Lipid Oxidation in Fish Meal Stored under Different Conditions on Growth, Feed Efficiency and Hepatopancreatic Cells of Black Tiger Shrimp (*Penaeus monodon*)

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Five isonitrogenous diets containing fish meal (FM) stored under different conditions were used: diet 1 (control), freshly produced FM (TBARS = 7.87 mgMAD/kg FM), diet 2, low rancidity-non ethoxyquin FM stored at 4^{o}C for 1.5 month (TBARS = 15.02 mgMAD/kg FM), diet 3 moderate rancidity-ethoxyquin treated FM stored at ambient temperature for 4.5 months (TBARS = 22.52 mgMAD/kg FM), diet 4, moderate rancidity-non ethoxyquin FM stored at ambient temperature for 3 months (TBARS = 25.67 mgMAD/kg FM), diet 5, high rancidity-non ethoxyquin FM stored at ambient temperature for 4.5 months (TBARS = 62.31 mgMAD/kg FM). A 60-day feeding trial was carried out in a semi-closed system with 30 glass aquaria, each containing 25 shrimps with an average initial weight of 0.25 g. The lowest final weight, percentage weight gain and specific growth rate and the highest percentage abnormality (50%) of hepatopancreatic cells were found in the shrimp fed diet 3 ($p<0.05$) followed by those fed diet 5. Growth performance, feed utilization efficiency and hepatopancreatic cells of shrimp fed diet 2 were not different from those of the control group ($p>0.05$). Survival of shrimps was in the range of 87.33± 6.89-92.00±7.15% and not significantly different among the treatment groups.

Key words: Fish meal, Lipid oxidation, Black tiger shrimp, Growth performance, Hepatopancreatic cell

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1. Introduction

An increasing pressure from a steady declined supply and higher price of fishmeal has led aquafeed producers search for alternative protein sources. However, fishmeal is still a very vital dietary ingredient for many carnivorous species due to its unique nutritional qualities. It has an almost ideal available amino acid profile for most cultured species, excellent palatability making diets very attractive leading to maximum intake, unidentified growth factors and is rich in polyunsaturated fatty acids required by aquatic species. In spite of this, a satisfactory fish growth may not be achieved if low quality fishmeal is used in a diet. Rancidity from oxidation of lipid occurring during storage is one of several factors affecting fishmeal quality . The highly unsaturated fatty acid characteristic of fish lipid including those with five (20:5n-3) and six (22:6n-3) ethylenic bonds are readily susceptible to oxidation due to the greater chain lengths and the greater number of unsaturated carbon-carbon bonds along the fatty acid chains (Ackman and Gunnlaugsdottir, 1992). The mechanism of lipid oxidation begins with auto-oxidation involving the direct reaction of lipids with molecular oxygen to form hydroperoxides. This is followed by secondary reactions yielding diperoxides if further oxidation takes place, or ketoglycerides if the hydroperoxides are dehydrated. Fission of hydroperoxides yield products containing carbonyl and hydroxy groups which will react further to form other products. These products of secondary oxidation of lipids contribute to off flavour and include toxic compounds frequently-associated with rancidity (Chow, 1980; Halliwell and Chirico, 1993). Furthermore, carbonyl groups produced by the fission of aldehydic hydroperoxides can react with the *epsilon* - amino group of lysine, thereby reducing the nutritive value of the protein. For stored feedstuff, rate of lipid oxidation is increased by factors such as the presence of lipoxidase, hematin, peroxides (products of auto-oxidation of lipids which catalyze the oxidation of lipids), light (involving in the photolysis of peroxides),

high temperature, moisture, trace metal notably iron, copper, cobalt, and zinc (Chow, 1980; Dabrowski and Guderly, 2002; Sutton et al., 2006).

The negative effects of oxidative rancidity mainly in polyunsaturated fish oils and diets on growth, feed intake and health have been demonstrated in several fish species. Smith (1979) reported growth depression, microcytic anaemia and liver lipoid degeneration in 13 g rainbow trout fed a rancid diet. Low red blood cell numbers, haemoglobin content and haematocrit and increased haemolysis, polychromatocyte development, splenic haemosiderosis and hepatic ceroidosis were also illustrated in rainbow trout fed highly or extremely oxidized oils (Moccia et al., 1984). Koshio et al. (1994) fed diets containing either herring oil or canola oil with peroxide values ranging from < 1, 14, and 40 meq/kg of oil to Atlantic salmon and found that oil type was not an important factor affecting fish survival and growth, but the weight gain of fish fed the diet containing mildly oxidized oil (40 meq/kg of oil) was significantly less than that of fish fed the diets containing fresh oil. In juvenile hybrid tilapia (*Oreochromis nilotica* x *O. aureus*), poor growth performance was observed in the group of fish fed the diet containing oxidized oil with peroxide value of 98 meq/kg oil without antioxidant supplementation (Huang and Huang, 2004). Eurasian perch showed lower feed intake and the poorest growth when fed with the diet containing high concentration (18%) of unstabilized menhaden oil. Fatty acid composition of perch muscle , viscera and liver was also significantly affected showing low concentrations of lenolenic, eicopentaenoic and docosapentaenoic acids (Kestemont et al., 2001).

