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Full Length Research Paper

Effect of B-Mannanase Supplementation on the Growth and Apparent Digestibility of Red Tilapia Fed Formulated Diets Containing Palm Kernel Cake

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This study was conducted to evaluate the influence of β-mannanase supplementation on the growth performance, apparent nutrient digestibility (ADCs), meat and carcass content in tilapia fed palm kernel cake (PKC). Three diets containing 40% PKC supplemented with 1% freeze dried β-mannanase (FD), and 1% Hemicell® (Hemi); and control without enzyme inclusion. The final body weight, weight gain, and FCR were significantly different (P<0.05) in tilapia fed control diet compared to diet containing enzyme. β-Mannanase supplementation led to an increase (P<0.05) in the ADCs of crude protein, ash and fiber compared with the control. Dietary treatment had significant effects (P<0.05) only on fat composition in meat quality in the fish fed FD diet had lower than the other treatments. In conclusion, supplementation of diet containing PKC with β-mannanase could improve the growth performance, energy, and nutrient digestibility of tilapia, thus increasing the nutritional value of PKC as potential feed ingredients.

Keywords: Digestibility; palm kernel cake; tilapia; β-mannanase

INTRODUCTION

Towards large scale production of aquaculture, it needs a development of cost-effective and digestible formulated diets for it to become economical. Knowledge of the nutritive value in feed is important to animal nutrition in order to make a cost effective feed. Thus it is important for ingredients in the feed to perform the same as actual value of its chemical composition, in which all nutrients is successfully been digesting and absorbing by the animal.

In line with this, the optimization of fish feed is important as it will affect the growth of the fish muscle (Halver and Hardy, 2002). Development of digestible diet is also needed for maximizing diet utilization and minimizing wastage that can bring negative impact to water quality and possibly fish health. The utilization of carbohydrate instead of protein as a source of energy could give additional impact by reduction of nitrogenous waste of culture system (Miller et al., 2005). The efficiency of fish in utilizing carbohydrate, lipid, and protein from the diet for their energy source varies considerably (NRC, 1993). It has been well established that protein to energy ratios in

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aquafeeds have significant impacts on fish performance (Azevedo et al., 2004; Cho et al., 2005; Lee et al., 2000). Maximizing the utilization of dietary protein for growth is related to both the dietary inclusion level of protein and the availability of non-protein energy sources, namely lipid and carbohydrate. Fish feed act to satisfy the energy requirements, and if dietary energy is insufficient, i.e. a high protein to energy ratio, feed consumption will be increased (Mathis et al*.,* 2003), and dietary protein will be utilized for metabolic energy, not only resulting in an inefficient use of an expensive dietary component, but also contributing to nitrogenous wastes in effluent waters. Protein sparing effect happened when non-protein energy has been used instead of protein for catabolism to provide energy and for growth. Protein sparing by non-protein energy sources has been documented in Atlantic salmon (Azevedo et al., 2004). Although lipid is recognized as the major non-protein energy provider for fish, the carbohydrate is more preferable because of its low cost and ready availability thus more economical (Millikin, 1983). Manipulating the knowledge of nutrient digestibility of the various feed ingredients in formulating fish feeds is desirable so that effective substitution of one ingredient for another might be achieved (Köprücu and Özdemir, 2005).

Due to high levels of poorly digestible components, PKC have limited value as feed ingredients for tilapia diets. The supplementation of β-glucanase or protease in a diet containing 344 g/kg soybean meal significantly improved the apparent nutrient digestibility of juvenile rainbow trout (*Oncorhynchus mykiss*) (Dalsgaard et al., 2012). The addition of β-mannanase potential might increase the apparent nutrient digestibility of PKC in fish feed. Thus, this study was aimed to investigate the influence of enzyme supplementation in diet containing high concentration of PKC, a locally abundant by product in Malaysia, by determining the growth, apparent digestibility (crude protein, crude lipid, ash, amino acid, cellulose, hemicellulose and lignin), and carcass characteristics of diets fed to red tilapia.

MATERIALS AND METHODS

Enzyme Source

The β-mannanase preparation used for this study was obtained from the fermentation of PKC by *Bacillus subtilis*. It was produced using batch fermentation process, in which the enzyme was separated from the fermentation residue and the source of microorganism at the end of fermentation period. The major active enzyme of the preparation was β-mannanase, although it also contained other enzymes including α-galactosidase, endoglucanase, and β-mannosidase in minor concentration. The enzyme was then freeze dried using Labconco Freezone 4.5 liter Benchtop at a condenser temperature of -50°C for 24 h at a pressure of 1.5-3.0 mmHg. A reference enzyme (Hemicell®) was also used in this study. The Hemicell® was claimed to contain several types of active enzymes including α-galactosidase, endoglucanase, and β-mannosidase, however only β-mannanase activity was tested in this study. The Hemicell® contained 15,575 nkat/g of β-mannanase. The preparation of freeze dried enzyme was assayed and having 34,215 nkat/g of β-mannanase. One unit of enzyme activity was expressed as 1 nmol of reducing sugar (mannose) formed within 5 min per g reaction at 50°C, and pH 6.0 i.e. as nkat/g.

Experimental Dietary Preparation

Three pelleted diets were formulated to produce the dietary treatments of freeze dried β-mannanase (FD), commercial enzyme Hemicell® (Hemi), and control (without enzyme addition). Formulation of the experimental diets is shown in Table 1. Sinking pellets (diameter 3.2 mm and length 4 mm) were made using a mini pelleting machine, single screw extruder. The pelleted diets were dried in a convection oven at 60^{o}C for 12 h and then stored at 9^{o}C prior usage for feeding trials.

Experimental Procedures

Growth Study

About 500 pieces of tilapia were harvested from Fisheries Research Institute, FRI Glami Lemi's pond and transferred to 5 tonnes circular fiberglass tank. Fish were acclimatized there for two weeks and fed with commercial diet (Dindings, 32% crude protein). A system consisted of 9 rectangular fiberglass tanks; each containing 1.5 tonnes of freshwater was used in the study. Each tank was aerated with air stones connected to an air supply system. The fiberglass tank was a flow through system. Prior to the experiment started, the sexes of fish were determined, and only male fish were selected for the experiment. The average initial wet weight of red tilapia (48.3 ± 2.1 g) was determined by weighing the fish individually, using an analytical balance (Sartorius; 0.1 g sensitivity). Thirty fish were randomly distributed to all tanks, with 3 tanks per treatment. Dietary treatments were randomly assigned to the triplicate groups of fish. Fish were hand fed twice daily (09:00 and 16:00 h) until apparent satiation for 12 weeks. Each group of fish was individually weighed fortnightly. No feed was offered to the fish on the day they were weighed.

