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Original Article

Effects of zero fishmeal diet on growth performance and immune response of Pacific white shrimp (*Litopenaeus vannamei*)♣

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Abstract

As industries worldwide focus on sustainable businesses, it has to be adapted using alternative protein feedstocks in animal feed. Therefore, this research was interested in the zero percent fishmeal diets and supplementing it with trace minerals (TM), compared to 15% of fishmeal diets. The diets were assigned in three treatments; the first treatment is a control group with fishmeal 15%, the second and third are the zero fishmeal diets with supplementing TM 0.12% for adjusted TM level equal to fishmeal 15% (T1) and TM 0.18% for increasing the level of TM in the diet two times of fishmeal 15%. The shrimp (0.6940-0.7060 g body weight) were fed by each type of experimental diets, and growth and immune parameters were investigated at the end of 4-week trial. The growth, total hemocyte count, superoxide dismutase, and glutathione showed no difference between fishmeal 15% and fishmeal 0% groups (P>0.05), except phenoloxidase activity exhibited a higher response when increasing the TM concentration (P<0.05). These results indicated the potential to reducing fishmeal in the white shrimp diet to zero percent without any adverse effect on growth and immune response based on TM supplementing at the growing state or four weeks.

Keywords: trace minerals, total hemocyte count, phenoloxidase enzyme, superoxide dismutase, glutathione

1. Introduction

Today, businesses and industries worldwide aim to do business in a sustainable and environmentally friendly manner. Similar to the aquaculture and feed industry, it has been adapted for more than 30 years, such as stopping the use of un-regulation catching fishmeal in animal feed formulations, using alternative animal protein sources, using alternative plant protein sources, and used industrial processing wastes such as tuna by-product meal and dried distillers grains (DDGS). Although fishmeal is a good source of protein due to its high amino acid value (Cho & Kim, 2011; Tacon & Metian, 2008), and rich in minerals and vitamins (Riche, 2015), however, to make fisheries and aquaculture sustainable, research on the reduction of fishmeal usage and the use of alternative raw materials in feed formulas remains essential, and research is ongoing.

One of the world's most important industries is the Pacific white shrimp (*Litopenaeus vannamei*) industry, which has a large production capacity of nearly five million tons per year (Food and Agriculture Organization of the United Nations [FAO], 2020); in particular, Southeast Asia and China have the most extensive production (Global Aquaculture Alliance [GOAL}, 2017). However, the reduction of fishmeal in the animal feed resulted in decreased nutrients such as trace minerals. Insufficient trace elements can affect these enzyme activities, resulting to weaken animals and lower their yield production. Therefore, trace mineral supplementation must be considered and must be balanced to meet the animal's needs.

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This research focuses on the effects of trace minerals (Cu, Zn, and Se) supplementation in zero fishmeal diets compared to the normal 15% fishmeal diet for Pacific white shrimp (*L. vannamei*) on growth and immune response.

2. Materials and Methods

2.1 Feed production

All raw materials were analyzed proximate items, amino acid profile, and minerals content for balanced the nutrient composition in every formula. Three experimental feeds were formulated to be isonitrogenous (37% protein) and isolipidic (5% lipid) based on NRC (2011) requirements. The first diet contained fishmeal 15% represented normal levels of fishmeal in the feeds as the control group (T1). The second and third feeds removed fishmeal from the formula and substituted it with poultry meal, soy protein concentrate, and squid liver powder meal to enhance the attractant property. Importantly, trace minerals were graded levels of concentration of 0.12% (80 mg/kg Zn, 11 mg/kg Cu, 0.6 mg/kg Se) to adjust the mineral balance level to be equal to the control diets (FM15%) and 0.18% (120 mg/kg Zn, 16.5 mg/kg Cu, 0.9 mg/kg Se) in the second (T2) and third (T3) diets, respectively. Fish oil, cholesterol, methionine, monosodium phosphate, and calcium carbonate were added to balancing the nutrient between treatments at the same levels (Table 1). Raw materials were ground and mixed, then pelleted by a mincer machine and dried in a hot air oven at 90 °C to a moisture content of 9% - 10% after that sealed in plastic bags and stored at room temperature until used. Proximate analysis results of the experimental feeds analyzed by the AOAC (2000) method were given in Table 1. The calculation for nitrogen free extract (NFE) is 100 % - (% crude protein + % crude lipid + % crude fiber + % ash). The gross energy (GE) is calculated from the method of Blaxter (1989).

