Digestibility and effective level of meat and bone meal in practical formulated diet for milkfish, *Chanos chanos* Forsskal, in freshwater and seawater

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Abstract

The efficient level of MBM for good growth and survival of milkfish, *Chanos chanos* Forsskal, without histological changes in tissues (liver, intestine, muscle, kidney and brain) was determined in both freshwater (FW) and seawater (SW) culture conditions. To determine this level, growth and digestibility experiments were conducted in FW and SW together with observations on histology of tissues. In the growth experiments, fish (wet weight less <5g) in triplicate groups were fed for three months with either of the six test diets formulated to be isonitrogenous at 36% with varying dietary levels of MBM at 0%, 7.5%, 15%, 22.5%, 30% and 37.5%. The apparent digestibility coefficients of crude protein and crude fat in diets were determined using a dietary indicator (Chromic Oxide). Intestinal content of fed fish (wet weight, 85g – 65g) were collected by stripping the last posterior third of the intestine. Milkfish survival was 100% in all treatments in FW. Protein of MBM was more digestible to milkfish in SW (93.64%) than in FW (67.34%). Based on growth efficiencies, survival data, and histology of tissues examined, milkfish was able to utilize dietary level of MBM at 30% in FW and at 22.5% in SW. Assimilation of organic matter in test diets that contained MBM at 7.5% to 30% were high (88% - 93%) in both FW and SW.

Extended Abstract

The efficient level of meat and bone meal (MBM) for good growth and survival of milkfish fingerling without histological changes in tissues was verified in freshwater (FW) and seawater (SW). Two feeding experiments were conducted in FWand SW: growth experiment and digestibility experiment. For the growth experiment, a Control Diet and five test diets were formulated to be isonitrogenous (37% crude protein). The Control Diet or Diet 1 has no MBM while Diets 2, 3, 4, 5 & 6 contained MBM at 7.5%, 15%, 22.5%, 30% and 37.5%, respectively. These MBM levels replaced fish meal (FM) protein in the Control Diet from 14.4%, 28.8%, 43.2%, 57.6% and 72%, respectively.

For the growth experiment in FW, the six formulated diets were fed to triplicate groups of milkfish fingerlings (fish initial average weight, 4.46g) for 12 weeks. Survival rates of milkfish were 100% in all treatments. Percent weight gain (PWG) of milkfish fed the Control Diet was highest (669%) and did not differ with the PWG of fish fed Diets 3 (598%) and 5 (644%) which contained 15% and 30% MBM, respectively ($P<0.05$). Fish fed diet with 37.5% MBM has significantly low PWG (353%). Specific growth rates (SGRs) and PWG results have the same trend. Feed conversion ratios (FCR) of fish fed highest MBM level was significantly the lowest. Condition factor (K), viscerasomatic index (VSI), and hepatopsomatic index (HSI) were similar in all treatments. Hematocrit was highest in fish fed the Control Diet.

The same test diets were fed to triplicate groups of milkfish fingerlings (fish initial average weight, 3.24g) in SW for 14 weeks. The PWG (753% - 537%), survival rates (85% - 76%), SGR (2.18 - 1.89), and FCR (3.84 - 4.65) were similar in all treatments. There were also no differences noted on VSI, HSI and hematocrit values in all treatments, however, Condition Factor (K) was significantly highest in fish fed the Control Diet and diet with 37.5% MBM ($P<0.05$). However, at 12 weeks fish feeding on Diet 6 had significantly poor PWG, SGR and FCR.

The apparent digestibility coefficients of crude protein (ACPDC) and crude fat (ACFDC) of diets with different MBM levels (0% to 30%) were determined in FW and SW using an inert indicator, Chromic Oxide. Intestinal contents of fish (wet weight, 85g – 65g) were collected by stripping the last posterior third of the intestine. The CP of diets with 0% to 30% MBM were consistently digested well by fish in FW (69.45% - 58.51%) compared to that in SW (58.65% - 30.14%). In SW, the ACPDC of diet with 7.5% MBM was lowest. When ACPDC of MBM was calculated as a single ingredient, results showed that CP of MBM was more digestible in SW (93.64%) than in FW (67.34%). The ACFDC of diets in FW were higher (94.68% - 90.33%) than in SW (92.14% - 78.79%). However, in SW the ACFDC was significantly lowest at 7.5% MBM. The assimilation efficiencies (U) of diets in FW ranged from 94.83% to 89.54%, while in SW the ranged was from 93.56% to 90.92%. Within FW or SW, diets were decreasingly assimilated with increasing dietary MBM.

Milkfish carcass increased in crude protein and crude fat content and the average carcass Ca:P ratio was 2.61 in FW and 1.94 in SW. There were no changes in the histology of fish liver, intestine, muscle, kidney and brain tissues, except for small lipid vacuoles noted in fish fed Diet 2 (MBM 7.5%) in SW.

Based on growth efficiencies, survival data, and histology of tissues examined, milkfish was able to utilize MBM in practical diet formulations at dietary level of 22.5% when grown in SW, and at 30% when grown in FW. These MBM levels substituted 45% and 60% of fish meal protein, respectively.

Introduction

Milkfish is one of the most important food fish of Southeast Asia. In the Philippines it is the number one aquaculture species in terms of production volume and money value. This fish is euryhaline and grown intensively in brackish water ponds and in sea cages. Milkfish fry is increasingly produced in hatcheries in the Philippines and in Indonesia. The rearing of milkfish in low stocking density which is dependent on natural food has now shifted to high density culture systems in ponds and cages. High stocking density culture of milkfish requires a formulated feed that is efficient.

A major aquafeed component is fishmeal which is under pressure due to increased global aquaculture production. With an appropriate economic and regulatory motivation, the use of alternative feed ingredients from various plant- and animal-based sources may increase according to Naylor et al. (2009). A suitable alternative aquafeed ingredient is Meat and Bone Meal (MBM) which is an animal protein and a by-product of the meat processing and canning industry. MBM is used as feed ingredient and evaluated as a protein source to partly substitute fish meal in dietary formulations of commercially popular species for culture (Hu et al., 2008; Ai et al., 2006; Wang et al., 2006; Bharadwaj, et al., 2002; Millamena et al., 2002; Wu et al., 1999; Kikuchi et al., 1997) but there was none on milkfish. The work of Sumagaysay-Chavoso and Diego-McGlone (2003) on milkfish feeding in ponds used a practical diet formulation with an inclusion level of MBM at 8%. This present proposal aims to determine effective level of MBM in milkfish practical diet formulation and apparent digestibility values of crude protein and crude ash of diets with MBM in milkfish reared in freshwater and in seawater.

Materials and Methods

I. Growth Experiments

Experimental Animals and Set-up

Milkfish fry from a hatchery and nursed in brackishwater ponds were transported at fingerlings size to the laboratory and reared in formulated maintenance diet prior to feeding experiments. The fish were stocked in 250 L circular fiberglass tanks that were equipped with aeration and flowing water at 2-3 exchanges per day. In the freshwater (FW) experiment, milkfish fingerlings (average body weight, 4.46 ± 0.11g) were stocked in 3 replicate groups at 15 fish/tank, while for the seawater (SW) experiment, milkfish fingerlings (average body weight, 3.24 ± 0.04g) were stocked in 4 replicate groups at 25 fish/tank.

Dietary Treatments

Six practical isonitrogenous diets at 36% were formulated with increasing levels of meat and bone meal (MBM) in Table 1. The Control Diet or Diet 1 contained 26% protein from Danish and Peruvian fish meals. The other diets were Diet 2, Diet 3, Diet 4, Diet 5 and Diet 6 which contained 7.5%, 15%, 22.5%, 30% and 37.5% MBM, respectively, which substituted fish meal protein at 15%, 30%, 45%, 60% and 75%, respectively.

Test diets were prepared according to Catacutan et. al.,(2001). Dry diets were broken and sieve to size 20 mesh size for the first few weeks of feeding and from 3 to 6 mm in length for the bigger size fish. The diets were placed in plastic containers with caps and stored in a cold room at 20 ^{o}C.

Feeding Experiment

Initial weight of fish were taken at the start of feeding. During sampling which was every two weeks, fish were weighed in bulk per tank to adjust feeding rate accordingly. Either of the following anaesthetics was used, MS-222 or 2-phenoxy ethanol was used during weighing. Fish were fed twice daily and feeding rate range from 10% to 3% as fish increased in weight. Growth and survival rates were noted in each treatment every sampling.

Termination of Feeding

Feeding was terminated after 12 weeks in FW and at 14 weeks in the SW experiment. Carass samples were analyzed for proximate nutrient content including Ca and P levels. Viscera and liver weights were measured in fish samples and also hematocrit levels.

The following were calculated at termination of feeding: survival rate, Percent Weight Gain (PWG), Specific Growth Rate (SGR), Feed Conversion Ratio (FCR), Protein Efficiency Ratio (PER), and Apparent Protein Retention (APR). The following indicators were also measured: Condition factor (K), Viscerasomatic Index (VSI), Hepatosomatic Index (HSI) and Hematocrit level.