Table 1. Formulation of the experimental diets (g/kg of diet) for tilapia

Experimental diets	Control	FD	HEMI
Fish Meal	130	130	130
Soybean Meal	250	250	250
PKC	400	400	400
Rice bran	112	112	112
Squid oil	55	55	55
Skim milk	10		
Freeze dried enzyme		10	
Hemicell®			10
DCP (dicalcium phosphate)	10	10	10
Palm oil	18	18	18
Masterqube	20	20	20
Vitamin premix[b]	3	3	3
Vitamin C	1	1	1
Mineral premix[c]	4	4	4
Celite[a]	5	5	5

Celite ® (acid-washed diatomaceous silica) is a source of acid-insoluble ash. Inert marker for calculation of apparent digestibility.
[b]Provides per kg of diet: retinyl acetate, 3750 IU; cholecalciferol, 3000 IU; all-rac-α-tocopheryl acetate, 75 IU; menadione sodium bisulfite, 1.5 mg; L-ascorbic acid (Stay C), 75 mg; cyanocobalamine, 0.03 mg; D-biotin, 0.21 mg; choline chloride, 1500 mg; folic acid, 1.5 mg; niacin, 15 mg; D-calcium pantothenate, 30 mg; pyridoxine HCl, 7.5 mg; riboflavin, 9 mg; thiamin HCl, 1.5 mg; Astaxanthin (Carophyll-Pink, Hoffman-La Roche), 75 mg.
[c]Provides per kg of diet: sodium chloride (NaCl, 39% Na, 61% Cl), 1200 mg; ferrous sulfate ($FeSO_4.7H_2O$, 20% Fe), 13 mg; manganese sulfate ($MnSO_4$, 36% Mn), 32 mg; zinc sulfate ($ZnSO_4.7H_2O$, 40% Zn), 60 mg; copper sulfate ($CuSO_4.5H_2O$, 25% Cu), 7 mg; potassium iodide (KI, 24% K, 76% I), 8 mg.

Apparent Digestibility Test

The test was conducted in the hatchery at the same place as above in 300 l cylindroconical tanks. The fish were acclimated to the experimental system for 7 days before the experiment started to attain normal feeding. Fecal collection commenced, 5 days after changing to the experimental diets to allow evacuation of all previously ingested material. Red tilapia (175 ± 25 g), were transferred to the indoor tanks, and the apparent digestibility were performed based on the formulated diets stated in Table 1. Each experimental diet was tested in triplicate, using groups of 15 of fish each, randomly distributed to 9 tanks. The nutrient concentration in all diets, and faeces were determined, and compared to those of an indigestible marker (0.5% Celite®) in acid insoluble ash method (AIA).

Fecal samples were collected using method as described by Goddard and McLean (2001). During the trial, fish were fed twice daily (09:00 and 16:00 h) at the rate of 3% of their body weight. About 30 min after every feeding session, all tanks were carefully cleaned, and all uneaten feed residues were removed. Intact faeces strands were siphoned carefully onto a fine mesh, and rinsed once with distilled water. To reduce any loss of nutrients or marker from the faeces due to leaching, and to prevent bacterial contamination, faeces were collected immediately after they were observed in the tanks (Goddard and McLean, 2001). Fecal matter was collected in the morning of the next day and evening, before the next feeding. The collected faeces were centrifuged at 5000 x g for 15 min, in order to remove water, and the supernatant discarded. Fecal samples from each treatment tanks were pooled at the end of the experiment, consequently freeze dried for 48 h, and finely ground using a mortar and pestle. The faeces were stored in a desiccator until required for analysis. Fecal collection was continued for 20 days until it was judged to be sufficient sample had been collected for chemical analysis.

Analytical Procedures

Biochemical Analysis

Before starting the experiment, 6 fish of the same population were killed, and analyzed for initial body composition. At the end of experiment, whole fish (3 fish/ tank) from each tank was dried in a convection oven, grounded, and analysis for proximate. In addition scale of the fish was carefully removed, and the muscle was dissected. For each tank three fish were sacrificed by lethal anesthetic dose (clove oil) after 24 h of fasting period, the dorsal muscle parts of fish fillets were pooled, and then freeze dried and homogenized. In addition,

Chemical Analyses

The proximate analysis of crude protein, crude lipid, crude fiber, ash, gross energy, and amino acid, NDF, ADL and ADF for diets and faeces collections from the digestibility study were determined. Samples of finely ground raw ingredients, diets and whole body fish were analyzed in duplicate by standard laboratory methods in accordance with AOAC (1995). Crude protein was determined by automatic (Tecator) Kjeldahl technique and multiplying N by 6.25, total lipid by Soxtec Tecator System (petroleum ether extracted, b.p. 40 - 60^{o}C) and crude fibre by Fibretec M Hot and Cold Extraction Tecator System. Dry matter was calculated from weight loss after 24 h of drying at 105^{o}C. Ash was determined using an oven at 600^{o}C to constant weight.

The determination of acid insoluble ash (AIA) was done according to a method as described by Choct (1998) with slight modifications. The AIA content of diet and digested samples were measured after ashing the samples, and treating the ash with boiling 4 M HCl. Samples (2.0 - 3.0 g for diets and 0.5 g - 1.0 g for faeces) were weighted into porcelain crucibles, and dried for 24 hours at 105^{o}C in drying oven. The crucibles were than cooled in a desiccator, and weighted. The dried samples were then going through combustion for ashing at 600^{o}C for 4 h. After ashing, crucibles were cooled in desiccators and weighted after 3 h of cooling. The samples were transferred in a new filter bag, and both weights (emptied and filled with samples) were recorded. The bags were sealed, and labeled. The bags were placed in a glass bowl (Pyrex), and boiled with 4 M HCl for 1 h in fume hood. HCl was removed, and the residue in the bag was rinsed with hot water for 3 times. The procedure was repeated until the samples appeared white. The bags were then oven-dried (105^{o}C) and re-weighed.