Table 1. Experimental diet formulations and proximate composition of experimental diets (% dry matter)

Ingredients (%)	T1	T2	T3
Fishmeal [1]	15.00	0.00	0.00
Animal protein [2]	5.50	12.50	12.50
Soybean meal [3]	40.00	40.00	40.00
Soy protein concentrate [4]	0.00	8.00	8.00
Wheat flour	34.130	32.125	32.065
Fish oil	2.00	2.50	2.50
Lecithin	0.50	0.50	0.50
Cholesterol	0.00	0.005	0.005
Methionine	0.05	0.15	0.15
Multivitamin [5]	0.50	0.50	0.50
Betaine	0.30	0.30	0.30
Choline chloride	0.50	0.50	0.50
Monosodium phosphate	0.00	0.50	0.50
Calcium carbonate	0.70	1.60	1.60
Magnesium sulfate	0.30	0.30	0.30
Potassium chloride	0.30	0.30	0.30
Organic iron	0.10	0.10	0.10
Trace minerals mixture [6]	0.12	0.12	0.18
Moisture (%)	10.1	9.88	10.6
Crude protein (%)	37.6	37.0	36.7
Crude lipid (%)	4.46	5.30	5.80
Crude fiber (%)	1.46	2.09	2.06
Ash (%)	7.80	7.02	6.91
NFE (%)	38.58	38.71	37.93
GE (kcal/g)	4.00	4.15	4.15

[1]Fishmeal: moisture, 7.86%; crude protein, 60.4%; crude lipid, 7.22%. [2]Animal protein: moisture, 7.98%; crude protein, 57.9%; crude lipid, 12.4%. [3]Soybean meal: moisture, 10.7%; crude protein, 48.9%; crude lipid, <1%. [4]Soy protein concentrate: moisture, 6.06%; crude protein, 61.8%; crude lipid, <1%. [5]Multivitamin: Vit. A, 4,500 IU/kg; B1, 8 mg/kg; B2, 20 mg/kg; Calcium Pantothenate, 40 mg/kg; B6, 20 mg/kg; B12, 0.03 mg/kg; C, 110 IU/kg; K, 6 mg/kg; D, 1,100 IU/kg; E, 95 IU/kg; niacin, 40 mg/kg; folic acid, 2 mg/kg. [6]Trace minerals mixture: 0.12% (80 mg/kg Zn, 11 mg/kg Cu, 0.6 mg/kg Se), 0.18% (120 mg/kg Zn, 16.5 mg/kg Cu, 0.9 mg/kg Se)

2.2 Feeding trials in Pacific white shrimp

The animal ethics committee of Institutional Care and Use Committee of Kasetsart University accredited by Institute of Animals for Scientific Purpose Development (IAD), National Research Council Thailand (NRCT) (protocol code ACKU-60-FIS-050). The 1,800 healthy Pacific white shrimp, *L. vannamei*, was obtained from commercial hatcheries with an average weight of 0.6999 ± 0.0041 grams and randomly transferred into 30 cages (width 1 m x length 1 m x height 1.2 m) at a density of 60 shrimp per cage, which the 100% of ponds area were paved with PE. Water quality parameters were monitored and maintained at salinity 15 g/L, dissolved oxygen was higher than 5 mg/L, pH 7.5-8.5, water temperature ranged from 28 to 30 °C, and nitrite was lower than 0.5 mg/L, analyzed by test kit (Advance Pharma Co., Ltd., Bangkok, Thailand) and changing the water by gradually draining the 20% of old water in the pond and adding 20% new water every two days. Feeding time was set up at five times per day (8:00, 10:00, 14:00, 16:00, and 20:30 h) throughout four weeks of the culture. The feed given was calculated from 12% of body weight at the start; after that, check trays were used to monitor the feed consumption of shrimp in each cage every day.