In comparison with those reported in fish, only few studies on effects of lipid oxidation were carried out in shrimp. Bautista and Subosa (1997) examined a tolerable level of black tiger shrimp (*Penaeus monodon*) fed oxidized feed fortified with increasing levels of tetraethoxypropane (TEP, the oxidation product). The unsaturated fatty acid content of the diets decreased as its TEP content increased. Shrimp fed the diet the containing 100 g TEP/

kg diet with TBA value of 1262 mg malonaldehyde/ kg fat showed signs of physical deterioration and slowest growth after 6-8 weeks. They further investigated the effect of butylated hydroxytoluene (BHT) on the quality of shrimp diet stored at various temperatures and found that growth and survival of *P. monodon* fed diets with BHT after 10 weeks of rearing were significantly better than those fed the diet without BHT (Bautista and Subosa, 1999).

For sub-tropical and tropical regions with high humidity and temperature throughout the year especially in rainy and summer season, lipids in either feedstuff or diets are readily susceptible to oxidation if storage is prolonged. This study is an endeavor to examine an effect of lipid oxidation in fish meal stored under different conditions on growth, feed efficiency and hepatopancreatic cells of black tiger shrimp.

2. Materials and methods

2.1 Fish meal

Forty kilograms of freshly produced premium grade fish meal (72 % protein) were divided into two equal portions, one of which was treated with ethoxyquin at a concentration of 200 ppm. Each portion was then equally divided and stored at 4^{o}C and ambient conditions for 0, 1.5, 3.0 and 4.5 months with two replications/storage condition. At each storage interval, samples were taken and analysed for thiobarbituric acid reactive substances (TBARS), anisidine value (AnV) and free fatty acid (FFA) according to AOAC, (1990), Buege and Aust (1978); IUPAC (1979) and Uchiyama (1973), respectively. Representatives of low, moderate and high rancidity fish meal to be used in experimental diets was made based on the mentioned analysis results. To avoid an experimental error, fish meal from each storage treatment was immediately incorporated into a diet with antioxidant (BHT)

supplementation, packed in a sealed dark bag and stored at -20°C for further use in a feeding trial.

2.2 Experimental diets

Five experimental diets containing FM varying in degrees of rancidity as shown in Table 1 were used: diet 1 (control)-freshly produced premium grade FM, diet 2-low rancidity FM without ethoxyquin stored at 4^{o}C for 1.5 months, diet 3-moderate rancidity FM treated with ethoxyquin stored at ambient conditions for 4.5 months, diet 4-moderate rancidity FM without ethoxyquin stored at ambient conditions for 3 months and diet 5- high rancidity FM without ethoxyquin stored at ambient conditions for 4.5 months. Experimental diets were formulated to be isonitrogenous and isocaloric composing of basal ingredients as shown in Table 2. Dry ingredients were mixed thoroughly using Hobart mixer, then the oil and water added after which mixing was continued for 20 minutes until all ingredients were well blended. The mixture was cold-pelleted using a 2-mm die attached to the mixer. The resulting pellets were dried at 60°C for up to 36 h, then stored at -20°C until further use. Proximate analysis of the finished diets was performed according to AOAC (1990). Fatty acid composition of the diets was analysed using gas chromatography.

2.3 Shrimp

Five thousand post larvae-15 (PL15) were nursed for one month in a 2-m^3 cement tank by feeding artemia in the first two weeks after which time they were gradually trained to feed commercial shrimp diet. They were then transferred to thirty 200-litre aquaria at forty shrimps/aquarium and fed with the control diet for ten days in order to acclimatize them to the experimental diets.

2.4 Experimental system

Thirty 200-l glass aquaria equipped with aeration devices were used. Continuous seawater supply was from semi-circulating system with water flow rate of 0.8 l/min, salinity ranged between 27-34 ppt and pH between 6.5-8. Water quality was monitored daily. Ammonia and nitrite concentration in the culture system was determined every two weeks.

2.5 Feeding trial and sample collection

Twenty-five shrimps of approximately 0.25 g/shrimp were sorted, weighed and stocked into each of thirty aquaria. Six aquaria were then randomly assigned to each experimental diet. The shrimps were fed respective diets four times daily at 7.00, 12.00, 17.00 and 23.00 h for 60 days. To obtain the actual feed intake, excess feed in each aquarium was collected, dried, weighed and subtracted from the amount given to the shrimp. Feeding was adjusted when appropriated by observing feeding behavior and molting cycle. Shrimp health and mortality was monitored and recorded regularly. Molted shells and carcasses were collected as soon as noticed to prevent an error from scavengering.

One hundred grams of shrimp at the beginning of the feeding trial were collected for initial proximate composition analysis. Upon completion of the feeding trial, shrimps were individually weighed and ten shrimps were removed from each aquarium for final carcass proximate composition (AOAC, 1990). Shrimp samples were oven-dried at 60^{o}C for 36 h, fine ground and kept at -20^{o}C until analysis. Percentage weight gain, specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER), productive protein value (PPV) and survival rate were calculated accordingly.