Histology and TEM

Milkfish liver, muscle, distal intestine, kidney, and brain tissues were processed to determine histological changes at termination of the experiment in FW, while in the SW experiment only the liver, muscle and brain were sampled. Fish brain samples taken on the same location or area of the brain were also submitted for TEM analysis. However, samples of the brain after processing by light microscopy did not show any significant changes among treatments so samples for TEM analysis were done only on FW samples.

Water Analyses

Total Hardness as ppm CaCO3 were determined in SW and FW samples while the following were analyzed in the SW experiment: pH, DO (mg/ L O_2), NH_3-N (ppm), NO_2-N (ppm) and PO_4-P (ppm).

Proximate analysis

Samples of test diets, fecal or intestinal samples, and initial and final milkfish carcass samples were analyzed for proximate nutrient content including Ca and P levels. Moisture was by oven drying at 105^{o}C, Crude Protein or CP by Kjeldahl Method (using Kjeltec 2300) using the factor 6.25, Crude Fat or CF by Soxtec Method, Crude Fiber or CFr by Fibertec Method, Crude Ash or CAs by AOAC 923.03 (modified), and Nitrogen Free Extract or NFE was determined by difference.

II. Digestibility Experiment

Dietary Treatments

The apparent digestibility coefficients of CP (ACPDC) and CF (ACFDC) of diets were determined in FW and SW using an inert indicator chromic oxide (Cr_2O_3) at 1% (Table 2). The diets were the Control Diet or Diet 1 (0 % MBM), Diet 2 (7.5% MBM), Diet 3 (15% MBM) and Diet 4 (22.5% MBM) which were the same in composition in the growth experiment. To determine the ACPDC of MBM as a single feed ingredient Diet 5 was formulated to contain 30% of MBM and 70% of Control Diet as the Reference Diet or RF. Test diets were prepared similarly as in the growth experiment and cut into 3-5 mm suitable for bigger fish. Diets were placed in plastic containers with caps and stored in a cold room at 20 ^{o}C.

Experimental Animals and Feeding

Milkfish were stocked in 250 L tanks and fed test diets at 3% given twice daily. Average weight of fish in the SW experiment was 67g and were stocked at 8-9 fish/tank while in the FW experiment the average weight of fish was 81g and stocked at 10 fish/tank. After 10 days (Ferraris et al., 1986) of feeding fish were fed to satiation and then killed almost all at the same time in each tank by applying a blow in the head using a PVC pipe. Intestines were immediately dissected and contents were collected only from the last posterior third of the intestine by stripping (Ferraris et al., 1986). Samples were immediately placed in Petri dishes positioned on top of crushed ice. After samples of intestinal content have been collected from all fish a tank, the labeled Petri dish was placed in a freezer. Samples were pooled for each treatment and freeze dried prior to chromic oxide and nutrient analyses.

Chemical Analysis

Chromic oxide levels in the intestinal content and in test diets was analyzed using perchloric acid digestion (Carter et al., 1960). Crude protein and crude fat were determined in the intestinal samples with similar methods in carcass analysis in the growth experiments.

The ACPDCs and ACFDCs were calculated in the Control Diet and Diets 2 to 5. The following formula was used as described by Spyridakis et al., (1989):

$$ADC_{CP}\ (\%) = 100 - (100\ X\ \%\ Cr_2O_{3\ diet} / Cr_2O_{3\ feces}\ X\ \%\ CP_{feces} / \%CP_{diet})$$

The ACPDC of MBM as a single ingredient was calculated according to Forster (1999):

$$ADC_{CP}\ (\%) = 100/30\ X\ [\ Test - \{\ (70/100)\ X\ \ RF\}\ ]$$

Where: Test = ADC of CP in the test diet, RF = ADC of CP in RF.

Assimilation Efficiencies

The assimilation of organic matter by milkfish in the test diets were determined according to Conover (1966). The organic matter in the feed and fecal matter were determined by ashing. The assimilation efficiency U was calculated as:

$$U = (F-E) / (1-E)\ (F)$$

Where F is organic matter in feed (%), and E is organic matter in fecal matter (%).

Statistical Analysis

Data from the experiments were analyzed with ANOVA and the Duncan Multiple Range Test. Diffrences berween means were considered significant at $P<0.05$.

Results

<u>Growth Experiments</u>

Freshwater

The survival rates of milkfish fry were 100% in all treatments after 12 weeks of feeding (Table 3). The PWG and SGR of milkfish fed Diet 1 was highest (669%) and similar with fish fed Diets 3 (598%) and 5 (644%). The PWG, SGR, and FCR of milkfish fed with dietary MBM levels of 7.5% to 30% were similar and significantly higher than fish fed Diet 6 (37.5% MBM) ($P<0.05$). Moreover, the K, VSI and HSI of milkfish were the same in all treatments while hematocrit levels of fish fed Diet 1 was highest at (62.60±4.91%) compared with the rest of the treatments which were below 50% (Table 4).

Fish fed highest level of MBM was noted to decrease in PWG on the 6th week of feeding (Fig.1). However, the PWG of fish fed second highest level of MBM at 30% was not noted to decrease in all sampling periods.

At the start of feeding, milkfish carcass contained 70.60% CP and 10.62% CF (Table 5). However, after 12 weeks of feeding on Diets 1 to 6, the levels of CP in the carcass decreased (<63%) while that of CF increased (>24%). The levels CP (62.73%) and CF (24.29%) in the carcass of fish fed Diet 6 were significantly different from the rest of the treatments ($P<0.05$). Levels of P (6.14 - 4.93) and Ca (16.39 – 12.06) in milkfish carcass were similar in all treatments with an average Ca: P ratio of 2.61.

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Seawater

The survival rates of milkfish fry after 14 weeks of feeding ranged from 85% to 76% and were not significantly different in all treatments (Table 6). Similarly, there were no significant differences noted in milkfish PWG, SGR and FCR. However, results of sampling conducted on the 12th week, these parameters showed significant differences (Table 7), where PWG, SGR and FCR of fish fed Diet 4 (22.5% MBM) were significantly better than those of fish fed Diets 5 (30% MBM) and 6 (37.5% MBM). The PWG of fish fed with 37.5% MBM was noted to decrease starting on the 8th week of sampling (Fig. 2).

The VSI, HSI and hematocrit of fish were similar in all treatments, but K was highest in fish fed Diet 1 (1.83) and Diet 6 (1.69) (Table 8).

The levels of CP and CAs in milkfish carcass did not vary at different dietary levels of MBM (Table 9). However, CF and CFr levels varied significantly and also carcass Ca and P. The average Ca: P ratio in SW was 1.94.

<u>Digestibility Experiments and assimilation of diets</u>

The ACPDCs and ACFDCs of test diets were not similar between fish grown in FW and SW (Table 10). The CP of diets containing 0% to 30% MBM were 69.45% to 58.51% digestible to milkfish in FW as compared to only 58.65% - 30.14% in SW. In SW, the ACPDC of diet with 7.5% MBM was lowest at

30.14%, but the same diet has the highest ACPDC in FW at 69.45%. Also in SW, CP of diet with 30% MBM was the most digestible to milkfish at 58.65%. When the ACPDC of MBM was calculated as a single ingredient, results showed that CP of MBM was more digestible in SW (93.64%) compared to that in FW (67.34%).

The level of crude fat in the diets was about 9% and the estimated crude fat contribution of MBM was increasing and ranged from 0.48% to 1.91% (Table 10). The ACFDCs of diets in FW ranged from 94.68% - 90.33% while in SW it ranged from 92.14% - 78.79%. In SW, the ACFDC of Diet 2 (7.5% MBM, with 0.48% CF from MBM) was significantly lowest at 78.79%, while that of Diet 5 (30% MBM, with 1.91% CF from MBM) was the most digestible at 92.14%.

The assimilation efficiencies (U) of diets in FW ranged from 94.83% to 88.88%, while in SW the range was from 93.56% to 88.26%. The assimilation of Diet 1 (no MBM) was significantly highest in both FW and SW which tend to decrease as the amount of MBM increases in the diet.

<u>Water Parameters</u>

Water Analyses showed that Total Water Hardness (expressed as ppm $CaCO_3$) in SW was 5257 ppm and only 214 ppm in FW. The other water parameters monitored in SW: pH, 7.74-8.07; DO (mg/ L O_2), 5.88 -9.12; P (ppm), 0.03-0.12; NH_3-N (ppm), 0.01-0.16; NO_2-N (ppm), not detected except in one sample at 0.01 ppm.

3. Histology (liver, muscle, posterior third of the intestine, kidney and brain)

In FW, the liver, muscle, distal intestine, kidney and brain of milkfish showed normal histology in all treatments and also in the muscle and brain of fish in SW (Figs. 3, 4, 5 and 6). The liver of milkfish in SW fed a dietary level of 7.5% MBM has small lipid vacuoles not noted in fish fed with the other diets.

