). Phytic acid estimation was carried out by the modified photometric procedure of Vaintraub and Lapteva (1988) and the total saponin content was determined by the method of Hiai *et al.* (1976).

Calculation and statistical analysis

All calculations were performed for each fish individually. Growth performance was assessed in terms of the Body Weigh Gain (BWG) which calculated by subtracting final and initial body weight, Feed Conversion Ratio (FCR) was calculated as live weight gain/feed consumption (dry matter), and Metabolic Growth Rate (MGR) as live weight gain (g)/average metabolic live weight (kg $^{0.8}$)/day. The Average Metabolic Rate was calculated as mg oxygen consumed kg $^{0.8}$ /h on a weekly basis. The Standard Metabolic Rate (SMR) was taken as the lowest metabolic rate sustained for 2 h by the undisturbed fish that had been fasted for the preceding 24 h (Ultsch *et al.* 1980). This calculation was done using the oxygen consumption values recorded on the day during which the fish were starved, before experimental feeding started. Oxygen uptake (g) x 14.85 (kJ /g) gave the energy expenditure (EE) during the whole experiment (Huisman 1976) and the energy apparently metabolised (ME) was calculated by subtracting energy retention and energy expenditure of carcass (ER) from the gross energy of the feed consumed. Diet nutrient utilization was analysed in terms of Feed Intake (FI), Protein Production Value (PPV, %) was calculated as protein gain x 100/feed protein. Protein Efficiency Ratio (PER) was calculated as live weight gain (g)/ protein fed (g).

The data were subjected to ANOVA and statistical comparisons between the feeding groups were made using the Duncan's Multiple Range Test (Statistica for Windows, release 5.1 H, 97 edition). The significance of observed differences was tested at $p< 0.05$. the values presented in the text are Mean ± Standard Deviation.

Results

Respiration chamber experiment

All data of the experimental chamber was shown in Table 4. where from the statistic analysis there were no significance difference between treatment.

The data of Nile Tilapia's SMR were same with reported before which were around 44.4 mg/kg $^{0.8}$/d (Becker, 1990). The scope for spontaneous activity was a good measure of the energy available to the fish for body tissue synthesis. The RMR and SSA values in this experiment were also similar with reported before. Tilapia zillii has RMR and SSA were around 64.4 mg/kg $^{0.8}$/d and 111.2 mg/kg $^{0.8}$/d, respectively.

Table 4. Metabolic rates of the experimental fish

	Control	D-1	D-2
Standard metabolic rate (SMR):			
- mg/kg $^{0.8}$/h	42.10	49.43	55.81
Routine metabolic rate (RMR):			
- fasting (mg/kg $^{0.8}$/h)	62.65	73.43	67.44
Scope for spontaneous activity (SSA)			
- fasting (mg/kg $^{0.8}$/h)	105.24	108.42	103.6

2. Aquaria experiment

The chemical composition of the experimental fish was in Table 5. There was significant difference in crude lipid of control and treatments where were fishes fed with moringa and morus had low lipid ($P<0,05$). Total energy of body in D-2 was significantly lower than other treatments ($P<0,05$).

Table 5. Initial and final chemical composition of the experimental fish

	Initial Mean	Control Mean	D-1	D-2 Mean	Mean
DM (% of fresh)	20.24	27.99		28.42	25.62
CA (% DM)	10.71	16.34		16.25	17.52
CP (% of DM)	66.05	56.66		57.26	58.29
CL (% of DM)	12.38	22.79[a]		19.97[b]	18.21[b]
GE (kJ g-1)	15.57	21.17[a]		21.06[a]	19.95[b]

The calculated average values of nutrient utilization, FCR and biological value of diet are presented in Table 6. The BW gain value in D-1 was significantly higher than those of the control and D-2. This is because of high the initial BW and nutrient intake in that treatment. Palatability of the ration in D-1 was good for tilapia. As the consequenses utilization of nutrient in D-1 was better than D-2, but not significantly different with control. The highest FCR value was done in D-2 ($P<0.05$).