2.6 Pathological studies of hepatopancreatic tissue

Five shrimps were randomly sampled from each aquarium at the end of the feeding trial. Shrimps were fixed in Davidson's fixative followed the standard protocol (Bell and Lightner, 1988). The hepatopancreas was embedded in paraffin, sliced (3-4 µ) and stained with haematoxylin-eosin (Humason, 1972). Pathological examination was performed using light microscope (Olympus® Model AX-70).

2.7 Statistical Analysis

Data were subjected to analysis of variance (ANOVA) and mean differences were compared using Duncan's New Multiple Range Test at $p<0.05$.

3. Results

3.1 Fatty acid composition of diets

Fatty acid composition were similar among experimental diets. A slight decrease was observed in PUFA composition and ratio of PUFA to saturated fatty acids in diets 3, 4 and 5 (Table 3).

3.2 Growth performance and feed utilization

Final weight, percentage weight gain, specific growth rate, feed intake, feed conversion ratio (FCR), protein efficiency ratio (PER) and productive protein value (PPV) are shown in Tables 4 and 5. Growth parameters clearly demonstrate that shrimp fed the diets containing fishmeal stored at ambient temperature for 4.5 months (diets 3 and 5) had significantly inferior growth in comparison with those fed diets 1, 2 and 4 ($p<0.05$). The poorest growth responses were noted in shrimp fed diet 3 containing fishmeal with ethoxyquin supplementation stored at ambient temperature for 4.5 months. Survival of

shrimp were in the range of 88-92 % and not significantly different among treatments ($p>0.05$)

Feed intake of shrimp fed diet 3 was noticeably and significantly the lowest while those fed other diets were similar (Table 5). For the feed and protein utilization, diets 1, 2 and 4 showed significantly better FCR, PER and PPV than those of diets 3 and 4 ($p<0.05$).

3.3 Proximate composition of shrimp carcass

Crude protein, lipid contents and moisture of shrimp fed experimental diets were not significantly different ($p>0.05$). Although there was only a slight difference in the ash content of shrimp carcass, statistical analysis revealed the differences ($p<0.05$) as shown in Table 6.

3.4 Changes of Hepatopacreatic tissue

Oxidation of fishmeal lipid have an effect on shrimp hepatopancreas as shown in Table 7 and Figures 2-6. The highest degree of abnormality with heavy haemocytic infiltration, atrophy of tubular epithelial and nodule formation was detected in shrimp fed diet 3 followed by those fed diet 5 (Figures 2-5) with 50% and 60% normal cells, respectively. Light infiltration of haemocyte was also observed in hepatopancreas of shrimp fed diet 4 (Figure 6) with 80% normal cells. Shrimp fed diets 1 and 2 had 100 % healthy normal cells (Figure 1).

4. Discussion

Adverse effects of feeding oxidized oil and diets on growth and health status have been reported in several fish species such as common carp, rainbow trout, yellow tail, chinook salmon (Tacon, 1991), Atlantic salmon (Koshio et al., 1994), tilapia (Huang and Huang, 2004), African catfish (Baker and Davies, 1997) and Atlantic halibut (Martins et al., 2007). Growth depression, loss of appetite, poor feed conversion efficiency and high mortality are the common pathological signs observed in fish affected by dietary oxidative rancidity. In the present study, shrimp fed diet 5 containing FM without ethoxyquin (EQ) stored at ambient temperature (27-32^{o}C) for 4.5 months (TBARS = 62.31 mgMAD/kg) had significantly poorer final weight, percentage weight gain, and FCR as compared with those fed other diets except for the shrimp fed diet 3 with FM stored for the same period of time but treated with EQ (TBARS = 22.52 mgMAD/kg). The results implied that storing fish meal for up to 4.5 months without an antioxidant in the humid tropical climate might not be appropriate and that addition of EQ, though preventing lipid oxidation in FM, did not help promote good growth. Although EQ is a synthetic antioxidant being used in fish meal (Saxena et al., 2000) and feed industry (Murphy, 2000), shrimp fed diet 3 containing FM treated with EQ showed the poorest growth. This was due distinctively to lowest feed intake reflecting low diet palatability which might in turn be as a result of EQ presented in FM. Since the experimental diets were not supplemented with any attractant, suppression of feed intake indicated that *P. monodon* might be very sensitive to EQ even presented in a very minute amount. FCR, PER and PPV were in accordance with growth responses in that shrimps fed diets 5 and 3 were significantly inferior to those fed diets 1, 2 and 4. Despite better final weight, weight gain and SGR as compared with diet 3, effects of oxidative rancidity on feed and protein utilization were more pronounced in shrimp fed diet 5. This might be due to crosslinkage between amino acids and resulting aldehydes from lipid oxidation causing an insolubility of protein in

fishmeal (Kussi et al., 1975; Esterbauer et al., 1991) which directly affected protein digestibility and utilization. Growth and nutrient utilization of shrimp fed diets 2 and 4 were similar to those fed the control diet indicating that storing fish meal without antioxidant at ambient temperature in the humid tropic for up to three months is considered acceptable with regard to lipid oxidation effects on shrimp growth performances. Fatty acid composition of experiment diets were similar. However, a slight differences in the levels of PUFA and ratio of PUFA/saturated fatty acids were noticed. Oxidative rancidity has well known effects on reduction of PUFA (Baker and Davies, 1997) but such pronounced effect was not observed in the present study which might be due to lower amount of lipid in fish meal as compared with those experimented with fish oil.