4. TEM results

An initial brain sample of milkfish was subjected to TEM for baseline structure After the growth experiments in FW and SW, brain samples were obtained from fed fish and these were analyzed first under light microscopy for detection of histological changes. Since there were no observed abnormality in the structure, only samples from FW was done (Fig 7).

Discussion

Milkfish in FW was able to utilize MBM up to 30% of formulated practical diet based on growth, survival data, and histology of tissues examined. In SW, based on the 12^{th} and 14^{th} week sampling results, milkfish utilized up to 22.5% of MBM in the diet. There were no differences in the histology of tissues that were examined when fish were reared in FW (liver, muscle, distal intestine, kidney, and brain). In SW there were no differences noted in the histology of fish muscle and brain, however, liver tissues were normal except in fish fed diet with 7.5% MBM where there was vacuolation. Milkfish was not able to tolerate the highest MBM inclusion at 37.5%, and based on results of the bi-monthly sampling PWGs (Fig 1) of fish on this diet started to decrease on the 8^{th} week of feeding in both FW and SW.

The apparent CP digestibility of formulated diet with fishmeal at 45% as the major protein source is less than 70% in milkfish reared in FW or in SW, and this is only 55% when milkfish weigh less than 100g (Ferraris et., al., 1986). In this present study, the ACPDC of Control diet (36% fish meal) in sub-adult milkfish was 58.5% and 43.6% in FW and SW, respectively. This study confirmed that in SW (Table 10), milkfish digest significantly well the CP of MBM at 30% in the diet (58.65%) than that of fishmeal in the Control diet (43.61%). Furthermore, ACPDC of diets did not decrease as dietary level of MBM increased from 15% to up to 37.5% in both FW and SW.

Although the ACPDC of diet with 7.5% MBM was highest in FW, growth of fish after 12 weeks was significantly lower than the Control diet. In SW, the ACPDC of this diet was significantly lowest. Probably, that amino acid profile of diet with 7.5% MBM, which was a combination of all the other protein sources, was not best for milkfish. Also, the essential amino index (EAAI) (Penaflorida, 1989) of this diet was probably low for milkfish and which could explain why PWG of milkfish in the Control diet was similar with diets containing 15% and 30% MBM and not with diets containing 7.5% and 22.5% MBM. However, it should be noted that in SW the protein of MBM as a single ingredient was more digestible (93.64%) compared to that in FW (67.34%), and that the assimilation efficiencies of all diets were not different between FW and SW.

The ACFDC of diet containing 7.5% MBM was significantly lowest in SW although CF coming from MBM was minimal. Fish fed this diet showed vacuolation in the liver only in SW and not noted in other treatments especially in diet with higher levels of MBM. The design of this experiment, however, will not be able to explain this result. Probably food motility changes when there is alterations in osmoregulatory proces when fish is in seawater and that affects the ability of milkfish to digest (Ferraris et al., 1966). In this study total hardness (expressed as ppm $CaCO_3$) of SW was 5257 compared to only 214 in FW.

In both FW and SW, carcass of milkfish increased in CP and CF which is what is generally noted in cultured fish. However, milkfish on the same dietary treatments but grown in FW or SW, showed a remarkable difference in the amount of Ca in the carcass (Tables 5 and 7). The levels of P in the carcass were almost the same in both FW and SW grown fish, however, Ca level was elevated in fish grown in FW with a Ca:P of 2.61, and 1.94 in SW.

Based on growth efficiencies, survival data, and histology of tissues examined, milkfish was able to utilize MBM in practical diet formulations at dietary level of 22.5% when grown in SW, and at 30% when grown in FW. These MBM levels substituted 43.2% and 57.6% of fish meal protein, respectively.

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Table 1 Composition of test diets for the growth experiments.

Feed Ingredients	**Diet 1**	**Diet 2**	**Diet 3**	**Diet 4**	**Diet 5**	**Diet 6**
	(Control Diet)					
Danish FM	18	15.25	12.25	9.25	6.25	3.25
Peruvian Fish meal	18	15.25	12.25	9.25	6.25	3.25
Meat & Bone meal *	0	7.5	15	22.5	30	37.5
Acetes	5	5	5	5	5	5
Squid liver powder	4	4	4	4	4	4
DSBM	8	10	12	14	16	18
Wheat pollard	2	3	2	2	2	1
Corn starch	18	18	18	18	18	18
SBO	2	2	2	2	2	2
Salmon Fish Oil	2	2	2	2	2	2
Lecithin	0.5	0.5	0.5	0.5	0.5	0.5
Mineral mix	1.5	1.5	1.5	1.5	1.5	1.5
Vit mix (Commercial)	2	2	2	2	2	2
Vitamin C	0.05	0.05	0.05	0.05	0.05	0.05
Dicalphos	1.75	1.75	1.75	1.75	1.75	1.75
Rice bran	17.2	12.2	9.7	6.2	2.7	0.2
TOTAL	100	100	100	100	100	100
Proximate Analysis (% Dry Matter)						
Crude Protein	37.78	38.07	38.89	39.08	39.56	40.09
Crude Fat	8.05	7.61	8.17	7.93	8.56	8.57
Crude Fiber	4.52	4.97	3.43	3.04	2.54	2.45
Crude Ash	14.01	14.99	16.25	17.01	17.17	18.12
NFE	35.64	34.36	33.26	32.94	32.17	30.77

*MBM: Moisture 6.24%; Based on DM, CP-53.77%, CrAsh-29.69%, Cr Fat-6.36%, CrFiber-1.2% & NFE-8.98%.

Table 2 Diet compositions for digestibility determination which include the Reference diet (RF) and the test Diet (TD) as the single ingredients.

Feed Ingredients	**Diet 1 or RF**	**Diet 2 (7.5% MBM)**	**Diet 3 (15% MBM)**	**Diet 4 (22.5% MBM)**	**Diet 5 or TD (70% RF Diet + 30% MBM)**
Danish FM	18	15.25	12.25	9.25	30 + 70 % Diet 1 (RF)
Peruvian Fish meal	18	15.25	12.25	9.25	
Meat & Bone meal	0	7.5	15	22.5	
Acetes	5	5	5	5	
Squid liver powder	4	4	4	4	
DSBM	8	10	12	14	
Wheat pollard	2	3	2	2	
Corn starch	18	18	18	18	
SBO	2	2	2	2	
Salmon Fish Oil	2	2	2	2	
Lecithin	0.5	0.5	0.5	0.5	
Mineral mix	1.5	1.5	1.5	1.5	
Vit mix Oversea	2	2	2	2	
Vitamin C	0.05	0.05	0.05	0.05	
Dicalphos	1.75	1.75	1.75	1.75	
Rice bran	16.2	11.2	8.7	5.2	
Chromic Oxide	1	1	1	1	
TOTAL	100	100	100	100	100
Estimated Crude Fat from MBM (%)	0	0.48	0.95	1.43	1.91
Proximate Analysis (% DM)					
Crude Protein	35.56	36.83	35.97	36.37	40.20
Crude Fat	9.62	8.85	8.56	9.04	8.49
Crude Fiber	0.33	1.44	1.17	1.18	0.43
Crude Ash	15.31	16.91	18.15	19.73	20.37
NFE	39.18	35.97	36.15	33.68	30.51

Table 3 The % Survival, % Weight Gain (PWG) , Specific Growth Rate (SGR) and Feed Conversion Ratio (FCR) of milkfish reared in freshwater fed different levels of meat and bone meal or MBM for 12 weeks*

Treatments	**% MBM**	**Init. Avg. Wt. (g)**	**Final Avg. Wt. (g)**	**% Survival** [1]	**PWG** [2]	**SGR** [3]	**FCR** [4]
1	Control	4.45 ± 0.05[a]	34.30 ± 2.10[c]	100.00 ± 0.00[a]	669.26 ± 30.74[c]	2.43 ± 0.05[c]	2.36 ± 0.16[a]
2	7.5	4.47 ± 0.04[a]	27.94 ± 1.47[b]	100.00 ± 0.00[a]	521.86 ± 38.82[b]	2.17 ± 0.07[b]	2.33 ± 0.09[a]
3	15.0	4.44 ± 0.04[a]	30.77 ± 1.44[bc]	100.00 ± 0.00[a]	598.45 ± 33.37[bc]	2.31 ± 0.06[bc]	2.52 ± 0.15[a]
4	22.5	4.46 ± 0.03[a]	27.93 ± 1.30[b]	100.00 ± 0.00[a]	526.07 ± 31.00[b]	2.18 ± 0.06[b]	2.66 ± 0.09[a]
5	30.0	4.46 ± 0.05[a]	33.18 ± 1.52[bc]	100.00 ± 0.00[a]	643.97 ± 39.36[bc]	2.39 ± 0.06[bc]	2.44 ± 0.05[a]
6	37.5	4.46 ± 0.04[a]	20.27 ± 0.54[a]	100.00 ± 0.00[a]	353.12 ± 18.54[a]	1.80 ± 0.05[a]	3.17 ± 0.08[b]

*Means of 3 replicate groups ± s.e.m. with the same superscript in each column are not significantly different (P<0.05).
[1] Survival (%) = 100 X (final no. of fish/ initial no. of fish)
[2] Wt gain (%) = [(Final wt.-Initial wt.) / Initial wt.] X 100
[3] SGR (specific growth rate) = 100 (Ln avg. final wt. – Ln avg. initial wt.) / No. of days.
[4] FCR (feed conversion ratio) = dry wt. feed (g) / wet wt. gain (g).