The amino acid determination in the diets, faeces and raw PKC was determined after acid hydrolysis, performic acid hydrolysis and alkaline hydrolysis using HPLC. The liquid chromatography (LC) analyzed derivatized amino acids using Waters Alliance 2695 Separations Module equipped with online degasser and autosampler, and connected to Waters 2475 multi-wavelength Fluorescence Detector (Waters, Milford, Massachusetts, USA). Briefly, the samples were hydrolysed with 6 N HCl for 24 h at 110^{o}C in a tightly screw capped glass tubes. Waters AccQ Tag column (3.9 x 150 mm) was used for amino acid separation. The method was referred from Waters AccQ Tag^{tm} method for hydrolysate amino acid analysis. The column was set at temperature of 36°C, and the injection volume was 5 µl. The AccQ Tag^{tm} Eluent A concentrate and 60% acetonitrile were filtered using a 0.45 µm regenerated cellulose membrane filter prior to injection onto HPLC system. A flow rate was set at 1 ml/min. Performic acid hydrolysis was done to measure the concentration of cysteine and methionine in the samples. Cysteine and methionine were analyzed as cysteic acid and methionine sulphone, respectively, by oxidation with performic acid for 16 h at 4^{o}C (in refrigerator) and neutralization with 48% hydrobromic acid (HBr) prior to hydrolysis. Methionine and cysteine were determined using the same method of acid hydrolysis after treatment with performic acid oxidation. Amino acids were identified and quantified using a standard amino acid mixture (Amino acid standard H, Pierce, Rockford, Illinois, USA). Tryptophan was measured after alkaline hydrolysis of samples with 4.3 N $LiOH.H_2O$ in a screw-capped tube for 16 h at 120^{o}C. 6 N HCl was added to the hydrolysates to adjust pH to pH 4.5 and ready for detection after samples filtration. A stock solution (5 µg/ml) of L-Tryptophan was prepared in 0.1 N HCl. A Waters Empower 2 for Microsoft Windows chromatographic software was used to control LC and detectors and for collect/ data acquisition.

The calculation of apparent digestibility coefficients (ADCs) for crude protein, crude lipid, crude fiber, ash, amino acids, and energy for the test ingredients and diets were calculated as follows (Cho et al., 1982):

$$ADC = 1-(F/D \times D_i/F_i) \quad (1)$$

Where: D = % nutrient or energy (MJ/kg gross energy) of diet; F = % nutrient or energy (MJ/kg gross energy) of faeces; D_i = % digestion indicator (Celite) in diet; F_i = % digestion indicator (Celite) in faeces.

Mannanase and endoglucanase activity was determined using the method of Sachslehner et al. (1998) and Ghose (1987); respectively. One nkat of β-mannanase activity and endoglucanase was defined as the amount of enzyme that produced 1 nmol of reducing sugars (as mannose and glucose) in 1 second under the assay conditions. Reducing sugars were determined by the dinitrosalicylic acid method of Miller (1959). α-galactosidase and β-Mannosidase activity was assayed using p-nitrophenyl-β-D-glycosides

(pNP-α-Gal and pNP-β-Man) (Growβwindhager et al., 1999). One nanokatal (nkat) of α-galactosidase and β-mannosidase activity was defined as that amount of enzyme required to catalyze the release of 1 nmol p-nitrophenol per second under the assay conditions.

Water Quality Analysis

Water parameters quality was monitored at two weeks interval. The water samples were collected for spectrophotometrical quantification (Hach DR-2010) of nitrate-N (HACH kit, program 351), nitrite-N (HACH kit, program 371), total ammonia-N (HACH kit, program 380), and phosphate (HACH kit, program 490). Total ammonia-N (NH_3-N), nitrite-N (NO_2^--N), nitrate-N (NO_3^--N), and phosphate (PO_4^{-3}) levels were measured according to ammonia salicylate method, diazotization method, cadmium reduction method and molybdovanadate reagent, respectively and by using HACH reagent kit (powder pillow detection kits).

Data Calculation and Statistical Analysis

Weight gain (WG), specific growth rate (SGR), feed conversion ratio (FCR), survival rate, protein efficiency ratio (PER), and protein productive value (PPV) were calculated as follows:

$$WG = \text{final weight (g)} - \text{initial weight (g)} \quad (2)$$

$$FCR = \text{total feed intake in dry basis (g)} / \text{weight gain (g)} \quad (3)$$

$$SGR = [LN(\text{mean final weight}) - LN(\text{mean initial weight}) / \text{days}] \times 100 \quad (4)$$

$$\text{Survival (\%)} = \text{final quantity} / \text{initial quantity} \times 100 \quad (5)$$

$$PER = \text{total weight gain (g)} / \text{protein intake (g)} \quad (6)$$

$$PPV = \frac{[\text{final fish body protein (g)} - \text{initial fish body protein (g)}]}{\text{total protein consumed (g)}} \times 100 \quad (7)$$

RESULT

Proximate and Amino Acid Composition of Experimental Diets

Diets were formulated to have approximately 30% of crude protein, 7.0% of crude lipid, and 17.0 MJ/kg of gross energy. Approximately the actual crude protein, crude lipid and gross energy, obtained from the pelleted diet matched with the predicted data, i.e., 30.4 - 31.5%, 6.4 - 6.7% and 16.58 - 17.63 MJ/kg; respectively (Table 2). The diets contained approximately 9.8 - 10.0% of crude fiber. All three diets were catogerized as isonitrogenous, isolipid, and isoenergetic, and had approximately the same profile of amino acid ($P>0.05$). The raw PKC have crude protein, crude lipid, crude fiber, ash, and gross energy of 15.3%, 2.1%, 18.9%, 4.8% and 15.62 MJ/kg, respectively.