Hepatopancreas (HP) is a vital and major organ of decapod that combines many of the functions of the liver, pancreas, intestine and other organs in the vertebrates. Proper functioning of the HP is therefore important to the health, growth and survival of cultured shrimp (Caceci et al., 1988, Vote et al., 1985). As for nutrient utilization, it is an excellent model for food digestion and cell secretion because of its primary role in the synthesis and secretion of digestive enzymes, final digestion of the ingested food and subsequent uptake of nutrients (Hu and Leung, 2007). The other important role of HP is detoxification such as toxin in feed and pollutants in environment (Boonyaratpalin et al., 2001: Bianchini et al., 2007: Vogt and Quintillo, 1994). Pathological examination of hepatopancretic tissues under light microscope demonstrated increasing degrees of cell abnormality in shrimp fed diets 4, 5 and 3, respectively. The highest degree of hemocytic infiltration, dystrophy of tubular epithelial and nodule formation in hepatopancretic tissues of shrimp fed diet 3 supported the poorest growth responses. Dystrophy detected in B-cell (for intracellular digestion and assimilation) and R-cell (as storage cell and resorption of metabolites) without nutrient storage in an aforementioned group of shrimps verified insufficiency of nutrients in

supporting good growth due to the lowest feed intake (50% less than that of the control group). Nodule formations found in shrimps fed diets 5 and 3 may have been a response to either toxic effects of peroxidation compounds causing cell injuries or accumulation of infiltration around shredded injured cells (Kinsella, 1987; Sanders, 1987; Lightner and Redman, 1985). Since nodular formation can be one of defense mechanisms to infection, bacterial infection was therefore examined. No bacterial infection was found in samples which was examined by microbiological techniques. Mild lipid oxidative compounds in FM incorporated into diet 4 may cause insignificant irritation in HP cells within a tolerable level allowing normal growth. On the contrary, shrimp fed diet 3 containing FM with EQ which effectively reduced lipid oxidation had the highest degree of HP destruction and poorest feed intake and growth indicating the negative effects of EQ on palatability, growth and cellular responses of shrimps. Bautista et al. (1992) also found a similar symptom in black tiger shrimp fed diets containing either EQ or propyl gallate as an antioxidant. EQ has been shown to have significant toxic effects on poultry, swine, rats and mice such as weight loss, increase in liver weight and hepatic cell proliferation, and inhibition of energy metabolism and ATP production (Saxena et al., 2000). Błaszczyk (2006) also found that EQ induced DNA damage in human lymphocytes in a dose-dependent manner. Mortality of shrimps was similar in all groups indicating that oxidative rancidity does not have any effect on the mortality.

5. Conclusion

This study revealed effects of high degree of lipid oxidation in fishmeal and ethoxyquin on feed intake, growth, feed utilization and histopathology of black tiger shrimp. High rancidity-non ethoxyquin FM stored at ambient temperature for 4.5 months (TBARS = 62.31 mgMAD/kg FM) resulted in poor growth performance but ethoxyquin supplemented fishmeal showed the poorest performance and the highest percentage abnormality (50%) of

hepatopancreatic cells. The results implied that storing fish meal for up to 4.5 months without an antioxidant in the humid tropical climate might not be appropriate and that addition of EQ, though preventing lipid oxidation in FM, did not help promote good growth and health of shrimp.

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Table 1 Thiobarbituric acid reactive substances (TBARS), anisidine value (AnV) and free fatty acid (FFA) of fishmeal used in experimental diets

Diet	Storage conditions	TBARS (mgMAD/kg FM)	FFA (Unit)	AnV (%)
1 Control	Freshly produced FM	7.87±0.60	5.65±0.19	16.36±0.58
2 LR-EQ*	No ethoxyquin, stored at 4°C for 1.5 months	15.02±0.90	12.55±0.08	29.58±0.14
3 MR+EQ	Ethoxyquin treated, stored at ambient temperature for 4.5 months	22.5±0.88	22.63±0.42	20.55±0.20
4 MR-EQ	No ethoxyquin, stored at ambient temperature for 3 months	25.67±0.30	17.46±0.25	28.80±0.63
5 HR-EQ	No ethoxyquin, stored at ambient temperature for 4.5 months	62.31±0.62	26.20±0.22	36.87±0.79

*LR-EQ = low rancidity fishmeal without ethoxyquin, MR+EQ = moderate rancidity fishmeal with ethoxyquin, MR-EQ = moderate rancidity fishmeal without ethoxyquin, HR-EQ = high rancidity fishmeal without ethoxyquin

Table 2 Composition of experimental diets (as-fed basis) modified from Boonyaratpalin et al. (2001)

Ingredient	g/kg
Fish meal[1]	420.0
Squid meal	80.0
Wheat gluten	60.0
Wheat flour	225.0
Rice flour	119.0
Lecithin	20.0
Vitamin premix[2]	3.3
Ascorbic acid	1.0
Vitamin E	1.5
Cholesterol	5.0
Zeolite	15.0
Mineral mix[3]	40.0
Carboxymethyl cellulose	10.0
BHT	0.2
Proximate composition[4]	
Protein	42.40-43.63
Lipid	7.28-7.86
Ash	7.98-8.57