Table 6. Growth performance and nutrient utilization of the experiment fish

	C Mean	SD	D-1 Mean	SD	D-2 Mean	SD
Initial BW (g)	16.5b		18.9a		18.0a	
Final BW (g)	29.3b		38.9a		31.4b	
BW Gain (g)	12.8b		20.0a		13.4b	
Feed offered (g)	16.3b		21.1a		19.5a	
Protein intake (g)	5.9b		7.7a		7.0ab	
Lipid intake (g)	1.3b		1.7a		1.4b	
Energy intake (kJ)	311.5b		396.5a		387.1a	
FCR	1.0b		1.1b		1.5a	
PER	2.7a		2.6a		1.9b	
PPV (%)	46.6b		55.3a		45.8b	
ER (%)	40.9a		44.6a		35.1b	

The complete energy budget of the fish in the different experimental groups was set up in Table 7. There were differences in

the GE intake and energy retention (% GE) between the treatments. In control treatment was showed efficiency in energy utilization with low energy intake but high percent of energy retention, while in the D-2 treatment was in oppossite where high energy intake produced low energy retention. The best energy utilization was happened in D-1 treatment.

Table 7. Energy budget of fish in different experimental group

	C Mean	SD	D-1 Mean	SD	D-2 Mean	SD
Initial GE of carcass (kJ)	39.7		39.7		39.7	
Final GE of carcass (kJ)	170.6ab		166.6b		185.0a	
GE intake (kJ)	387.1a		311.5b		396.5a	
EE (% GEI)	24.3		25.5		24.9	
ER (% GEI)	35.1b		40.9a		44.6a	
AUE (% GEI)	48.5		31.4		38.9	
ME (% GEI)	59,4		66,4		69,5	

Discussion

All the experimental fish consumed the feed provided completely and there were no mortality of fish during the experiment. Diet containing moringa and mulberry leaves were palatable for fish. Energy budget in tilapia fed with extracted moringa and morus have same heat production which around 25 %, while the energy retained in morus group was lower than another treatments. Protein efficiency ratio (PER) in D2 showed the worst. Mulberry leaves which still contained secondary compound (saponin, phytic and tannin) was affected to the performance. The value of PER in diet control and D1 were same with reported before (Francis *et al.*, 2002), while the value of PPV (45 – 55%) was higher in this experiment compared to fish fed with quillaja saponin which had PPV around 32- 36 % (Francis *et al.*, 2002). The

physiological effect of saponins on fish has been controversial, some authors reporting positive and others negatives influences. Roy et al. (1990) reported that saponin depressed blood parameters such as hematocrit, haemoglobin and red blood cell in several species of fish. On the other hand Francis et al. (2002) reported that supplementation of quillaja saponin in diet carp resulted high oxygen consumption and indicating higher metabolic activity.

There is usually an increase in body fat and energy content with increasing body size in fish fed maximum rations (Cui *et al.*, 1996). In present study, body size had significant effects on the content of fat and energy of Nile tilapia. This is because of all rations has good palatability and quality. The ration was prepared as NRC requirement for Nile tilapia. Even though diet with mulberry leaves was not as efficient as other treatment. Theoretically the proportions of food energy in growing animals would be allocated to various organ target in the body and resulted by the size. Shouqi Xie *et al.* (1997) reported that in mature sexual fish resulted in reduced growth rate caused by the decrease in relative food intake.

Utilization of fresh moringa in fish diet was reported by Richter (2003) with very bad performance of the Nile tilapia. There is no information about morus on diet fish. Ekastuti (2006) reported utilization of kinds of morus leaves with different moisture levels on silk worm production. In this experiment showed that morus leaves without extraction resulted good performance in Nile tilapia although was not same as moringa methanol extracted.

Conclusion

1. Moringa extracted and morus leaf meal could substitute 30 % of fish meal in Tilapia diet.
2. Energy budget of Tilapia fed with extracted moringa have same pattern compare to
   Control, while morus treatment was different.
3. The energy expenditure and energy retention were around 25 % and 40 %, respectively.

Acknowledgement

I would like to thank to DAAD scholarship, Germany for short time research, Prof. Klaus Becker and Department of Animal Nutrition and Aquaculture, Institute for Animal Production in the Tropics and Subtropics, University of Hohenheim for guiding me to study on Fish Bioenergetic.

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