Table 4 Condition Factor (K) , viscerasomatic index (VSI), hepatosomatic index (HSI) and hematocrit of milkfish reared in freshwater fed different levels of meat and bone meal or MBM for 12 weeks *

Treatments	**% MBM**	**K** [1]	**VSI** [2]	**HSI** [3]	**Hematocrit (%)**
1	Control	1.42 ± 0.03[a]	8.12 ± 0.03[a]	1.05 ± 0.11[a]	62.60 ± 4.91[b]
2	7.5	1.47 ± 0.02[a]	7.92 ± 0.20[a]	1.17 ± 0.06[a]	41.34 ± 3.77[a]
3	15.0	1.42 ± 0.02[a]	7.19 ± 0.26[a]	1.00 ± 0.06[a]	44.61 ± 1.25[a]
4	22.5	1.14 ± 0.18[a]	7.54 ± 0.36[a]	1.03 ± 0.11[a]	43.11 ± 2.13[a]
5	30.0	1.34 ± 0.16[a]	7.95 ± 0.35[a]	1.23 ± 0.11[a]	44.56 ± 2.67[a]
6	37.5	1.41 ± 0.03[a]	8.32 ± 0.19[a]	1.26 ± 0.10[a]	41.88 ± 1.88[a]

*Means of 3 replicate groups ± s.e.m. with the same superscript in each column are not significantly different (P<0.05).
[1] Condition Factor (K) = 100 X (wt./length^3)
[2] Viscerasomatic Index (VSI) = 100 X (viscera wt. / body wt.)
[3] Hepatosomatic Index (HSI) = 100 X (liver wt. / body wt.)

Table 5 Level of nutrients, Ca & P (in dry matter basis) in milkfish carcass after 12 weeks of feeding in freshwater with different dietary levels of meat and bone meal or MBM and also the nutrient levels at the start of feeding *

Dietary Treatments	Crude protein	Crude Fat	Crude Fiber	Crude Ash	NFE	Ca	P
Initial	70.60	10.62	1.09	14.34	3.36		
Diet 1 (Control Diet)	56.83 ± 0.55[a]	31.70 ± 0.16[b]	0.85 ± 0.00[bc]	10.15 ± 0.25[a]	0.48 ± 0.47[a]	13.63 ± 1.01[a]	5.20 ± 0.25[a]
Diet 2	57.10 ± 0.52[a]	30.49 ± 1.21[b]	1.01 ± 0.04[c]	10.49 ± 0.36[a]	0.92 ± 0.30[a]	15.13 ± 0.50[a]	5.65 ± 0.52[a]
Diet 3	57.42 ± 0.45[a]	30.43 ± 0.43[b]	0.74 ± 0.09[b]	10.38 ± 0.04[a]	1.04 ± 0.11[a]	13.88 ± 0.26[a]	5.30 ± 0.35[a]
Diet 4	56.61 ± 1.95[a]	30.30 ± 0.59[b]	0.48 ± 0.06[a]	10.34 ± 0.14[a]	2.28 ± 1.17[a]	16.39 ± 1.28[a]	5.79 ± 0.12[a]
Diet 5	57.01 ± 0.72[a]	30.57 ± 0.78[b]	0.46 ± 0.04[a]	9.89 ± 0.11[a]	2.07 ± 0.09[a]	12.06 ± 1.57[a]	4.93 ± 0.20[a]
Diet 6	62.73 ± 0.72[b]	24.29 ± 0.24[a]	0.40 ± 0.02[a]	10.90 ± 0.11[a]	1.70 ± 0.36[a]	15.14 ± 0.50[a]	6.14 ± 0.05[a]

*Means of 2- 3 replicates with the same superscript in each column are not significantly different (P<0.05).

Table 6 The % Survival, % Weight Gain, Specific Growth Rate (SGR) and Feed Conversion Ratio (FCR) of milkfish reared in seawater fed different levels of meat and bone meal or MBM for 14 weeks*

Treatments	**% MBM**	**Initial Avg. Wt. (g)**	**Final Avg. Wt. (g)**	**% Survival** [1]	**PWG** [2]	**SGR** [3]	**FCR** [4]
1	Control	3.24 ± 0.04 [a]	28.44 ± 2.07[a]	76.00 ± 4.62[a]	632.11 ± 61.35[a]	2.03 ± 0.09[a]	4.15 ± 0.44[a]
2	7.5 %	3.21 ± 0.04 [a]	24.17 ± 1.08[a]	85.33 ± 1.33[a]	652.43 ± 23.33[a]	2.06 ± 0.03[a]	4.18 ± 0.30[a]
3	15.0 %	3.19 ± 0.04 [a]	23.75 ± 0.27[a]	82.67 ± 7.06[a]	644.06 ± 14.63[a]	2.05 ± 0.02[a]	4.25 ± 0.05[a]
4	22.5 %	3.28 ± 0.05 [a]	28.44 ± 3.32[a]	66.00 ± 10.00[a]	753.69 ± 110.70[a]	2.18 ± 0.13[a]	3.84 ± 0.53[a]
5	30.0 %	3.28 ± 0.03 [a]	22.37 ± 1.24[a]	80.00 ± 2.31[a]	645.92 ± 26.67[a]	2.05 ± 0.04[a]	4.42 ± 0.01[a]
6	37.5 %	3.27 ± 0.04 [a]	20.59 ± 1.04 [a]	76.00 ± 4.00[a]	537.00 ± 24.46[a]	1.89 ± 0.04[a]	4.65 ± 0.41[a]

*Means of 3 replicate groups ± s.e.m. with the same superscript in each column are not significantly different (P<0.05).

[1] Survival (%) = 100 X (final no. of fish/ initial no. of fish)

[2] Wt. gain (%) = [(Final wt.-Initial wt.) / Initial wt.] X 100

[3] SGR (specific growth rate) = 100 (Ln avg. final wt. – Ln avg. initial wt.) / No. of days.

[4] FCR (feed conversion ratio) = dry wt. feed (g) / wet wt. gain (g).

Table 7 The % Survival, % Weight Gain, Specific Growth Rate (SGR) and Feed Conversion Ratio (FCR) of milkfish reared in seawater fed different levels of meat and bone meal or MBM for 12 weeks*

Treatments	**% MBM**	**Initial Avg. Wt. (g)**	**Final Avg. Wt. (g)**	**% Survival** [1]	**PWG** [2]	**SGR** [3]	**FCR** [4]
1	Control	3.24 ± 0.04^{a}	20.87 ± 0.89^{ab}	76.00 ± 4.62^{a}	553.09 ± 25.00^{abc}	2.23 ± 0.05^{bc}	3.73 ± 0.42^{a}
2	7.5	3.21 ± 0.04^{a}	21.67 ± 0.48^{bc}	88.00 ± 2.31^{a}	574.99 ± 8.89^{bc}	2.27 ± 0.02^{bc}	4.15 ± 0.21^{abc}
3	15.0	3.19 ± 0.04^{a}	20.86 ± 0.43^{ab}	90.67 ± 5.81^{a}	553.73 ± 22.26^{abc}	2.23 ± 0.04^{bc}	4.35 ± 0.13^{abc}
4	22.5	3.28 ± 0.05^{a}	25.45 ± 1.79^{c}	73.33 ± 4.81^{a}	677.70 ± 59.71^{c}	2.43 ± 0.09^{c}	3.80 ± 0.29^{ab}
5	30.0	3.28 ± 0.03^{a}	20.16 ± 1.85^{ab}	81.33 ± 1.33^{a}	515.07 ± 55.19^{ab}	2.15 ± 0.11^{ab}	4.76 ± 0.29^{bc}
6	37.5	3.27 ± 0.04^{a}	17.19 ± 1.03^{a}	81.33 ± 1.33^{a}	426.91 ± 36.56^{a}	1.97 ± 0.09^{a}	5.08 ± 0.33^{c}

*Means of 3 replicate groups ± s.e.m. with the same superscript in each column are not significantly different ($P<0.05$).
[1] Survival (%) = 100 X (final no. of fish/ initial no. of fish)
[2] Wt gain (%) = [(Final wt.-Initial wt.) / Initial wt.] X 100
[3] SGR (specific growth rate) = 100 (Ln avg. final wt. – Ln avg. initial wt.) / No. of days.
[4] FCR (feed conversion ratio) = dry wt. feed (g) / wet wt. gain (g).