[1]Fish meal in diet 2 = 420 g/kg, diet 3 = 425 g/kg, diet 4 = 440 g/kg, diet 5 = 450 g/kg

[2] Vitamin premix (amount in kg of premix): vit. A 3500,000 IU; vit. D 800,000 IU; vit. E 40 g; vit. K 15 g; vit. B_1 20 g; vit. B_2 15 g; vit. B_6 20 g; vit. B_{12} 10 mg; niacin 40 g; panthothenic acid 40 g; folic acid 4 g; biotin 400 mg; inositol 150 g

[3]Mineral mix (g/kg of premix): K_2HPO_4, 40; $Ca_3(PO_4)_2$, 5.5; $MgSO_47H_2O$, 6.1; NaH_2PO_4 $2H_2O$, 16; cellulose 828

[4]From proximate analysis

Table 3 Relative fatty acid compositions (% of total fatty acids) of experimental diets

Fatty acid	Diet				
	1 Control	2 LR-EQ	3 MR+EQ	4 MR-EQ	5 HR-EQ
C14:0	3.44	3.32	3.35	3.42	3.54
C15:0	1.03	0.95	0.93	1.03	1.06
C16:0	24.96	24.17	25.09	25.00	24.96
C16:1 n-7	3.79	3.63	3.53	3.94	4.07
C17:0	1.20	1.26	1.30	1.37	1.24
C18:0	7.75	7.74	7.99	7.88	7.96
C18:1 n-9	8.95	9.00	8.92	8.90	8.85
C18:1 n-7	2.24	2.21	2.04	2.23	2.30
C18:2 n-6	17.90	18.17	18.40	17.64	17.88
C18:3 n-3	1.89	2.05	1.86	1.88	1.95
C18:3 n-6	0.34	0.47	0.37	0.34	0.35
C18:4 n-3	0.34	0.32	0.19	0.34	0.35
C20:0	0.34	0.47	0.37	0.51	0.35
C20:1 n-7	0.17	0.16	0.19	0.17	0.18
C20:1 n-9	0.34	0.47	0.37	0.34	0.35
C20:2 n-6	0.17	0.16	0.19	0.17	0.18
C20:4 n-6	2.58	2.53	2.60	2.74	2.65
C20:4 n-3	0.17	0.16	0.37	0.17	0.18
C20:5 n-3	3.79	3.63	3.53	3.77	3.54
C21:5 n-3	-	0.16	-	-	-
C22:0	0.34	0.47	0.56	0.51	0.35
C22:1 n-9	-	0.16	-	-	-
C22:4 n-6	0.52	0.47	0.56	0.51	0.53
C22:5 n-3	0.86	0.95	0.93	1.03	0.88
C22:5 n-6	1.03	1.11	1.12	1.03	1.06
C22:6 n-3	12.22	11.37	11.71	11.82	11.50
C24:0	0.34	0.47	0.37	0.34	0.35
C24:1	0.34	0.16	0.19	0.17	0.18
Unidentified	2.93	3.79	2.97	2.74	3.19
Saturated FA	39.41	39.02	39.96	40.07	39.82
Monoene FA	15.83	15.64	15.24	15.75	15.93
n-3 FA	19.28	18.64	18.59	19.01	18.41
n-6 FA	22.55	22.91	23.23	22.43	22.65
Total PUFA[1]	41.83	41.55	41.82	41.44	41.06
PUFA:Saturated FA	**1.0611**	**1.0648**	**1.0465**	**1.0342**	**1.0311**

*LR-EQ = low rancidity fishmeal without ethoxyquin, MR+EQ = moderate rancidity fishmeal with ethoxyquin, MR-EQ = moderate rancidity fishmeal without ethoxyquin, HR-EQ = high rancidity fishmeal without ethoxyquin

Table 4 Initial weight, final weight, weight gain, specific growth rate and survival rate of black tiger shrimp fed diets with fish meal differed in degrees of rancidity for 60 days

Diet	Initial weight (g/shrimp)	Final weight (g/shrimp)	Weight gain[2] (%)	SGR[3] (%/day)	Survival rate[4] (%)
1 Control	0.25±0.03[1]	4.15±0.30 [a]	1556.70±158.38 [a]	4.67±0.16 [a]	92.00±7.15
2 LR-EQ*	0.25±0.02	4.21±0.26 [a]	1610.78±208.39 [a]	4.72±0.20 [a]	88.67±7.34
3 MR+EQ	0.24±0.02	2.33±0.38 [c]	885.27±139.23 [c]	3.79±0.24 [c]	88.00±12.13
4 MR-EQ	0.25±0.01	4.12±0.39 [a]	1584.43±208.69 [a]	4.67±0.19 [a]	92.00±2.53
5 HR-EQ	0.24±0.02	3.28±0.46 [b]	1306.14±242.69 [b]	4.38±0.31 [b]	87.33±6.89

*LR-EQ = low rancidity fishmeal without ethoxyquin, MR+EQ = moderate rancidity fishmeal with ethoxyquin, MR-EQ = moderate rancidity fishmeal without ethoxyquin, HR-EQ = high rancidity fishmeal without ethoxyquin

[1] Values are means of three replications ± SD, means within the same column sharing the same superscripts are not significantly different ($p > 0.05$).