2.3 Harvest and growth performance evaluation

At the end of 4 weeks, Pacific white shrimp were harvested and evaluated the growth parameters such as final body weight, total production, percent weight gain (WG), average daily growth (ADG), specific growth rate (SGR), feed conversion ratio (FCR) and protein efficiency ratio (PER).

2.4 Digestive enzyme activity

The hepatopancreas of six shrimp per cage was randomly collected at 4 weeks. Shrimp's hepatopancreas was cut into small pieces, then added Tris-HCl buffer at pH 7.5 in the sample to a buffer ratio of 1: 3 (w/v) and mashed the sample thoroughly using a homogenizer, then precipitated by centrifugation at 4 °C on 3,000 rpm for 15 minutes. The supernatant was collected and then centrifuged at 4 °C at 14,000 rpm for 15 minutes. The clear supernatant was collected and stored at -20 °C for digestive enzyme activity analysis, proteases activity (Bezerra *et al*., 2005) and amylase activity (Hashini, Reshmi, & Sreekumar, 2003). The determination of protein content of enzyme extract was carried out by Lowry, Rosebrough, Farr, and Randall (1951).

2.5 Immune response analysis

At the end of four weeks, the hemolymph of fifty surviving shrimp per experimental treatment (five shrimp per replicate) was randomly collected to analyze total hemocyte count; use 400 µL of trypan blue solution, added hemolymph 100 µL, and count the hemocyte under the light microscope, hemolymph protein (Lowry *et al*., 1951), phenoloxidase enzyme activity (Encarnacion *et al*., 2012), superoxide dismutase enzyme activity, and total glutathione by Assay Kit of SIGMA-ALDRICH.

2.6 Hepatopancreas histopathology

At the end of four weeks, five random white shrimp per cage were collected to determine hepatopancreas health. White shrimp were similarly stunned, injected with Davidson's fixative, then surface sterilized with 70% ethanol before removing small portions of hepatopancreas tissue stained with hematoxylin and eosin (H&E) by standard methods for histological examination of tissue sections by light microscopy (LM).

2.7 Statistical analysis

Results were presented as means ± standard deviation. A completely randomized design (CRD) was assigned. The data in percentage was transformed to Arcsine as Given by C.I. Bliss before conducting the variance analysis. All data were tested for normality and were analyzed by one-way ANOVA (analysis of variance). Duncan's procedure was used for multiple comparisons on the differences between the treatment means. Differences were regarded as significant when $P<0.05$. The alphabetical notation was used to mark the differences at a significant level of alpha of 0.05.

3. Results and Discussion

3.1 Harvest and growth performance evaluation

No significant ($P>0.05$) differences were found for final body weight, total production, weight gain (WG), average daily gain (ADG), specific growth rate (SGR), feed conversion ratio (FCR), and protein efficiency ratio (PER) among the treatments for four weeks (Table 2).