Table 8 Condition Factor (K), viscerasomatic index (VSI), hepatosomatic index (HSI) and hematocrit of milkfish reared in seawater fed different levels of meat and bone meal or MBM for 14 weeks*****

Treatments	**% MBM**	**K**[1]	**VSI** [2]	**HSI**[3]	**Hematocrit (%)**
1	Control	1.83 ± 0.04^{b}	7.85 ± 0.65^{a}	1.15 ± 0.05^{a}	52.07 ± 3.69^{a}
2	7.5 %	1.52 ± 0.00^{a}	7.64 ± 0.14^{a}	1.18 ± 0.08^{a}	56.22 ± 1.09^{a}
3	15.0 %	1.50 ± 0.02^{a}	7.35 ± 0.26^{a}	1.21 ± 0.06^{a}	50.16 ± 1.88^{a}
4	22.5 %	1.53 ± 0.07^{a}	7.06 ± 0.32^{a}	1.06 ± 0.02^{a}	55.80 ± 3.32^{a}
5	30.0 %	1.50 ± 0.01^{a}	7.15 ± 0.10^{a}	1.21 ± 0.05^{a}	48.68 ± 3.69^{a}
6	37.5 %	1.69 ± 0.02^{b}	6.98 ± 0.04^{a}	1.14 ± 0.08^{a}	50.87 ± 2.90^{a}

*Means of 3 replicate groups ± s.e.m. with the same superscript in each column are not significantly different ($P<0.05$).
[1] Condition Factor (K) = 100 X (wt./length^3)
[2] Viscerasomatic Index (VSI) = 100 X (viscera wt. / body wt.)
[3] Hepatosomatic Index (HSI) = 100 X (liver wt. / body wt.)

Table 9 Level of nutrients, Ca & P (in dry matter basis) in milkfish carcass after 14 weeks of feeding in seawater with different dietary levels of meat and bone meal or MBM and also the nutrient levels at the start of feeding*

Dietary Treatments	Crude Protein	Crude Fat	Crude Fiber	Crude Ash	NFE	Ca	P
Initial	68.78	4.56	1.10	18.30	7.21		
Diet 1 (Control Diet)	57.04 ± 0.62^{a}	29.60 ± 0.45^{bc}	0.39 ± 0.02^{d}	10.58 ± 0.24^{a}	2.40 ± 0.04^{a}	9.71 ± 0.42^{b}	5.32 ± 0.10^{c}
Diet 2	59.01 ± 1.71^{a}	29.05 ± 0.23^{ab}	0.01 ± 0.00*	10.21 ± 1.44^{a}	1.73 ± 0.51^{a}	9.23 ± 0.17^{ab}	5.73 ± 0.06^{d}
Diet 3	58.99 ± 1.19^{a}	30.08 ± 1.51^{bc}	ND	10.32 ± 1.35^{a}	2.29 ± 0.00*	8.34 ± 0.06^{a}	4.39 ± 0.09^{a}
Diet 4	62.23 ± 1.49^{a}	26.87 ± 0.19^{a}	0.05 ± 0.01^{a}	10.85 ± 0.00^{a}	1.31 ± 0.00*	13.59 ± 0.02^{c}	5.56 ± 0.02^{cd}
Diet 5	58.10 ± 0.41^{a}	31.92 ± 0.13^{c}	0.09 ± 0.01^{b}	9.87 ± 0.31^{a}	0.61 ± 0.00*	9.92 ± 0.75^{b}	4.90 ± 0.16^{b}
Diet 6	59.05 ± 1.01^{a}	27.69 ± 0.88^{ab}	0.17 ± 0.04^{c}	10.80 ± 0.04^{a}	2.30 ± 0.05^{a}	9.94 ± 0.29^{b}	5.41 ± 0.02^{cd}

*Means of 2- 3 replicates with the same superscript in each column are not significantly different (P<0.05).

Table 10 Apparent Digestibility Coefficients (%) of crude protein (ACPDC) and crude fat (ACFDC), and Assimilation Efficiencies (U) of test diets with different levels of meat and bone meal (MBM) in milkfish reared in seawater and freshwater *

Test Diets	% MBM	ACPDC FW	ACPDC SW	ACFDC FW	ACFDC SW	U FW	U SW
Diet 1	0	58.51± 0.14 ^{a}	43.61± 17.7 ^{b}	94.07 ± 0.03 ^{b}	86.66 ± * ^{b}	94.83± 0.01 ^{c}	93.56 ± 0.00 ^{e}
Diet 2	7.5	69.64± 3.00 ^{b}	30.14± 1.44 ^{a}	94.68 ± 0.19 ^{b}	78.79± 0.92 ^{a}	92.50 ± 0.03 ^{b}	93.00 ± ^{d}*
Diet 3	15	63.84± 2.31 ^{a}	43.99± 0.84 ^{b}	93.44 ± 0.41 ^{ab}	85.76± 2.58 ^{b}	91.72 ± 0.16 ^{b}	92.38 ± 0.01 ^{c}
Diet 4	22.5	61.98± 1.14 ^{a}	42.59± 5.98 ^{b}	93.71 ± 0.02 ^{b}	83.62± 0.00 ^{b}	89.54 ± 0.45 ^{a}	90.92± 0.01 ^{b}
Diet 5	30	61.22 ± 14.63 ^{a}	58.65 ± 3.48 ^{c}	90.33 ± 3.48 ^{a}	92.14 ± ^{c}	88.88 ± 2.55 ^{a}	88.26± 0.16 ^{a}
Diet 5 (70% RF Diet + 30% MBM)	30	67.34	93.64				

- * one replicate only

*Means of 2-3 replicates with the same superscripts in each column are not significantly different (P<0.05)

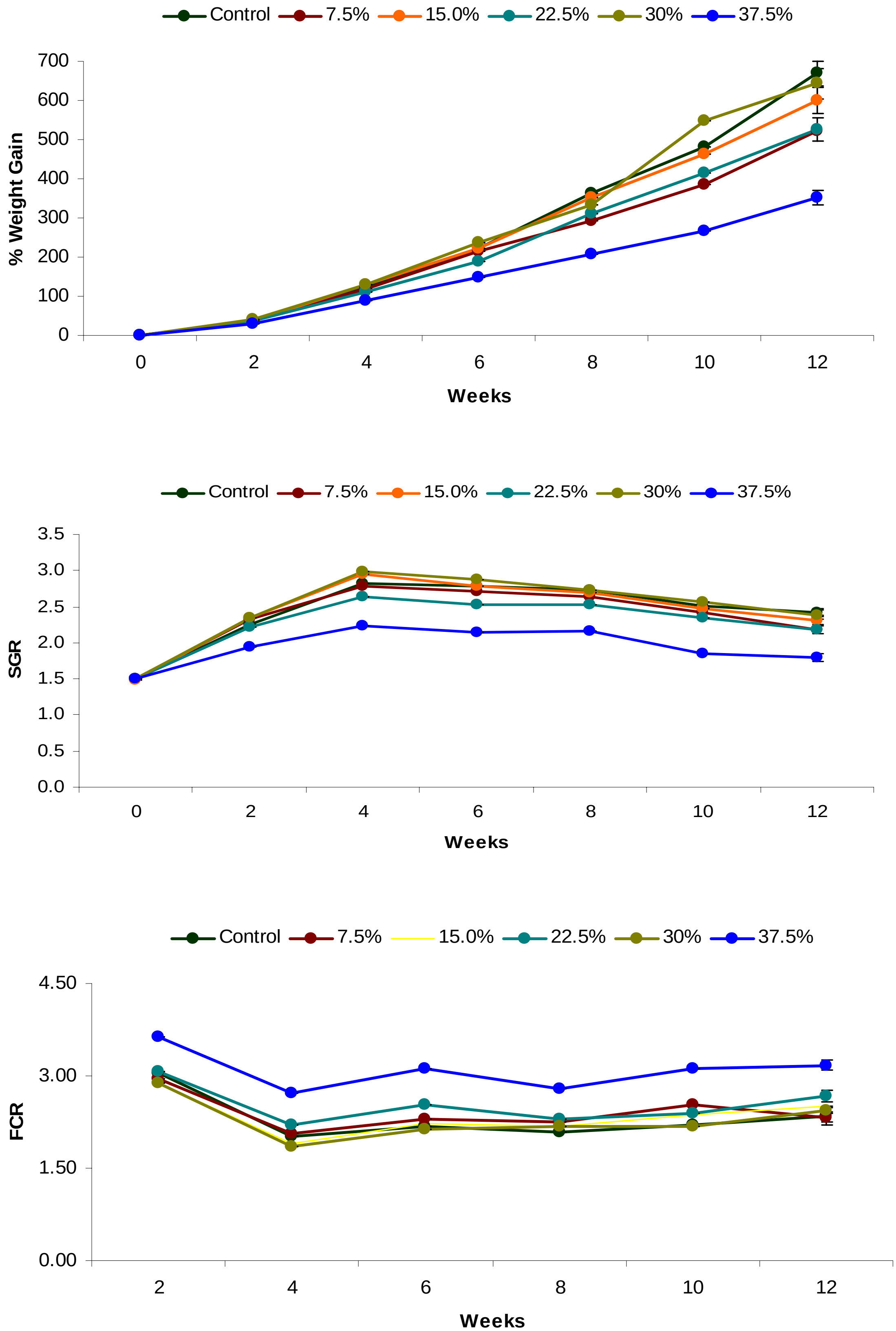


Fig. 1 Percent weight gain (PWG), specific growth rate (SGR) and feed conversion ratio (FCR) of milkfish fed different dietary levels of MBM (Control diet or Diet 1 - 0%, Diet 2 - 7.5%, Diet 3 - 15%, Diet 4 - 22.5%, Diet 5 - 30%, Diet 6 – 37.5%) in freshwater for 12 weeks.