[2] Percentage weight gain = (Final weight – Initial weight) / Initial weight x 100

[3] Specific growth rate = $(\ln W_2 - \ln W_1) / (t_2 - t_1) \times 100$

[4] Survival rate = Number of shrimp at the termination / Number of shrimp at stocking x 100

Table 5 Feed intake, feed conversion rate, protein efficiency ratio and productive protein value of black tiger shrimp fed diets with fish meal differed in degrees of rancidity for 60 days

Diet	Feed intake (g/shrimp)	FCR[2]	PER[3]	PPV[4] (%)
1 Control	9.72±0.81[1a]	2.16±0.28 [ab]	0.88±0.06 [ab]	35.02±3.07 [ab]
2 LR-EQ*	9.21±0.70 [a]	1.95±0.20 [a]	0.94±0.08 [a]	37.49±3.23 [a]
3 MR+EQ	5.63±0.67 [b]	2.29±0.37 [bc]	0.81±0.12 [bc]	31.91±5.18 [bc]
4 MR-EQ	9.39±0.88 [a]	2.08±0.19 [ab]	0.91±0.08 [ab]	36.11±3.17 [ab]
5 HR-EQ	9.19±1.02 [a]	2.59±0.19 [c]	0.73±0.11 [c]	28.79±2.10 [c]

*LR-EQ = low rancidity fishmeal without ethoxyquin, MR+EQ = moderate rancidity fishmeal with ethoxyquin, MR-EQ = moderate rancidity fishmeal without ethoxyquin, HR-EQ = high rancidity fishmeal without ethoxyquin

[1] Values are means of three replications ± SD, means within the same column sharing the same superscripts are not significantly different ($p > 0.05$).

[2] Feed conversion ratio = Feed intake (g)/Weight gain (g)

[3] Protein efficiency ratio = Weight gain (g)/Protein intake (g)

[4] Productive protein value = Protein gain (g)/ Protein intake (g) X 100

Table 6 Proximate composition (% dry matter basis) of black tiger shrimp fed diets with fish meal differed in degrees of rancidity for 60 days

Diet	Protein	Lipid	Ash	Moisture
Initial shrimp	67.37±0.48[1]	5.58±0.46	16.64±0.63	77.91±0.47
1 Control	69.02±0.55	6.03±0.67	16.25±0.68 [b]	75.08±1.06
2 LR-EQ*	69.47±0.54	6.77±1.07	15.94±0.54 [ab]	74.53±1.18
3 MR+EQ	68.57±1.40	5.87±0.39	15.81±0.93 [ab]	74.90±1.63
4 MR-EQ	69.37±1.68	6.33±0.80	16.58±0.54 [b]	74.65±0.84
5 HR-EQ	68.92±1.10	6.22±1.00	15.17±0.63 [a]	74.88±1.22

*LR-EQ = low rancidity fishmeal without ethoxyquin, MR+EQ = moderate rancidity fishmeal with ethoxyquin, MR-EQ = moderate rancidity fishmeal without ethoxyquin, HR-EQ = high rancidity fishmeal without ethoxyquin

[1] Values are means of three replications ± SD, means within the same column sharing the same superscripts are not significantly different ($p > 0.05$) whereas those without superscripts indicate no statistical difference.

Table 7 Percentage abnormality of hepatopancreatic tissues of black tiger shrimp fed diets with fish meal differed in degrees of rancidity for 60 days

Diet	Normal	Infiltration	Atrophy	Nodule
1 Control	100	-	-	-
2 LR-EQ*	100	-	-	-
3 MR+EQ	50	30	10	10
4 MR-EQ	80	20	-	-
5 HR-EQ	60	20	10	10

*LR-EQ = low rancidity fishmeal without ethoxyquin, MR+EQ = moderate rancidity fishmeal with ethoxyquin, MR-EQ = moderate rancidity fishmeal without ethoxyquin, HR-EQ = high rancidity fishmeal without ethoxyquin

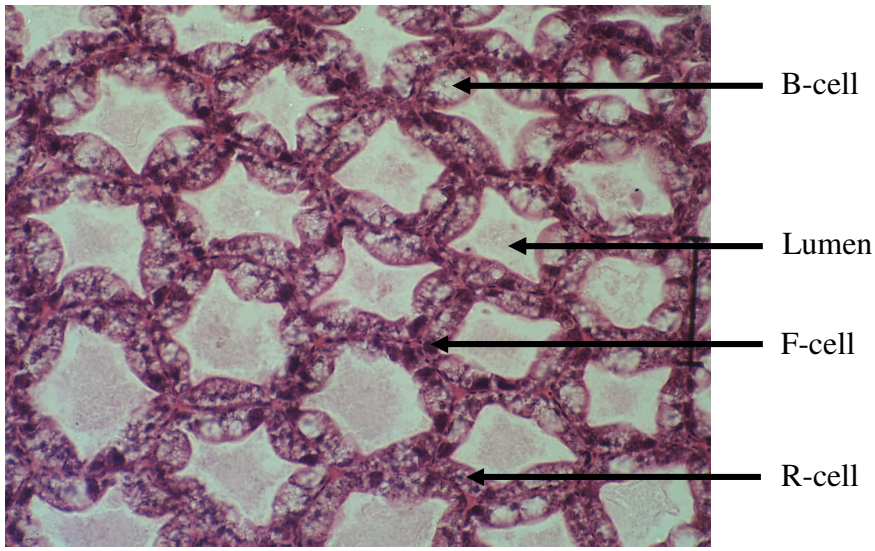

Figure 1 Normal hepatopancreas of *P. monodon* fed control diet showing regular structure and healthy B-cell, F-cell, R-cell as a star shape-like lumen (H&E, Bar = 100 μm).