Growth Performance

PER and PPV represent the protein efficiency ratio and protein productive value based on eviscerated fish weight (Table 3). The fish fed well on the experimental diets with no obvious objection to high level of PKC inclusion, and no physical irregularities were observed. Survival was high in all treatments, averaging 86 - 90% at harvest and not affected by inclusion of enzyme. Final mean body weight, WG, FCR and PPV in fish fed the control diet were significantly different ($P<0.05$) from fish fed diet containing enzymes. On the other hand, the PER, SGR, and TGC for fish fed the control diet were not significantly different, but lower than the fish fed diet containing enzyme. The water quality during the experimental period had total ammonia nitrogen (NH_3-N) of 0.53 - 1.81 mg/l, and phosphorous 0.61 - 1.56 mg/l. Data from chemical analysis of the experimental tanks indicated it had moderate concentration of total ammonia and phosphate (Table 3).

Apparent Digestibility Coefficients

The ADCs of CP, ash and DE, were significantly higher ($P<0.05$) in FD diet compared to the control diet (Table 4). On the other hand, Hemi diet showed higher CP, ash and DE compared to control but only ash, and DE had significant differences ($P<0.05$). A slightly higher CL ADCs was also found in the diet containing enzyme compared to control but the difference was not statistically significant ($P>0.05$). The cellulose content was not digested in all diets tested except for FD diet although limited digestion was noted. Hemicellulose and lignin digestibility were higher in diet containing enzyme, and both didestibility values were significantly different for FD diet compared to control. The digestible energy value was statistically higher ($P<0.05$) in diet containing enzyme compared to control. The ADCs of average amino acid were slightly higher in diet containing enzymes but all individual values were not significantly different compared to control diet. The digestibility of cystein, a non essential amino acid in the control diet showed the lowest value among all diets. The essential amino acid (EAA) were at moderate digestibility from 68% to 87%. The lowest EAA digestibility recorded was threonine and the highest was tryptohan. Although the data presented in this study suggest a reasonable agreement between ADCs of protein, and average amino acid; ADCs of the individual amino acids within different diets are varies (52% - 87%).

Whole Body and Muscle Composition

In this research, the diet contains high level of PKC with or without enzyme gave no effect on the muscle compositions (Table 5). The composition of the resulting whole body,

Table 2. Nutrient composition of the experimental diets (% dry matter)

Chemical composition	Control	Hemi	FD	PKC
Crude protein (%)	31.2	30.4	31.5	15.3
Crude fiber (%)	9.8	10.0	10.0	18.9
Crude lipid (%)	6.5	6.4	6.7	2.1
Ash (%)	10.7	10.9	11.1	4.8
NFE (%)	41.8	42.3	40.7	58.9
Dry matter (%)	92.0	98.0	92.5	89.7
Gross energy (MJ/kg)	16.58	17.47	17.63	15.62
Amino acid				
Arginine	4.254	4.052	4.129	3.536
Histidine	1.117	1.053	1.102	0.553
Isoleucine	2.138	2.017	2.079	1.007
Leucine	3.745	3.51	3.642	1.885
Lysine	1.914	1.886	1.933	0.806
Methionine	1.09	1.074	1.125	0.740
Phenylalanine	2.401	2.284	2.356	1.211
Threonine	1.947	1.831	1.916	0.943
Valine	2.579	2.422	2.505	1.437
Alanine	2.499	2.313	2.403	1.187
Aspartic acid	4.757	4.367	4.575	2.306
Cysteine	0.78	0.728	0.788	0.338
Glutamic acid	8.335	7.67	7.958	5.211
Glycine	4.147	2.902	3.049	1.358
Proline	3.142	2.894	3.033	1.119
Serine	2.779	2.596	2.723	1.303
Tryptophan	0.161	0.136	0.171	0.097
Tyrosine	1.272	1.30	1.221	0.605

NFE- Nitrogen - free extract=100–(% ash + % protein+ % lipid + % fiber)

Table 3. Initial mean body weight, final mean weight, WG, FCR, SGR, survival rate, PER, PPV, total ammonia-N (NH_3-N) and phosphate (PO_4^{-3}) of red tilapia fed 3 different diets for 12 weeks

Productivity index/ Growth performance	Control	Hemi	FD
Initial body weight (g)	47.26 ± 2.18	48.86 ± 2.02	48.87 ± 2.33
Final body weight (g)	129.37 ± 6.98[b]	152.22 ± 10.44 [a]	152.73 ± 0.97 [a]
WG[a] (g)	82.11 ± 6.08[b]	103.11 ± 13.57[a]	102.99 ± 3.73[ab]
FCR[b]	2.13 ± 0.15[a]	1.53 ± 0.13[b]	1.53 ± 0.17[b]
SGR[c] (%/d)	1.20 ± 0.05	1.35 ± 0.16	1.34 ± 0.07
Survival rate	86.7 ± 7.6	90.0 ± 0.0	88.3 ± 2.9
PER[d]	2.74 ± 0.36	3.55 ± 0.82	3.73 ± 0.76
PPV[e]	22.11 ± 2.92[b]	32.82 ± 1.55[a]	30.16 ± 2.81[a]
NH_3-N (mg/l)	1.81 ± 0.07[a]	0.55 ± 0.0[b]	0.53 ± 0.03[a]
Phosphate (PO_4^{-3}) (mg/l)	1.56 ± 0.04[a]	0.82 ± 0.02[b]	0.61 ± 0.05[c]

Values in each row with different superscripts have significant differences ($P<0.05$).

[a] Weight gain (WG) = final weight (g) – initial weight (g)

[b] Feed conversion ratio (FCR) = total feed intake in dry basis (g) / weight gain (g).

[c] Specific growth rate (SGR) = [Ln (mean final weight) – Ln (mean initial weight) / days]×100.

[d] Protein efficiency ratio (PER) = total weight gain (g) / protein intake (g).