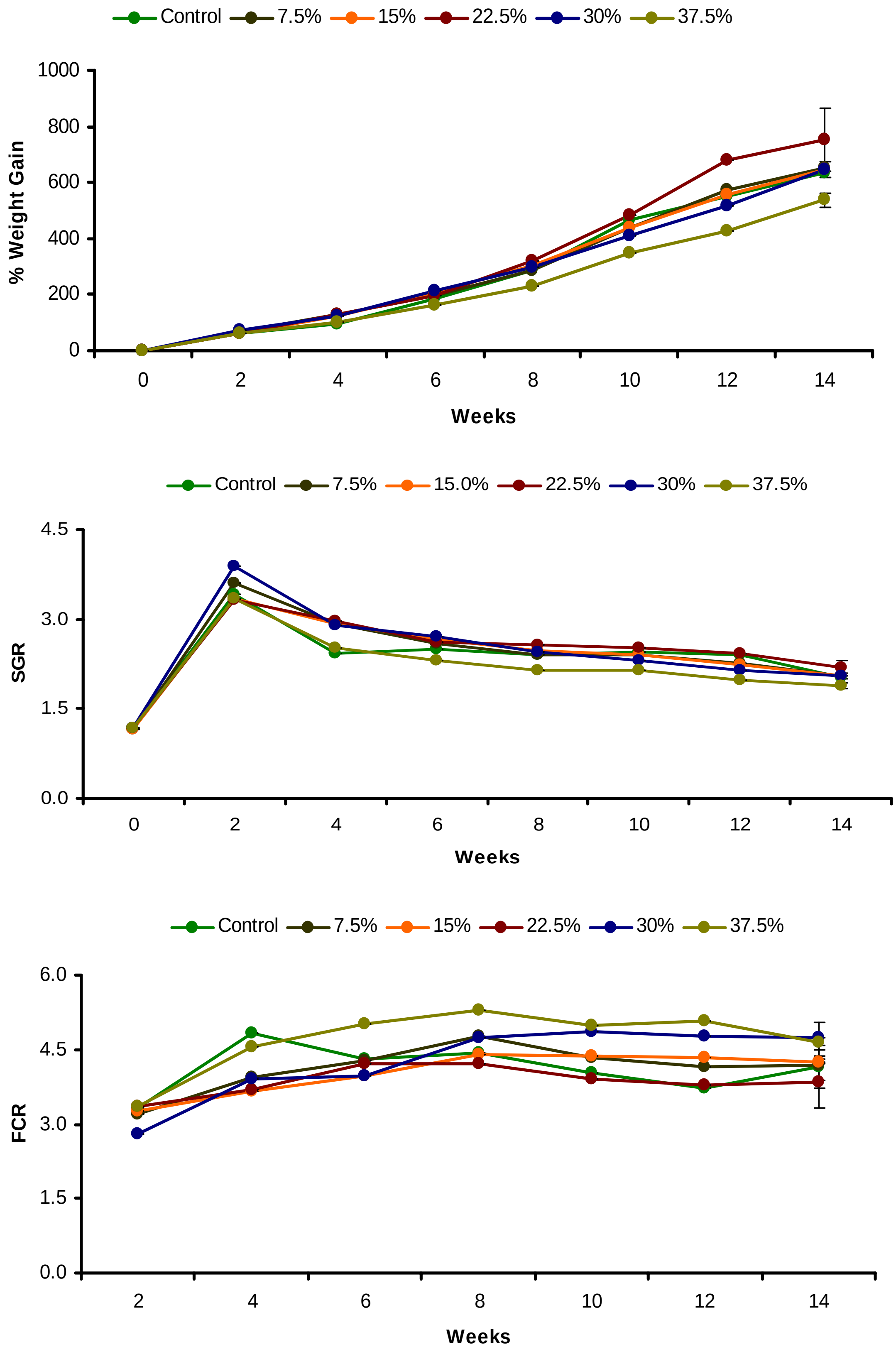
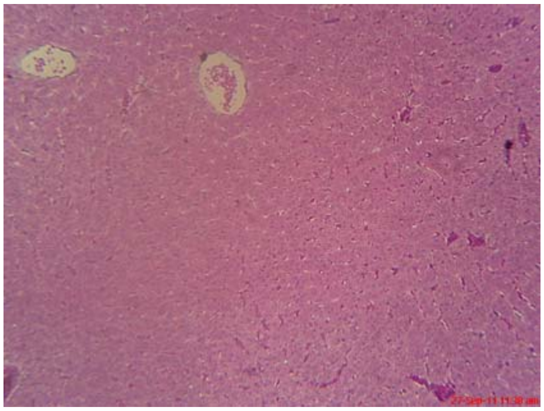
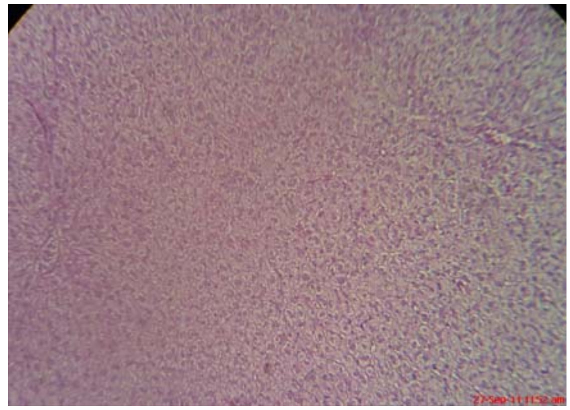


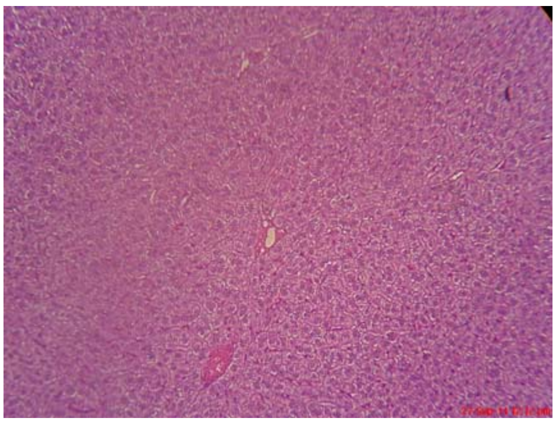
Fig. 2 Percent weight gain (PWG), specific growth rate (SGR) and feed conversion ratio (FCR) of milkfish fed different dietary levels of meat and bone meal or MBM (Control Diet or Diet 1 - 0%, Diet 2 - 7.5%, Diet 3 - 15%, Diet 4 - 22.5%, Diet 5 - 30%, Diet 6 – 37.5%) in seawater for 14 weeks.



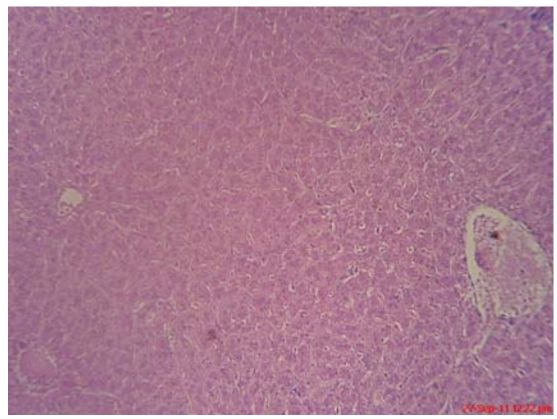
Diet 1 - FW liver ; normal liver ; H&E ; x20



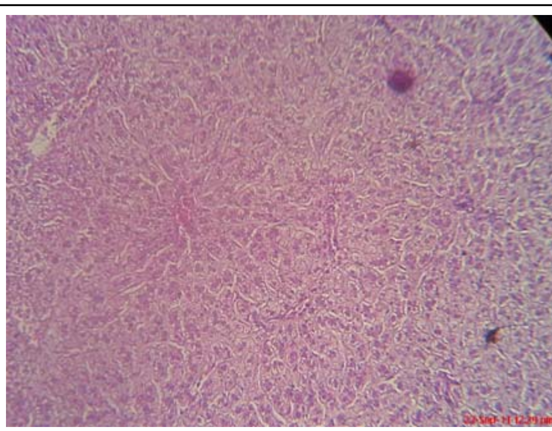
Diet 2 – FW liver ; normal liver ; H&E ; x20