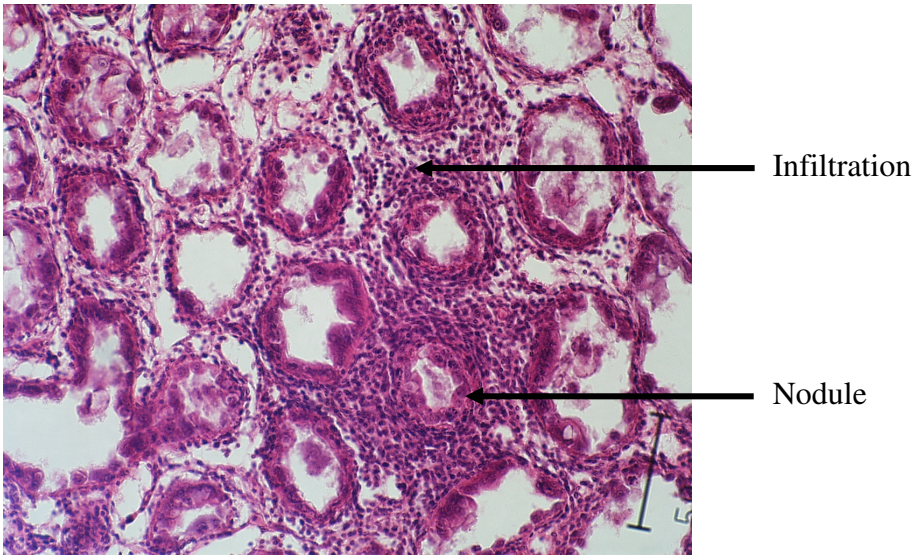

Figure 2 Abnormal hepatocytes of *P. monodon* fed diet 3 (moderate rancidity FM with ethoxyquin) for 60 days showing haemocytic infiltration and nodule formed around the degenerative hepatopacreatic tubule (H&E, Bar = 100 μm).

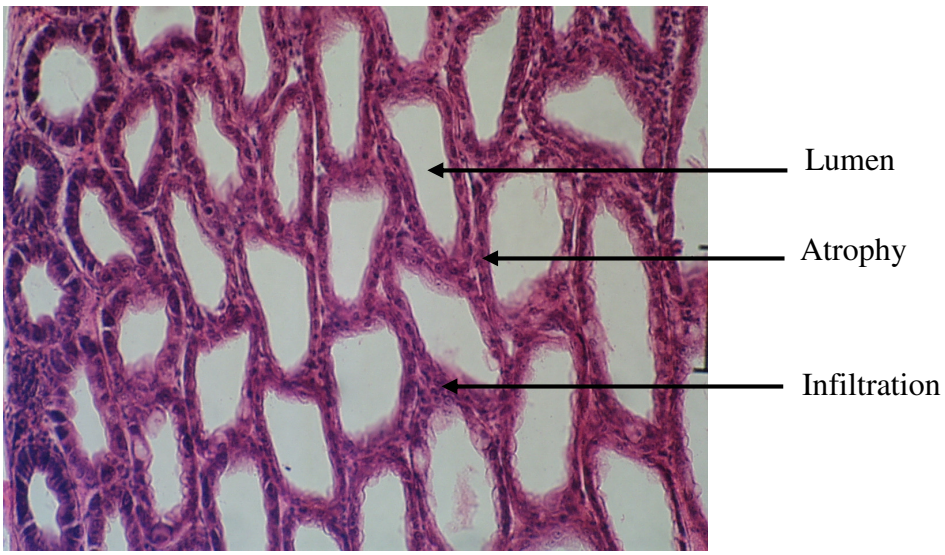

Figure 3 *P. monodon* fed diet 3 for 60 days showing severe atrophic changes of hepatopancreatic tubule with a small number of cells and moderate infiltration (H&E, Bar = 100 µm)