The points of this research can be categorized into two issues: 1. Effect of reducing fishmeal and substituting it with other raw materials on shrimp growth at growing state (4 weeks), and 2. Effect of trace mineral supplementation in the zero-fishmeal diets to maintain the growth levels of shrimp to be normal. Numerous studies have been conducted to reduce the use of fishmeal in animal feed formulas and used soy products instead, such as soybean meal, soy protein concentrate (Chen, Li, Xu, Sun, & Leng, 2017; Ghorbani, Abolhasani, Ghorbani, & Matinfar, 2017; Jatobá *et al*., 2017; Ray *et al*., 2020; Yun *et al*., 2017), poultry meal, and poultry by-product meal (Chi, Tan, Mai, & Zheng, 2009; Cruz-Suarez *et al*., 2007), which have yielded satisfactory results with no negative effects on growth. Interestingly, it was found that when fishmeal was removed from the recipe to zero percent and replaced with alternative raw materials, the growth of white shrimp was normal and also did not differ from the cultured shrimp with high fishmeal diets. According to Elkin, Davis, and Rouse (2007), the shrimp fed by the diets contained zero percent fishmeal (replaced the fishmeal with soybean meal and corn gluten meal) showed no difference in the final weight, weight gain, FCR, and survival rate compared with the shrimp fed by 9% fishmeal diets. However, some studies have shown that when reducing fishmeal content, growth was significantly lower. Suárez *et al*. (2009) found weight gain and specific growth rate of white shrimp fed 0% fishmeal diets displayed the lowest values and significant differences from other treatments (FM6%, FM10%, FM15% diets). From this research, a zero fishmeal diet with 0.12% supplementation of trace minerals (T2) was enough for the shrimp to survive and grow as well as the control group ($P>0.05$). Related to the research, copper (Cu) supplemented with 10 mg/kg, 20 mg/kg (Lee & Shiau, 2002), 32 mg/kg (Davis, Lawrence, & Gatlin, 1993a), 52 mg/kg, and 83 mg/kg (Bharadwaj, Patnaik, Browdy, & Lawrence, 2014) had significantly greater growth performance ($P<0.05$). For zinc (Zn) supplementation, Davis, Lawrence, and Gatlin (1993b) informed that 33 mg/kg showed maximized zinc storage in the hepatopancreas of shrimp. Katya *et al*. (2016) found that by adding 0.5% of trace mineral (Cu, Mn, and Zn) premix in *L. vannamei* diets, the final body weight and weight gain were significantly improved ($P<0.05$). Also, it was found that the use of organic minerals was significantly better than that of inorganic minerals (Lin, Lin, Yang, Li, & Luo, 2013; Yuan, Jin, Xiong, & Zhou, 2019). Supplementing and

Table 2. Growth performance and feed utilization of white shrimp (*Litopenaeus vannamei*) fed vary trace minerals concentration feed for 4 weeks (mean ± SD)

Parameters	T1	T2	T3	*P*-value
FBW (g/shrimp)	6.00 ± 0.40	6.04 ± 0.38	5.77 ± 0.67	0.475
TP (g)	317.8 ± 23.9	320.5 ± 23.0	304.1 ± 40.4	0.475
WG (%)[1]	659.7 ± 57.2	662.8 ± 54.9	624.2 ± 96.2	0.482
ADG (g/shrimp/day)[2]	0.18 ± 0.01	0.18 ± 0.01	0.17 ± 0.02	0.488
SGR (%/day)[3]	7.15 ± 0.22	7.18 ± 0.21	7.01 ± 0.40	0.423
FCR[4]	0.95 ± 0.08	0.94 ± 0.08	1.02 ± 0.17	0.356
PER[5]	2.82 ± 0.25	2.89 ± 0.23	2.73 ± 0.43	0.583

Remark: Data without superscript letters in the same row indicates no significant difference ($P>0.05$). FBW = Final body weight, TP = Total production. [1]Percent weight gain = 100 × (final body weight − initial body weight) /initial body weight. [2]Average daily gain = final body weight − initial body weight / days. [3]Specific growth rate = 100 × (Ln final body weight − Ln initial body weight) / days. [4]Feed conversion ratio = feed consumed (g) / total production (g). [5]Protein efficiency ratio = total production (g) / protein intake (g)

balancing minerals to meet the needs of animals is essential. For example, adjusting the mineral concentration of low fishmeal diet (12% fishmeal inclusion) close to the high fishmeal diet (30% fishmeal inclusion) found that growth rates, survival rates, levels of copper, manganese, and zinc accumulation of the shrimp fed by both diets were not significantly different (Huang, Wang, Zhang, & Song, 2017). According to the FAO, the optimal content of minerals for shrimp is 80-120 mg/kg for zinc, 8-12 mg/kg for copper, and 0.17-0.25 mg/kg for selenium (Tacon, 1987).