[e] Protein productive value (PPV) = [(final fish body protein (g) – initial fish body protein (g))/ total protein consumed (g)] x 100

Table 4. Apparent digestibility coefficients of crude protein (CP), crude lipid (CL), gross energy (GE), hemicellulose, lignin, cellulose, and amino acid (AA) for tilapia fed 3 experimental diets

	Apparent digestibility (%)		
	Control	**Hemi**	**FD**
Crude Lipid (CL)	90.49 ± 0.25	92.05 ± 0.25	91.54 ± 0.25
Ash	19.86 ± 0.89[b]	35.06 ± 2.45[a]	39.11 ± 4.06[a]
Gross Energy (GE)	46.40 ± 0.97[b]	57.02 ± 1.36[a]	60.24 ± 2.16[a]
Hemicellulose	76.11 ± 0.81[b]	82.30 ±1.70[a]	84.44 ± 0.46[a]
Lignin	31.90 ± 0.54[b]	43.96 ± 7.42[ab]	51.31 ± 0.94[a]
Cellulose	0.00 ± 0.00[b]	0.00 ± 0.00[b]	3.69 ± 1.91[a]
Crude Protein (CP)	73.07 ± 1.21[b]	78.23 ± 1.39[ab]	80.09 ± 2.68[a]
Essential amino acid (EAA)			
Arginine	81.82 ± 1.16	84.21 ± 2.43	84.33 ± 1.65
Histidine	82.26 ± 0.75	84.36 ± 2.13	84.22 ± 1.37
Isoleucine	74.41 ± 1.94	78.07 ± 3.84	76.98 ± 2.91
Leucine	74.65 ± 1.89	77.81 ± 3.49	77.62 ± 2.40
Lysine	77.27 ± 1.76	82.06 ± 3.02	81.22 ± 1.70
Methionine	74.33 ± 19.09	78.97 ± 5.99	77.42 ± 5.75
Phenylalanine	77.26 ± 1.76	80.43 ± 3.46	79.80 ± 2.42
Threonine	68.42 ± 2.99	73.08 ± 4.49	72.75 ± 3.44
Valine	71.65 ± 2.07	75.02 ± 3.71	74.77 ± 2.79
Tryptophan	85.55 ± 20.44	87.17 ± 18.14	78.74 ± 3.80
Non-essential amino acid (NEAA)			
Alanine	73.47 ± 2.22	76.34 ± 3.40	77.54 ± 2.00
Aspartic acid	79.87 ± 1.61	82.04 ± 1.72	82.76 ± 3.48

Table 4. Contnue

Cysteine	52.21 ± 9.19	63.25 ± 4.83	66.40 ± 3.39
Glutamic acid	81.11 ± 1.25	81.57 ± 0.44	83.64 ± 1.19
Glycine	79.15 ± 1.34	74.14 ± 4.26	76.98 ± 1.84
Proline	70.94 ± 2.39	66.73 ± 4.64	75.04 ± 2.65
Serine	71.31 ± 2.90	74.57 ± 2.89	75.90 ± 1.76
Tyrosine	77.30 ± 1.20	82.49 ± 3.54	79.12 ± 2.83
Average EAA digestibility	76.76	80.12	78.79
Average NEAA digestibility	73.17	75.14	77.17
Total average digestibility	75.16	77.91	78.07

Table 5. The proximate composition of whole body, and muscle of tilapia fed experimental diets (% in dry basis) for 12 weeks

	Initial	Final		
Content (%)	Whole body	Whole body		
		Control	Hemi	FD
Moisture	79.27 ± 1.54	76.87 ± 3.54	78.11 ± 4.40	79.19 ± 2.14
Ash	20.43 ± 2.73	18.37 ± 2.28	16.89 ± 3.21	17.31 ± 1.80
Crude Protein	62.31 ± 0.41	57.77 ± 2.66	57.20 ± 1.60	61.54 ± 3.92
Crude lipid	13.97 ± 1.21	21.01 ± 3.42^{ab}	23.05 ± 1.50^{a}	17.85 ± 1.43^{b}
		Muscle		
Crude Protein		89.28 ± 2.98	89.84 ± 1.37	89.69 ± 0.98
Crude lipid		2.16 ± 0.18	2.26 ± 0.41	1.94 ± 0.15

with the exception of lipid content, was minimally affected by the dietary treatment. Body CL levels decreased significantly (P<0.05) in tilapia receiving diet with inclusion of FD enzyme compared to Hemicell®, but was not significantly different from control. Fish whole body CP content increased slightly with the inclusion of FD enzyme but the same result was not found for Hemi diet compared to control. Statistically, the moisture, ash, and CP content of tilapia fed diet containing enzyme did not show any appreciable variation compared to control (P>0.05).

DISCUSSION

Growth Performance

Fish fed diets containing enzyme grew faster by showing higher final weight, and weight gain (P<0.05) than those fed on the control diet (Table 3). The main factor for better growth performance in the diet containing enzyme might be due to the increased in feed digestion. This was not a consequence of higher feed intake, as fish fed all diets containing the same proportion of ingredients, and fed until visual satiation. Hence, the difference was likely to be a result of poor utilization of consumed formulated diet containing high amount of plant source, which negatively influenced the growth of fish fed control diet in comparison with those fed diets containing enzyme. The use of enzyme have shown to increase the degradation of many types of plant sources in the animal diet (Li et al., 2010; Mushtaq et al., 2009; Nahm, 2007; Zangiabadi and Torki, 2010). Ng et al. (2002) has found that tilapia fed PKC pre-treated with commercial feed enzymes consistently showed better growth, and feed utilization efficiency compared to fish fed similar levels of raw PKC, and PKC could be incorporated up to 30% in the diet without significantly depressing fish growth. The reduced growth of Japanese seabass fed 20% or more protein from canola meal is probably due to its lower digestibility of canola (Cheng et al., 2010). However, Thiessen et al. (2004) reported that there are no significant difference in the growth of rainbow trout with initial weight of 106 g when 30% of canola protein concentrate replaced the fishmeal protein. Inclusion of high plant protein in fish diet has resulted in low palatability, poor indispensable amino acid profiles, impaired phosphorus availability, and complex synergistic interactions among anti-nutritional

factors are perhaps responsible factors that resulted in poor growth performance of fish (Bonaldo et al., 2011; Dias et al., 2005). To ensure palatability of the diets due to high inclusion of PKC, and other plant sources, squid oil was added as an attractant. Another possible reason for low growth at high PKC inclusion levels in the control diet might be due to the cell wall constituents (neutral detergent fiber, NDF and acid detergent fiber, ADF). Richter et al. (2003) suggested that relatively high level of NDF, and ADF besides total phenolics, non haemolytic saponin and phytic in diet containing higher level of moringa (24-36%), might have contributed to the poor growth performance of Nile tilapia. In monogastric animals including fish, dietary inclusion of viscous non starch polysaccharides (NSPs) can delay intestinal absorption of glucose, possibly through a reduced rate of gastric emptying leading to delayed absorption (Bach, 2001). Treatment of defatted oilseeds such as soybean meal, rapeseed meal, and sunflower cake with exogenous enzymes is targeted on degradation of its NSPs, has been found to improve their nutritive value, depending on the complex nature of NSPs (Denstadli et al., 2011). As a conclusion the addition of enzyme (FD or Hemicell®) significantly influenced the growth of tilapia fed diet containing high plant inclusion, which might served to make nutrient availabily excess to the fish.