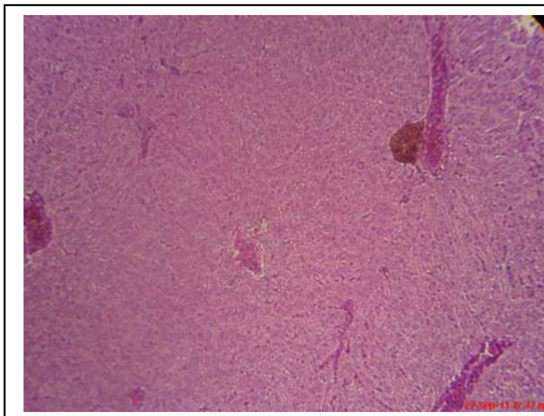
Diet 3 – FW liver ; H&E ; x20



Diet 4 – FW liver ; H&E ; x20

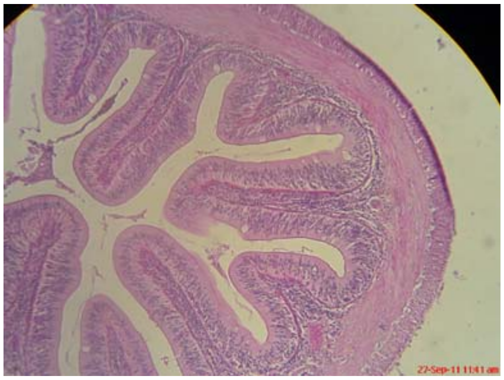


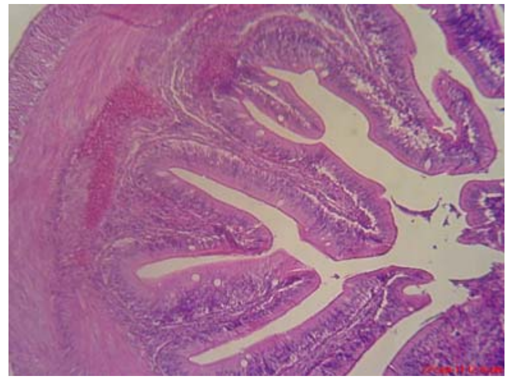
Diet 5 – FW liver ; H&E ; x20

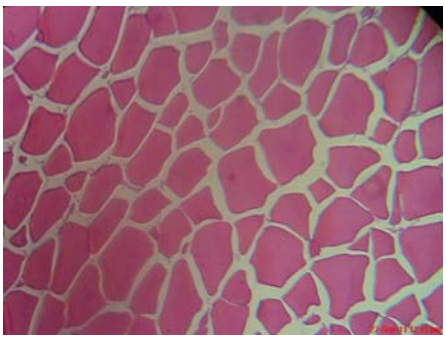


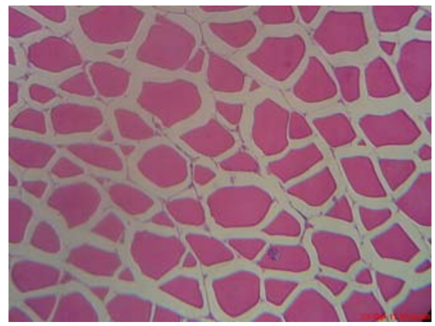
Diet 6 – FW liver ; H&E ; x20

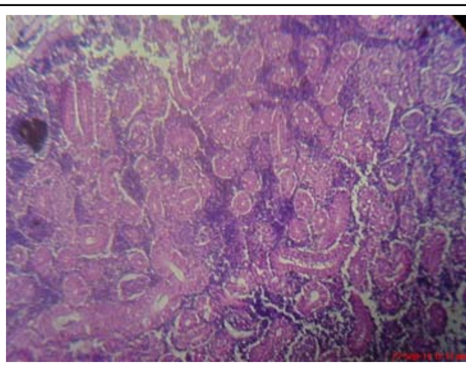
Fig. 3 The histology of milkfish liver tissues showing normal structures after feeding on diets with different levels of meat and bone meal or MBM (Diet 1 - 0%, Diet 2 - 7.5%, Diet 3 - 15%, Diet 4 - 22.5%, Diet 5 - 30%, Diet 6 – 37.5%) in freshwater (FW) for 12 weeks.


Diet 1-FW intestine ; H&E ; x20


Diet 3 – FW intestine ; H&E ; x20


D3- FW muscle ; H&E ; x20


Diet 4 – FW muscle ; H&E ; x20


Diet 3 – FW normal kidney ; H&E ; x20

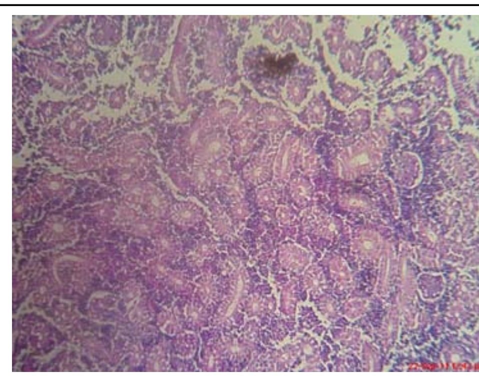
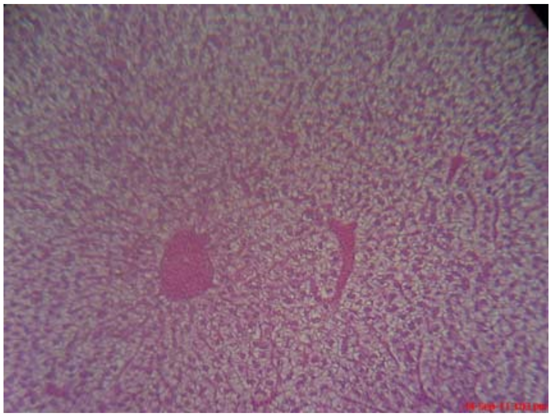
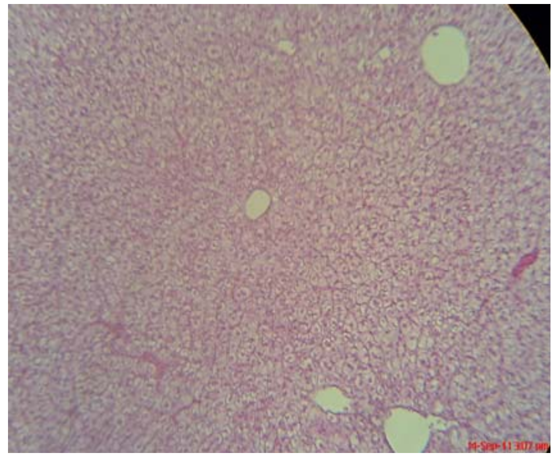

Diet 6 – FW normal kidney ; H&E ; x20

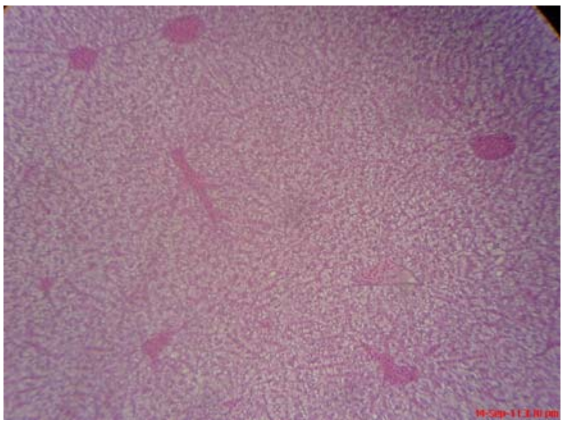
Fig. 4 The histology of milkfish tissues showing normal structures of the distal intestine, muscle, and kidney after feeding on diets with different levels of meat and bone meal or MBM (Diet 1 - 0%, Diet 2 - 7.5%, Diet 3 - 15%, Diet 4 - 22.5%, Diet 5 - 30%, Diet 6 – 37.5%) in freshwater (FW) for 12 weeks.