3.2 Digestive enzyme activity

The second parameter that is important and consistent with animal growth is the performance of digestive enzymes. Proteinase enzyme activity of *L. vannamei* shrimp fed diets with fishmeal containing 15% and 0%. It supplemented with the trace minerals at the 0.12% and 0.18% of the four weeks showed no significant difference with the range of 0.813±0.06 to 0.894±0.03 unit/mg protein (Table 3). Amylase enzyme activity of *L. vannamei* shrimp fed the feed containing 15% and 0%, and supplemented with trace minerals at the 0.12% and 0.18% of the four weeks showed no significant difference with the values range of 2.128±0.09 to 2.412±0.37 units/mg protein (Table 3). Typically, the digestive enzyme activities showed a corresponding change response to the nutritional status (Moullac, Klein, Sellos, & Wormhoudt, 1997), especially; protein and carbohydrate, which can influence digestive enzyme activities (Gamboa-Delgado, Molina-poveda, & Cahu, 2003). The level of protein in the diet had a more significant effect on the enzyme total activities in the large shrimp (17-30 g) than in the small shrimp (<10 g). When the specific activities (activity per mg of protein in the extract) of the fed shrimp were evaluated, small shrimp (<10 g) fed the 1:l animal/plant ratio diets displayed lower activities than those fed the 2:l ratio diet. So, the protein level influenced the enzyme activities in shrimp of all sizes. In contrast, the protein source had a more significant effect on the enzyme activities in small shrimp (<10 g) (Lee, Smith, & Lawrence, 1984). Interestingly, a significant impact was also not found on the protease activity in the shrimp's digestive tract fed the fishmeal 40%, fishmeal 12%, and fishmeal 6% diets (Bulbul *et al*., 2016). Chen *et al*. (2017) reported that 15% fishmeal with 5% soy protein concentrate (SPC) in diet did not affect the protease and amylase enzyme activity of white shrimp *L. vannamei* (P>0.05) (Chen *et al*., 2017) as same as this research, the 8% soy protein concentrate plus zero percent fishmeal in the diets showed no effect to these enzymes' activity compared with the 15% fishmeal diet (P>0.05) at four weeks. Considered trace minerals function, there was improved (P<0.05) growth performance in terms of body weight gain, average daily gain, and specific growth rate of Pangasius catfish fed supplemental ZnAA at different levels than fish fed supplemental $ZnSO_4$. White blood cell count, serum protein, immunoglobulin (IgM), hemoglobin, and superoxide anion in fish fed supplemental ZnAA was better (P<0.05) than fish fed inorganic $ZnSO_4$ (Jintasataporn, Ward, & Kattakdad, 2014).

3.3 Immune response analysis

The most basic and important parameter of the immune system is total hemocyte count or THC. From this research, shrimp fed by fishmeal 15% diet and fishmeal 0% diet had similar amounts of total hemocytes cells ranging from $13.8x10^4 \pm 8.81$ to $15.3x10^4 \pm 5.51$ cell/ml (Table 4) and were not statistically different (P>0.05). Low fishmeal did not affect total hemocyte count (P>0.05) in kuruma shrimp (Bulbul *et al*., 2016) and supplemented with zinc 35.0 mg/kg and 48.0 mg/kg (Shiau & Jiang, 2006) or selenium 0.3 mg/kg (Sritunyalucksana, Intaraprasong, Sanguanrut, Filer, & Fegan, 2011) could increase the THC values in white shrimp (P<0.05). The second parameter, phenoloxidase enzyme (PO), is part of the shrimp's innate immune system, an enzyme responsible for protecting the cells from foreign substances that enter the body by the process call melanization. This research showed treatment fishmeal 15% diet (T1) and fishmeal 0% diet with trace mineral 0.12% (T2) showed ranged 68.2 ± 6.64 to 73.3 ± 9.62 units/min/mg protein (Table 4), meaning that even though fishmeal was removed out from the formula, the values exhibited no difference on the base of the supplementing minerals content in the diets. 17.2% soy protein concentrate as the main protein source in *L. vannamei* shrimp diets showed phenoloxidase activity no difference from the shrimp fed high fishmeal diets (21% fishmeal) (Schleder *et al*., 2018). Moreover, treatment 3 (T3: fishmeal

Table 3. Digestive enzyme activity of white shrimp (*Litopenaeus vannamei*) fed for 4 weeks (mean ± SD)

Parameters	T1	T2	T3	*P*-value
Amylase (Unit/mg protein)	2.128±0.09	2.412±0.37	2.347±0.04	0.325
Proteinase (Unit/mg protein)	0.813±0.06	0.869±0.09	0.894±0.03	0.358