The reduction of FCR in fish fed diets containing enzyme coincided with the increased in protein utilization (protein efficiency ratio, PER and protein productive value, PPV), and in agreement with some studies evaluating the inclusion of enzyme in diet containing high level of plant sources (Ng et al., 2002; Vahjen et al., 2005). The present of higher level of digestible carbohydrate was thought as the main reason for different result obtained between control, and treatment diets (inclusion of enzyme). In contrast, no effect has been found when protease, and carbohydrase (xylanase, amylase, β-glucanase and cellulase) supplementation in diet containing high level of plant sources (soy, corn gluten, wheat) on growth, FCR and nutrient digestibilities of trout (Ogunkoya et al., 2006). The negative results obtained in their study might be due to several reasons, first the present of highly digestible ingredients may have diminished the possibilities of observing enzyme effects, and second, the low culturing temperature (15°C) is not an optimum temperature for enzyme to perform. Bonaldo et al. (2011) gave different views when high FCR result in turbot fish fed diets containing higher plant protein levels, as no association with the reduced digestibility of ingredients or alterations of gut histology, but due to unbalanced amino acid composition in the diet, thus resulting in the increase in the protein turnover. However, the tested diet used in this study had approximately the same level of protein, and plant source composition which would eliminate the beforementioned reason.

The plant cell wall especially in PKC, and soybean meal might have been digested by the enzymes, thus served as readily available carbohydrate source such as mannan oligosaccharide (MOS) to the fish. Rock lobster juvenile fed diet containing MOS has showed an improved growth (total weight, SGR and average weekly gain), and survival rate (Sang and Fotedar, 2010). The inclusion of MOS in the diet has also resulted in higher absorption surface of the gut indicated by the internal perimeter/external perimeter (Dimitroglou et al., 2008; Sang and Fotedar, 2010) as well as total bacteria count of the gut. SEM analysis suggests that MOS supplementation can produce more microvilli structures which have potential to improve nutrient capture (Dimitroglou et al., 2010). It is therefore be thought that the exogenous β-mannanase had increase the solubilization of mannan in PKC or soybean to low-molecular weight components (MOS, mannose), and decrease digesta viscosity thus leading to improved nutrient digestion and absorption.

The availability of high quality protein in the dietary ingredients is the key factor that give impact to the performance, and protein digestibility of fish. The dietary requirement for protein is in fact a requirement for a well-balanced mixture of essential or indispensable, and non-essential or dispensable amino acids (Wilson, 2002). The requirement for individual essential amino acids often varies among fish species (Akiyama et al., 1997; NRC, 1993). A deficiency of essential amino acid creates poor utilization of dietary protein, and therefore would caused growth retardation, poor live weight gain and feed efficiency (Halver, 2002; Khan and Abidi, 2007). The amino acid composition of the formulated diet obtained in this study (Table 2) had shown a deficiency in all ten essential amino acid except for leucine, and arginine when compared to the diet used for Nile tilapia (Santiago and Lovell, 1988). The arginine, histidine, isoleucine, leucine, valine, lysine, methionine, phenylalanine, threonine, and tryptophan requirements for Nile tilapia are 4.2%, 1.7%, 3.1%, 2.8-3.6%, 2.8%, 5.1%, 3.2%, 5.5%, 3.8% and 1.0% of amino acid, respectively. Inclusion of high concentration of raw PKC (40%) in diet, significantly depressed the catfish growth even with the addition of 1.2% dietary L-methionine. One possible reason would be that methionine is not the first limiting essential amino acid in the PKC based diets (Ng, 2004) and the other reason is the amino acid is not digestible. Methionine and lysine have been identified as the limiting amino acids for juvenile humpback grouper when fed with 100% poultry by product meal (PBM) as the sole dietary protein source (Shapawi et al., 2007). Enzyme supplementation has decreased the intestinal viscosity, thus increased the amino acid

digestibilities by 25% and improved the metabolizable energy levels, in broiler (Nahm, 2007). The addition of enzyme seems to improve the availability or digestibility of amino acid in this study.

Ammonia concentration was higher in treatment tanks containing fish fed control diet compared to diets containing enzyme (Table 3). Protein intake has a direct relationship with ammonia excretion in fish. The end product of amino acid catabolism is ammonia which contributed about 60 to 90% of the nitrogen excreted (Cowey and Walton, 1988). The use of enzyme may reduce the nitrogen excretion up to 40% in broiler diets (Nahm, 2007). The increased in ammonia concentration present in the cultured environment could be an indicator of reduced protein synthesis, expressed as lower growth, and protein retention of fish fed plant meal based diets, and might also because of the imbalance essential amino acid (Bonaldo et al., 2011; Fournier et al., 2004). The fingerling and advanced juvenile of Nile tilapia did not use the excess protein over 35% suggesting that some dietary protein might be deaminated, and produced ammonia (Abdel-Tawwab et al., 2010). However the protein content in the experimental diets was below 35% so that the excess protein might not be the reason for high ammonia in the environment fed control diet. Engin and Carter (2001) suggested an increased in the dietary level of non protein digestible energy would increased the nitrogen retention by decreasing nitrogen losses. It is parallel with higher PER and protein retention value, when diet contains high level of carbohydrate has been given to gilthead sea bream (Fernández et al., 2007). Shimeno et al. (1981) found the increasing of dietary carbohydrate has caused a reduction in the activities of amino acid-degrading enzymes in the hepatopancreas and thus resulted in a low nitrogen excretion rate and high PER. As a conclusion, inclusion of enzyme could reduced the released of ammonia to the environment due to the present of digestible non protein plant source.

