ENERGY AND PROTEIN BALANCE OF NILE TILAPIA FED WITH MORINGA AND MULBERRY LEAVES

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Abstract

The study was to evaluate energy and protein balance of diet that contain extracted moringa and mulberry leaf, as each 30% protein replacement for fish meal in nile tilapia diets. Three diets were control, prepared with fishmeal (C), diet 1 contained methanol extracted moringa (D-1) and diet 2 contained mulberry leaf (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. Prior to the experiment fish were measured standard metabolic rate (SMR), routine metabolic rate (RMR) and scope for spontaneous activity (SSA). Parameter measured were body weight, energy intake (GEI), energy expenditure (EE), energy metabolism (ME), energy retention (ER) and protein utilization. The data were subjected to analysis of variance and were continued using Duncan's Test. The results showed the average values of SMR, RMR and SSA were 49, 67 and 105 $mg.kg^{-0.8}.h^{-1}$, respectively. GEI for the control group was lower than the other groups, while final body weight in group D-1 was the highest. The ER (g) for group D-2 was the highest while value of Protein Efficiency Ratio and Protein Production Value in group D-1 were the highest. It was concluded that methanol extract of moringa are quite palatable and could replace 30% of protein fish meal in diets for nile tilapia.

Key words: moringa, mulberry, standard metabolic rate, routine metabolic rate, scope for spontaneous activity

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 the 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 side effects to the user. The other alternative forage which also contain high protein are *Moringa oleifera* and mulberry (*Morus* sp.) leaves.

M. oleifera is a tree which grows throughout in most of the tropic area and has several industrial and medicinal uses. They are not legumes and also not a gramineae, some people call it "The Miracle Tree". They have multifunction such as human food, water purification, medicinal products and animal and fish feed (Foidl *et al*. 2001). In Indonesia, in such area like Bali, Madura, North 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 *M. oleifera* plantation and uses for multi purposes. 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

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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 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, Morus nigra, Morus canva, Morus multicaulis* and *Morus alba* from Indonesia in different cutting stage had 15.71%-22.59% of CP, 3.70%-615% of CL, 8%-16.8% of CF and 3.5-4.6 Kal/kg GE. Those leaves also contained vitamin A, where *M. cathayana* posessed the highest (5671 and 5736 mg%, in young and old leaves respectively). The proximate analysis of sun-dried mulberry leaves were 20% and 22% of CP and CL respectively, and around 75% of DM digestibility (Phiny *et al.* 2003).

There is no information regarding the utilization of methanol extract 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 the energy balance and protein utilization of diet containing extracted moringa and mulbery leaf meal as 30% protein replacement for fish meal in diets for nile tilapia.

MATERIALS AND METHODS

Location and diet formulation

This study was done at Laboratory of Aquaculture, Department of Aquaculture System and Animal Nutrition, Institute for Animal Production in the Tropics and Subtropics, Hohenheim University, Germany. *M. 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 extract's were separated from the residues through filtered 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 (Table 1), while wheat meal and fish meal were analyzed by previous researcher (Richter *et al.* 2003).

Materials

Three diets, were designated as control diet(C), diet 1 (D-1) and diet 2 (D-2), were used in this experiment. The control diet was prepared with fish meal as the primary source of protein by mixing with various ingredients as shown in Table 2. 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. Proximate and amino acid composition, of experimental diets and amino acid requirements of nile tilapia is shown in Table 3.

A group of nile tilapia (11-24 g) fingerlings were used. There were two batches of experiment where experiment 1 as for evaluation of energy balance using respiratory boxes while experiment 2 as for evaluation of digestibility of diets containing methanol extract of 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.

Experimental environment

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

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

Chemical Composition	Wheat meal	Fish meal	MeOH moringa	Mulberry
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

fish could be measured continously (Focken *et al*. 1997). The boxes were illuminated 12 h on and off and water temperature was kept at 28.2 °C. During acclimatization (3 days) in the experimental set up, the fish received maintenance level of feed. In the prior of experiment fishes were fasted for two days according to measure standard metabolic rate. The standard rate of O_2 consumption (SMR) was determined when the fish had grown used to the respiration box approximately after 24 h, then the VO_2 values collected at the start of the experiments were tested for SMR which was attained when low metabolic rates had been measured constantly over 60 min. Routine VO_2 (RMR) is the O_2 consumption of a fasting fish over 24 hours including the VO_2 resulting from spontaneous activity, the highest VO_2 values recorded in the first 48 hours of experiment is the spontaneous activity (SSA) (Becker and Fishelson 1986).