Table 4. Immune response of white shrimp (*Litopenaeus vannamei*) fed for 4 weeks (mean ± SD)

Parameters	T1	T2	T3	*P*-value
THC ($x10^4$ cell/ml)	14.2±4.80	13.8±8.81	15.3±5.51	0.959
PO (unit/min/mg protein)	$68.2±6.64^a$	$73.3±9.62^a$	$109.0±4.24^b$	0.001
SOD (inhibition rate %)	90.33±0.06	68.89±18.90	59.34±27.11	0.207
Glutathione (nM)	0.55±0.09	0.31± 0.22	0.57± 0.03	0.113

Remark: Data without superscript letters in the same row indicates no significant difference (P>0.05). THC = Total haemocyte count, PO = phenoloxidase, SOD = Superoxide dismutase.

0% diet with trace mineral 0.18%) showed the highest values of 109.0 ± 4.24 units/min/mg protein (Table 4); maybe trace minerals could enhance the activity of the enzymes. The addition of 30 mg/kg zinc increased the activity of the phenoloxidase enzyme by 200% compared to the control group (Lin *et al*., 2013), and trace mineral-amino acid complex premix could improve phenoloxidase activity (Jintasataporn, Ward, Chumkam, & Jintasataporn, 2015). In addition to protecting the cells from pathogens, shrimp can also produce antioxidants to eliminate free radicals in the body, with two enzymes: superoxide dismutase (SOD) and glutathione. Superoxide dismutase (SOD) showed no significant differences (P>0.05) and had ranged from 59.34 ± 27.11 to 90.33 ± 0.06 % (Table 4). From the research of Xie, Liu, Zeng, Niu, and Tian (2016), no significant difference in SOD activity in white shrimp among the treatment that varies the fishmeal levels 5% to 25%. Yuan *et al*. (2020) reported that adding zinc 60 mg/kg in white shrimp diets could enhance Cu-Zn SOD activities. Lastly, a parameter is glutathione that needs selenium (Se) to be a cofactor (Rotruck *et al*., 1973). Selenium 1 mg/kg supplementation in *L. vannamei* diets showed no effect on glutathione activity (Wang, Wang, & Zhang, 2006) as same as the result of this experiment that had ranged from 0.31 ± 0.22 to 0.57 ± 0.03 nM (Table 4); this may be because the low-stress condition during culture shrimp in the trial condition causes the low free radical in the shrimp oxidative defensive system. On the other hand, the amount of selenium contained in the feed for the animal's needs is approximately 0.17-0.25 mg/kg (Tacon, 1987), therefore, 0.6 mg/kg selenium in experimental feed in normal conditions may be more than enough, but under the oxidative stress condition, shrimp requires more mineral. Moreover, zinc levels at 71.91 mg/kg, 83.14 mg/kg, and 112.32 mg/kg showed no difference (P>0.05) on glutathione activity in white shrimp (Musharraf & Khan, 2019) as same as the result of this experiment that showed the zinc level of the diets ranged from 120 to 180 mg/kg and glutathione exhibited no difference between the groups (P>0.05).

3.4 Hepatopancreas histopathology

The hepatopancreas is responsible for the synthesis and secretion of digestive enzymes, absorption of nutrients, storage of nutrients such as lipids, glycogen, minerals, and organic products mobilized and transported to muscle and other tissues according to growth and reproductive needs. Each hepatopancreas tubule has a lumen in the center comprised of four types of epithelial cells. Each plays a different role in hepatopancreatic function. E-cells (embryonic) found at the distal tips of each tubule with proximal nuclei and conspicuous nuclear bodies give rise to the other three cell types of the digestive gland. R-cells (resorptive), multi-vacuolated cells occur throughout the hepatopancreas and have absorptive, lipid, and glycogen storage functions. They also commonly sequester mineral deposits such as calcium, magnesium, phosphorus, sulfur, and others. B-cells are large (blister-like) primary secretor cells, are the primary producers of digestive enzymes in the hepatopancreas, and are responsible for nutrient accumulation, intracellular digestion, transport of digested material. F-cells (fibrillar) are responsible for protein synthesis and storage of minerals. The histology results of this experiment show hepatopancreas of treatment fishmeal 15% (T1) usually have shaped structures with high B-cell, R-cell, F-cell, and fat deposition in the cells (Figure 1, a). For treatment fishmeal 0% with TM 0.12% (T2), white shrimp had healthy hepatopancreas with abundant B-cell, F-cell, and regular shape (Figure 1, b). The fishmeal 0% with 0.18% (T3) showed all hepatopancreas cells are still in form with a typical structure of tubules and epithelial cells without damage or abnormal cell structures such as apoptosis or hemocyte leaching into hepatopancreas cells (Figure 1, c). Moreover, there was no infection of *Enterocytozoon hepatopenaei* (EHP) in the white shrimp body in all treatments.