Several studies have found that fish regulate their feed intake with respect to the energy content in the diet, that allowed the metabolic control contributing to spare protein when other energy sources are available (Fernández et al., 2007; Nankervis et al., 2000). Determination of protein to energy (P/E) ratio in fish diet is very important because the higher ratio, the better is the diet. If the dietary energy is too high (low protein to energy ratio) resulted in reduced feed intake which will eventually reduce growth, as reduce intake of other essential nutrients (Cho et al., 2005). Increases in dietary energy from 18 to 21 MJ/kg has resulted in higher growth rates and feed conversion ratio and a significant protein sparing effect of both carbohydrate and lipid in juvenile barramundi (Nankervis et al., 2000). The inclusion of enzymes (carbohydrase cocktail) has been detected to cause the breakdown of the NSPs in palm kernel meal and might help to provide energy (Ao et al., 2011) as also been speculated in this study. Carnivorous fish have limited ability to utilise fibrous structures as a source of energy. On the other hand, tilapia is an omnivorous fish which able to digest plant material better than carnivorous fish. Therefore, on the basis of gross energy, as well as P/E value, PKC are suitable for incorporation in fish diet to reduce the cost of fish feed with the addition of enzyme.

Apparent Digestibility Coeffiecients

Results of the study suggested that the protein digestibility coefficients was higher in the diet containing FD enzyme parallel with the average amino acid digestibility eventhough it was not significantly different for amino acid compared to control (Table 4). Supplementing of β-mannanase in the FD diet had a significant effect on the apparent digestibility of protein ($P<0.05$). The nutrient digestibility in control diet was lower, is most likely attributable to the large amount of poorly digestible fiber in plant sources. The addition of enzyme such as hemicellulases, β-glucanase, and protease in the plant based diet have bring improvements in the apparent digestibility of dry matter, crude protein, and gross energy of rainbow trout fish (Dalsgaard et al., 2012; Farhangi and Carter 2007).

The diet ADCs containing fish meal, soybean meal, PKC, and rice bran was 73.1 - 80.1% for protein, 90.5 - 92.1% for lipid, 19.9 - 39.1% for ash, and 46.4 -60.2% for energy. Köprücü and Özdemir (2005) obtained the ADCs value of diet containing anchovy meal, corn gluten meal, soybean meal, gammarid meal, and crayfish exoskeleton meal for protein is 71.0% - 90.5%, lipid is 72.0% - 97.5%, fiber or chitin is 69.3% - 96.1%; ash is 30.8% - 74.9% and energy is 54.8%-92.1% fed to Nile tilapia. The result obtained in this study was comparable to what has been obtained in their study and only slightly lower in ash, and energy digestiblity. While Sklan et al. (2004) obtained the ADCs of lipid and energy for tilapia are 72 - 90%, and 39 - 89%; respectively for formulated diets containing fish and poultry meals, corn gluten, soybean meal, rapeseed meal, sunflower seed meal, wheat, corn, sorghum, barley, and wheat bran. Different result obtained might be because different raw materials used contains different types of plant cell wall, some of which might even hard to be digested. The ADCs of gross energy was lower might be because the ingredients used in this study had a very low energy digestibility such as 40.4% for PKC, 44.3% for ricebran and 51.1% for soybean as has been determined by Laining et al. (2003).

The digestible energy of fish given diet containing enzyme had higher value than control. The apparent energy digestibility is higher for ingredients of animal origin

(anchovy meal, 92.1%) compared to plant origin (soybean meal, 83.7 - 88.1%; corn gluten, 82.7 - 89%; meal, gammarid meal, 65.6%; canola meal, 60.6% and flaxseed meal, 21.2 - 37.4%) (Köprücü and Özdemir, 2005; Tibbetts et al., 2006). Protein of both, plant and animal based feed ingredients are well been digested by humpback grouper (carnivorous fish), however the dry matter and gross energy of the protein rich animal feed ingredients are more digestible than the carbohydrate rich plant feed ingredients (Laining et al., 2003). Rahman et al. (2001) suggested the digestible energy value that are needed for *Oreochromis niloticus* is 17.7 MJ /kg. However the measured gross energy in the diets used in this study lie between 16.53 to 17.63 MJ/kg and the calculation of digestible energy was about 7.67 MJ/kg, 9.96 MJ/kg, and 10.62 MJ/kg; respectively for control, Hemi, and FD diet given to red tilapia used in this study. It is concluded that the digestible energy was lower in all diets than the suggested value, however the inclusion of enzyme gave a positive effect to the digestible energy value.

The ash digestibility was found to be significantly lower ($P<0.05$) in fish fed control diet while the highest was observed in FD diet (Table 4). Uncomplete degradation of carbohydrate to monosaccharides might lead to excreation by fish or they may possibly bind to other nutrients, and thus negatively interfere with the nutrient absorption like fat, protein and minerals (Francis et al., 2001; Glencross et al., 2003). Lower ash digestibility in control diet might be the effect of mineral had be excluded through faeces into the environment. Mineral that are present in higher concentration than 1 g/kg feed are define as macro elements such as Na, K, Mg, Ca, and P; while if feed contain less than 1 g/kg it will fall into micro or trace elements category such as Fe, Zn, Cu, Mn, Co, Ni, Pb, Cd, and Cr (Tacon and de Silva, 1983). Liebert and Portz (2005) have found that phytase supplementation increased the ash content via increasing apparent absorption of Ca, Mg, Cu, Fe, Sr, and Zn from phytate enriched diets. PKC contains high mineral content of micro, and minor mineral element such as Ca, P, Mg, Po, Na, Co, Zn, Fe, and Mg (Akpanabiatu et al., 2001). Thus it is concluded that the present of enzyme had enhanced the absorption of mineral by fish.