Experiment 2: In Aquaria

Another fifteen nile tilapia fingerlings (11-22 g) were kept in the 40-L of individual aquaria for acclimatization. The aquaria were integrated into a re-circulatory system at 26.6 °C. They were divided into three groups of 5 fishes each randomly and fed at around maintenance level (3 g/kg $^{0.8}$/day) with three kinds of diet containing approximately 35% protein, 7% lipid, 11% ash and a gross energy content of 19 kJ/g dry matter. After adaptation three fishes of the same average body weight

Table 2 Formulation of experimental diet (%DM)

Ingredients	Control	MeOH moringa	Mulberry
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
TiO_2	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)

Nutrient composition	Control	MeOH moringa	Mulberry	Tilapia's req.
Dry matter	94.30	97.50	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	8.00	10.74	6.99	-
Met + Cys	2.21	2.11	2.05	0.80
Isoleu	1.79	1.75	1.67	0.90
Leu	3.04	3.15	2.98	1.00
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

were killed and analyzed for initial body composition. During the experimental period, the fishes were fed at 10 g feed per metabolic body weight ($kg^{0.8}$) per day in four equal installments using an automatic feeder. Fishes were weighed individually every week. At the end of the eighth week of the experiment, fishes were weighed, sacrificed and analyzed for whole body composition. Prior to the chemical analysis, both the initial and experimental groups of fish were autoclaved at 120 °C for 30 min, homogenized, refrozen and freeze dried.

The proximate analysis of diet ingredients, diet and whole bodies of fishes (CP, CL, and ash) were based on the standard (AOAC 1995). 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 extract of moringa and raw morus leaves were determined according to the procedure described by Van Soest. An automated amino acid analyzer 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 and Becker (1996). Phytic acid estimation was carried out by the modified photometric procedure (Vaintraub and Lapteva 1988) and the total saponin content was determined by spectrophotometer.

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 the fishes 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 metabolized (ME) were calculated by subtracting energy retention and energy expenditure of carcass (ER) from the gross energy of the feed consumed. Diet nutrient utilization was analyzed 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 was subjected to analysis of variance (ANOVA) and statistical comparisons between the feeding groups were made using the Duncan's Multiple Range Test (Statistical for Windows, release 5.1 H, 97 edition). The significance of observed differences were tested at $p< 0.05$. The values that were in the text are Mean ± Standard Deviation.

RESULTS

Respiration chamber experiment

Metabolic rates of the experimental fish is shown in Table 4. The measurement of basal metabolism was done during fasting condition in a such group before the experimental was started. The data of nile tilapia's SMR was same with reported before which was around 44.4 mg/$kg^{0.8}$/d (Becker and Fishelson 1990). The routine metabolic rate (RMR) of nile tilapia was around 1.5 times comparing to standard metabolic rate (SMR). The scope for spontaneous activity (SSA) 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 around 64.4 mg/$kg^{0.8}$/d and 111.2 mg/$kg^{0.8}$/d, respectively.

Aquaria experiment

The chemical composition of the experimental fish was analyzed before and after experiment (Table 5). There was significant difference in crude lipid of control and

Table 4 Metabolic rates of the experimental fish

	Control	MeOH moringa	Mulberry
SMR (mg/kg $^{0.8}$/h)	42.10	49.43	55.81
RMR fasting (mg/kg $^{0.8}$/h)	62.65	73.43	67.44
SSA fasting (mg/kg $^{0.8}$/h)	105.24	108.42	103.6

Table 5 Initial and final chemical composition of the experimental fish

	Initial	Control	MeOH moringa	Mulberry
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]

Table 6 Growth performance and nutrient utilization of the experiment fish

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

treatments where were fishes fed with moringa and mullberry had low lipid ($p<0.05$). Total energy of body in D-2 was significantly lower than other treatments ($p<0.05$). 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 consequences 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 ($p<0.05$).

The complete energy budget of the fish in the different experimental groups was evaluated. There were differences in the GE intake and energy retention (% GE) among 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 opposite where high energy intake produced low energy retention. The best energy utilization was happened in D-1 treatment.

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 mullbery have same heat production which around 25%, while the energy retained in mulberry group was lower than another treatments. Protein efficiency ratio (PER) in D2 showed the worst. Mulberry leaves which still contained secondary compounds (saponin, phytic and

Table 7 Energy budget of fish in different experimental group

	Control	MeOH moringa	Mulberry
Initial GE of carcass (kJ)	39.7	39.7	39.7
Final GE of carcass (kJ)	166.6	185.0[a]	170.6[ab]
GE intake (kJ)	311.5[b]	396.5[a]	387.1[a]
EE (% GEI)	25.5	24.9	24.3
ER (% GEI)	40.9[a]	44.6[a]	35.1[b]
AUE (% GEI)	31.4	38.9	48.5
ME (% GEI)	66.4	69.5	59.4

tannin) were 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 saponin on fish has been controversial, some authors reporting positive and others negative 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 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. 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 *et al*. (2003) with very bad performance of the nile tilapia. There is no information about morus on diet fish. In this experiment showed that morus leaves without extraction resulted good performance in nile tilapia although was not same as moringa methanol extract.

CONCLUSION

Moringa extract and mulberry leaf meal could substitute 30% of fish meal in tilapia diet. Energy budget of tilapia fed with extract of moringa showed same pattern compare to control, while morus treatment was different. The energy expenditure and energy retention were around 25% and 40%, respectively.

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