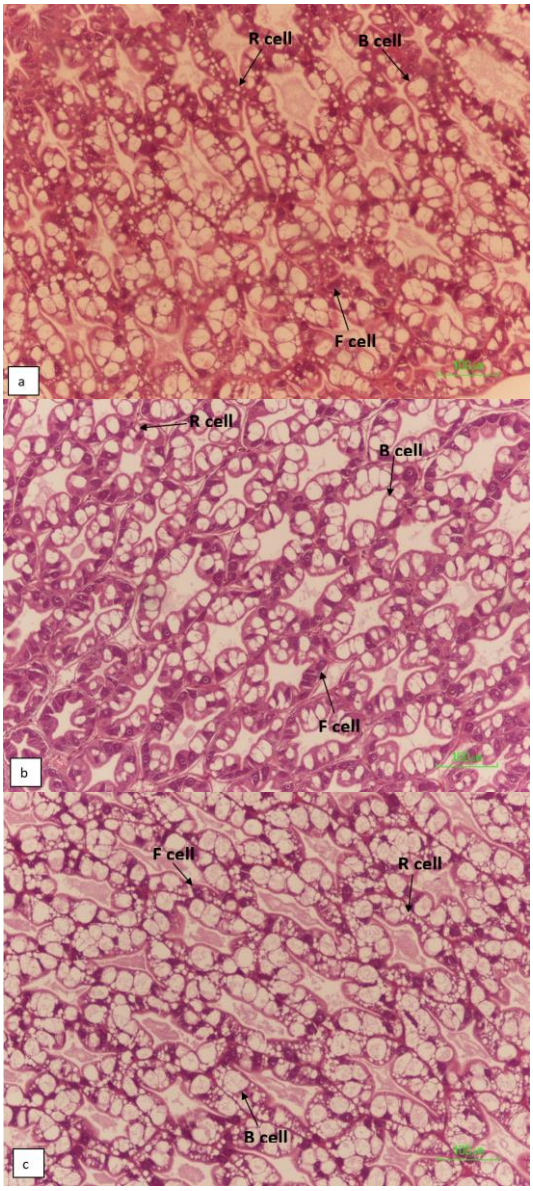

Figure 1. Histological section of hepatopancreas's white shrimp (*Litopenaeus vannamei*) age 4 weeks. By light microscope 10x magnification fed (a) 15% fishmeal diet, (b) 0% fishmeal with 0.12% trace minerals diet, (c) 0% fishmeal with 0.18% trace minerals diet showed abundant B-cells, R-cells, F-cells and no infected by EHP.

4. Conclusions

This research shows that non-fishmeal in white shrimp (*Litopenaeus vannamei*) recipes and substituted alternative protein feedstuffs such as poultry meal, squid meal, and soy products such as soybean meal and soy protein concentrate were practical. Growth performance and an immune response do not differ in all parameters (P>0.05). In contrast, it must be supplemented or balanced of minerals to meet the requirements or the same amount of high fishmeal formula to avoid adverse effects. It was clear that only 0.12% of the trace mineral supplementation was sufficient for the growth and immunity of white shrimp. There is no need to add up to 0.18% because the results are not different from other experiments. This experiment clearly showed that reducing the amount of fishmeal in white shrimp feed formulas can be done under nutrient balance conditions with added trace minerals.

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