The ADCs for lignin, and hemicellulose were significantly higher in diet containing enzyme, and only in fish fed FD diet, cellulose was digestible. PKC and soybean meal contains β-mannan and its derivatives. The treatment diets contains β-mannanase, an enzyme of which are able to cleave randomly within the 1,4-β-D- mannan chain of glucomannans, and galactomannans (McCleary and Matheson, 1986). Different enzyme are required to digest different types of plant composition in the diet because the composition of plant cell walls is extremely variable (Beauchemin et al., 2006). Treatment of soybean meal, rapeseed meal and sunflower cake with exogenous non starch polysaccharides (NSP) degrading enzymes has significantly gave effects on the reductions in total NSP in vitro, but the released substrates did not contribute to an improvement in fish performance (Denstadli et al., 2011). In conclusion, the present of FD enzyme helps to digest fibre in the cell wall of the plant source.

Carcass Analysis

The whole body protein, and moisture content of tilapia fed with diets containing enzyme were positively correlated with digestibility of protein eventhough it was not significantly different from control. Protein content was lower in fish fed control, and Hemi diet. Besides that, inadequate protein in the diet could also resulted in a reduction or cessation of growth, and a loss of weight due to withdrawal of protein from less vital tissue to maintain the functions of more vital tissue (Wilson, 2002). The results of higher weight gain might occur because the deposition of protein, and due to a balancing mechanism between protein anabolism and catabolism. An increase in fillet protein content seems to be a general effect of plant protein inclusion in the diet (de Francesco et al., 2004). The released of polysaccharide-bound protein is a result of enzymatic hydrolysis (cellulase, hemicellulase, pectinase and viscozyme L) in oat bran polysaccharide which would indirectly increase the protein recovery (Guan and Yao, 2008). Chee et al. (2012) has found that the enzymatic pre treatment method using extracted trypsin enzyme significantly resulted in more PKC protein (61.99 g/100 g) under the optimum conditions than did the alkaline (pH 9.5) method (10.21 g/100 g protein only). The used of proteolytic enzymes (proteases, pepsin, trypsin and bromelain) by Onuora and King (1985) has also increased the PKC protein (nitrogen) solubility by 34 - 62 g/100 g. The efficiency and protein retention values indicated that high carbohydrate diets (18% and 26% gelatinized cornstarch, GLC) performed significantly better in terms of protein compared with low inclusion (5% GLC) (Fernández et al., 2007). It is because the ability to utilize digestible carbohydrate rather than protein as an energy source, leads to an increase gain of ingested protein by anabolism thus could spare the protein. Even though the primary function of digestible carbohydrate is to provide energy but it is poorly utilized by fish (Nankervis et al., 2000). Feeding of European sea bass with diets containing various types, and levels of soybean derivatives replacing the fishmeal has also not affected the slaughter yield but significantly reduced liver weight (Brinker and Reiter, 2011; Tibaldi et al., 2006). The endogenous factors such as size, sex, stage of life cycle, and exogenous factors (diet composition, feeding frequency, temperature etc.) affect the body composition of fish (Hansen et al., 2007; Hepher,

1990). In this study all factors were maintained uniform, so that the composition of the diet was the only factor that could affect the chemical composition of the fish. The present of key enzymes that involved in the glycolysis pathway, and amino acid metabolism such as pyruvate kinase, 6-phosphofructo 1-kinase and alanine aminotransferase act as indicator of a protein sparing effect, with less protein being used to cover energy demands as more carbohydrate becomes available to fuel catabolic pathways (Fernández et al., 2007).

The whole body lipid content of the tilapia had an inversed result with protein content in fish fed FD diet. The significant different in lipid content between FD, and Hemi diet was found. This also indicated that protein was used in the catabolism (energy production) instead of anabolism (protein synthesis) in Hemi and control diet. The lipid content of fillets and visceral have been reduced in fish fed plant proteins (Brinker and Reiter, 2011; Dias et al., 2005; Wang et al., 2006). Oligosaccharides and non starch polysaccharides (NSPs) in soy preparations have been thought to give negative effect on the bioavailability of all nutrients, and energy through mechanisms involving a binding action with bile salts and/ or by an obstructing action on digestive enzymes coupled with changes in digesta viscosity, and transit rate (Francis et al*.,* 2001). Besides lower carcass lipid, high moisture and high ash content; reduced growth has also been observed in Cuneate drum fed with the diet containing soybean meal to replace the fish meal (Wang et al., 2006). However in this study the growth was the highest in fish fed FD diet and had lower carcass lipid content. An increase in carcass lipid content (in which dietary lipid, and energy levels has been held constant) with increasing dietary carbohydrate levels might suggest that the lipids has been synthesized from carbohydrates (Messina et al., 2013; Yang et al., 2002). The highest lipid content with the lowest protein content was observed in Hemi diet. The difference in lipid storage can be associated with differences in the energy/protein ratio of the diets, with higher ratios favouring lipid deposition (Fernández et al., 2007). The highest E/P ratio was in Hemi (0.59) followed by FD (0.56) and lastly control (0.53) diet. Diet supplemented with carbohydrase has resulted in similar fish growth as the control diet, decreased the levels of amylase, and total protease including protein depositions in body, and muscle of the fish (Thongprajukaew et al., 2011). Several factors could effect the changes in the animal's synthesis, deposition rate in muscle and/or different growth rates such as dietary protein level and initial body weight of fish (Abdel-Tawwab et al., 2006). The data gathered also suggests that the digestible carbohydrate level in FD diet was insufficient to provide energy for the fish, and thus, more dietary or reserved body lipid was metabolized to counteract the effect.

CONCLUSIONS

The addition of enzyme in a diet containing 40% of palm kernel cake (PKC) and 25% of soybean could improve the growth performance, energy, and nutrient digestibility, however had little or no effect on meat quality, digestive enzyme, and somatic index of the tested red tilapia. The supplementation of FD enzyme in the diet makes the carcasses had lower lipid, and high protein content. This type of diet might fullfilled the consumers need for lean, and high protein in fish products. Therefore, carbohydrates like PKC and soybean could be used at high concentration, and act as the main protein source for replacing fishmeal in the diet when added with enzyme. The water quality parameter was also better in term of low concentration of total ammnonia-N, and phosphate in the treatment tanks given diets containing enzyme. This will eventually cause the waste water from aquaculture industry would receive less nitrogen and lower the risk of eutrophization with enzyme supplementation. Thus, the contribution of plant source feed ingredients to the formulated diets could help to achieve cost effective diets as it may lead to a reduction of the overall feeding cost in tilapia production.

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