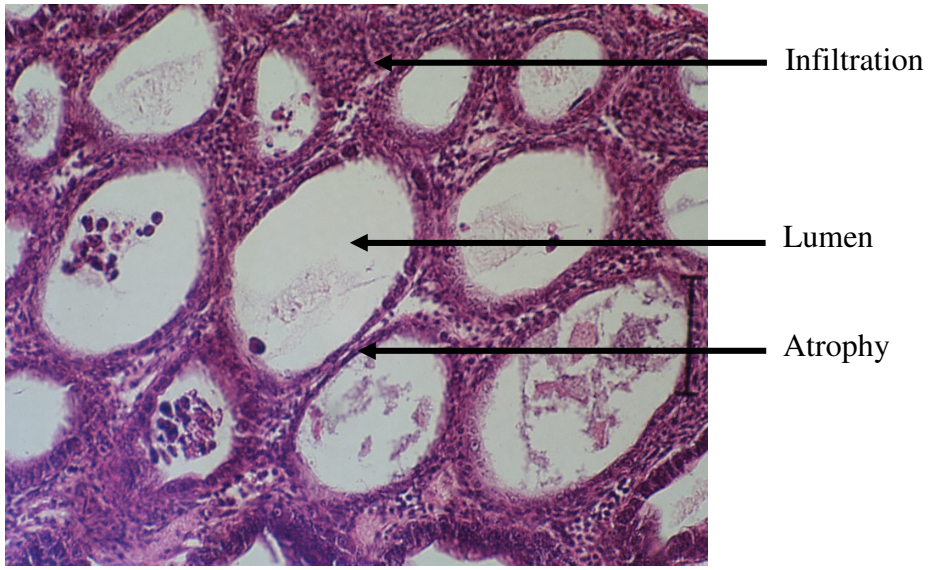

Figure 4 *P. monodon* fed diet 5 (high rancidity FM diet) for 60 days showing severe atrophy of tubular epithelium and degeneration (H&E, Bar = 100 µm).

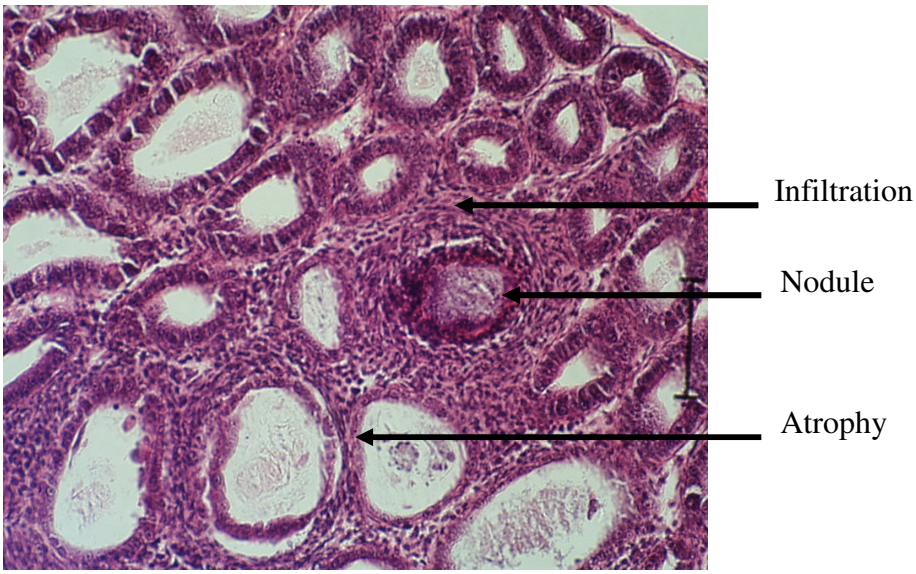

Figure 5. Heavy infiltration, atrophy and nodule formation observed in abnormal hepatocytes of *P. monodon* fed diet 5 for 60 days (H&E, Bar = 100 µm)

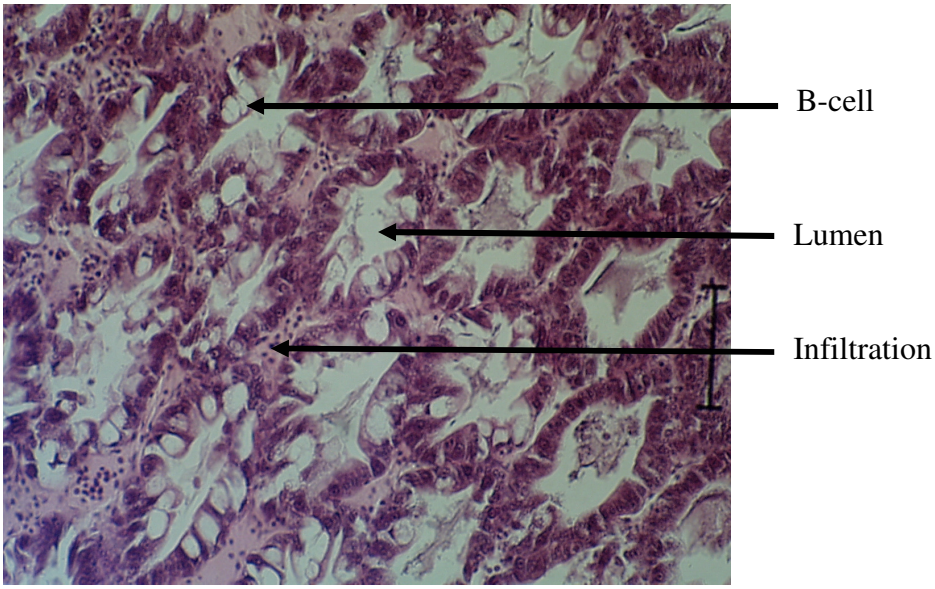

Figure 6 Light infiltration in hepatocytic cells of *P. monodon* fed diet 4 (modorate rancidity FM diet without ethoxyquin) for 60 days (H&E, Bar = 100 µm)