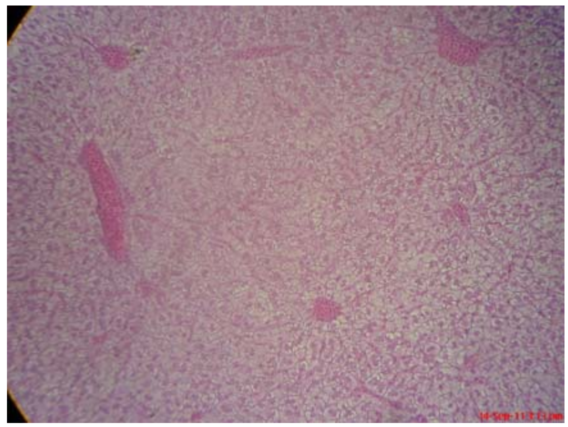
Diet 1 – SW liver ; H&E ; x20



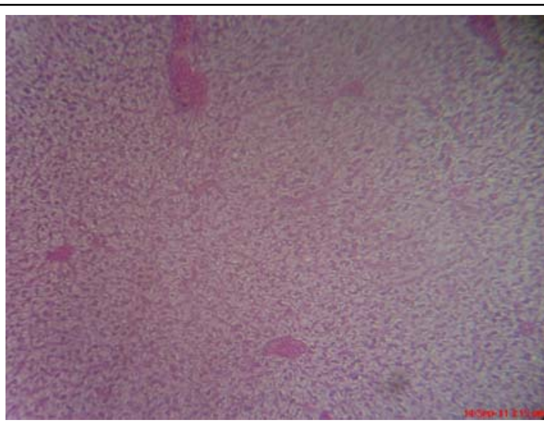
Diet 2 – SW liver; showing vacoulation ; H&E ; x20



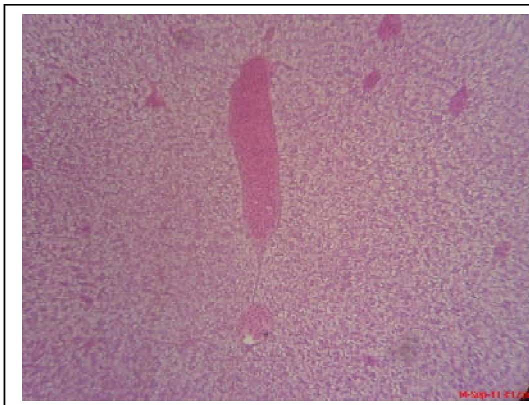
Diet 3 – SW liver ; H&E ; x20



Diet 4 – SW liver ; H&E ; x20

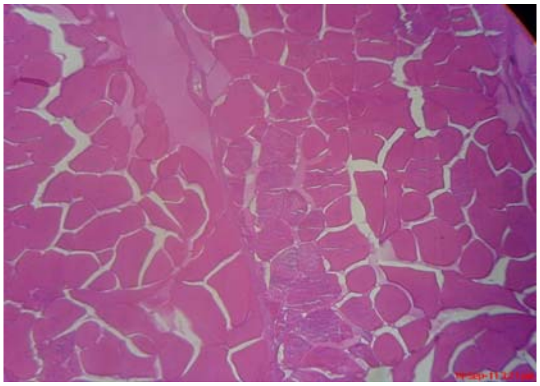


Diet 5 – SW liver ; H&E ; x20

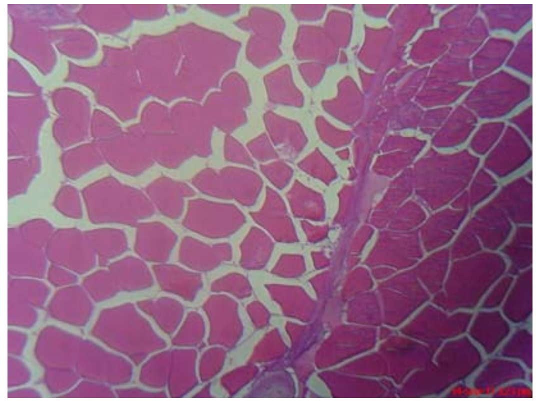


Diet 6 –SW liver ; H&E ; x20

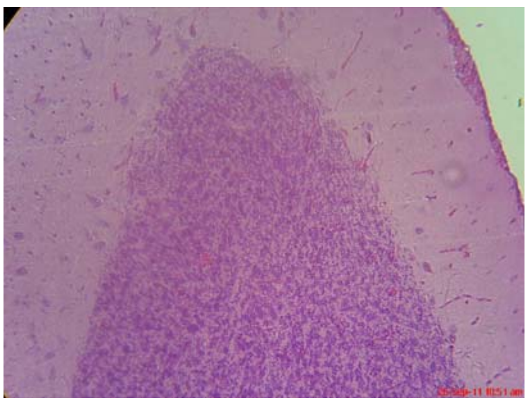
Fig. 5 The histology of milkfish liver tissues after feeding on diets with different levels of meat and bone meal or MBM (Diet 1 - 0%, Diet 2 - 7.5%, Diet 3 - 15%, Diet 4 - 22.5%, Diet 5 - 30%, Diet 6 – 37.5%) in seawater (SW) for 14 weeks. Except for Diet 2 which has small lipid vacuoles, the rest have normal structures.



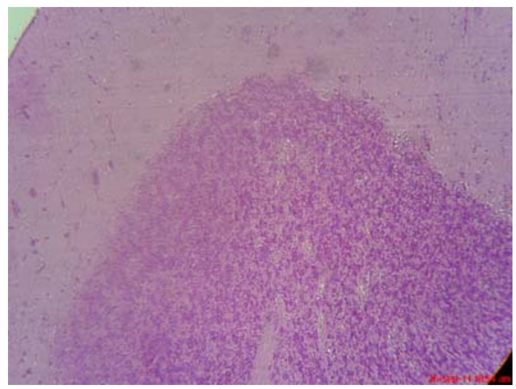
Diet 1 – SW muscle ; H&E ; x20



Diet 2 – SW muscle ; H&E ; x20

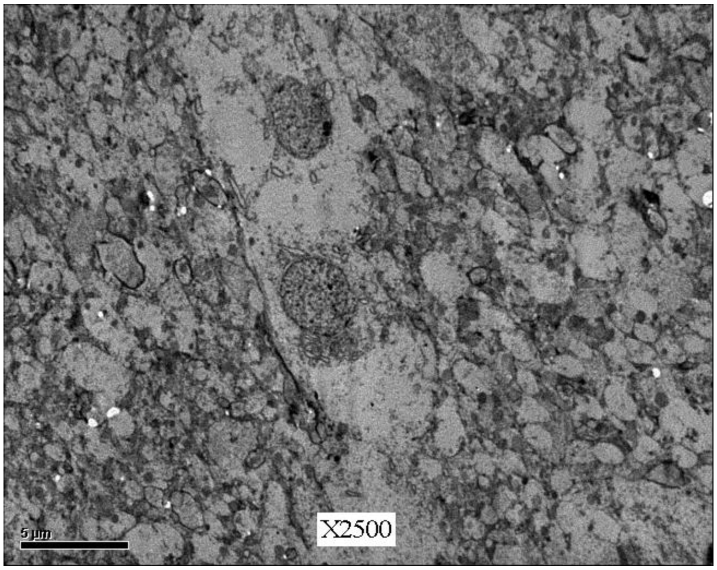


Diet 1 – SW Brain ; H&E ; x20

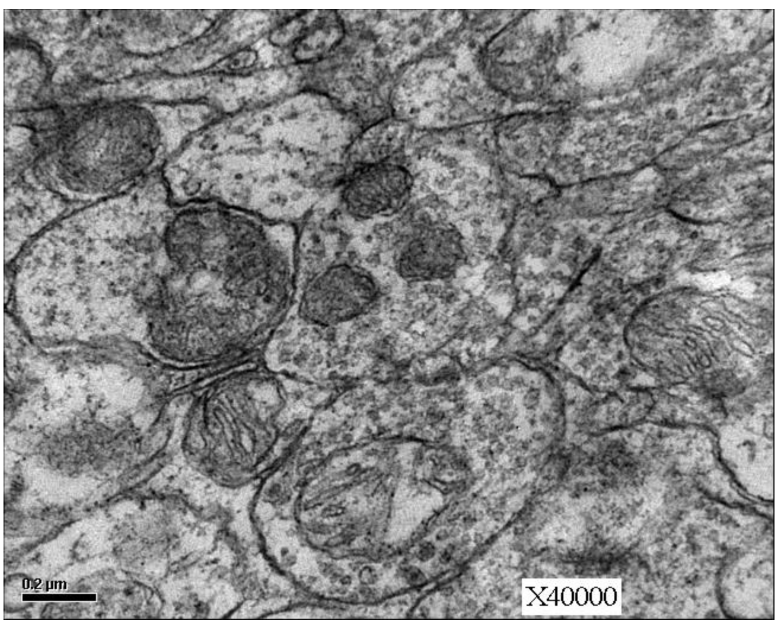


Diet 2 – SW Brain ; H&E ; x20

Fig. 6 The histology of milkfish muscle and brain tissues showing normal structures after feeding on diets with different levels of meat and bone meal or MBM (Diet 1 - 0%, Diet 2 - 7.5%, Diet 3 - 15%, Diet 4 - 22.5%, Diet 5 - 30%, Diet 6 – 37.5%) in seawater (SW) for 14 weeks. Tissues appeared to be normal in all treatments.



Diet 1 TEM Brain



Diet 6 TEM Brain

Fig. 7 The TEM results of milkfish brain tissues (in two magnifications) after feeding on diets with different levels of meat and bone meal or MBM (Diet 1 - 0%, Diet 2 - 7.5%, Diet 3 - 15%, Diet 4 - 22.5%, Diet 5 - 30%, Diet 6 – 37.5%) in freshwater (FW) for 12 weeks. Tissues appeared to be normal in all treatments.