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ARTICLE

Effect of dietary supplementation of minerals premix on growth performances and return in red tilapia (*Oreochromis nilloticus x Oreochromis mossambicus*)

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A B S T R A C T

Tilapia is a popular fish for consumers and aquaculture in every part of Thailand. This study was conducted to determine the level of mineral supplementation in the diet for the growth of red tilapia fingerlings, which gave the lower fish production cost. The fingerlings with an initial average weight of 3.1 ± 0.48 g and a length of 2.5±0.24 cm were raised in 3 m^3 concrete tank with 1.5 m3 of water volume at 20 fish/m2 stocking density. Fish were fed with pellets diet (28 % crude protein), which contained different levels of mineral supplements (0 (control), 20 and 40 g/Kg) in triplicate replications. The experiment was conducted within 80 days. The results showed that fish fed with diet3 had better average daily weight gain (ADG) and food conversion ratio (FCR) than diet2 and diet1 significantly (P<0.05). The ADG and FCR of fish fed with diet3 showed 0.70 ± 0.02, 1.72 ± 0.02 respectively, but there was a non-significant difference in survival rate from all treatments (P>0.05), and the fish production cost of diet 3 is lower than those in diet1 and diet2. Thus, this experiment concluded that minerals supplementation at the level 40 g/kg diet were suitable for tilapia aquaculture.

1. Introduction

Red tilapia is the fish that has been improved in terms of quantity and quality. Characteristics of red tilapia which differ from the others are the fish had a clear and white internal abdominal texture. The scales and skin are red, which are considered by the consumer because of the appetizing colors. The characteristic of red tilapia is matching the needs of both internal and external consumers. Also, red tilapia has a high potential for exportation, in the form of frozen and fillet. In the year 2013, the production of tilapia was 45.76% of freshwater aquaculture (Department of fisheries, 2013).

Moreover, during the year of 2016-2019, the Department of Fisheries of Thailand reported the quantity of tilapia aquaculture production was 176,463, 185,902, 191,019, and 211,368 tons respectively (Department of Fisheries 2016- 2019). It can be seen that production was increased every year. Moreover, the consumer prefers more quality fish in daily meals because fish are the protein sources for a healthy life in trendy. Therefore, red tilapia aquaculture has a high economic value in Thailand (Wanpho and Kamwongsa, 2000). The average market price of tilapia in the year 2019 at the size of 1-2 fish/kg was 41.66 baht/kg (Department of Fisheries, 2019), therefore; if the farmers can decrease the fish production cost, they will earn more profits and get the low risk to loss.

In the present, the freshwater fish industry is proliferating as the consumption rate increases. According to this event, many fish farmers expand their areas to increase their products continuously.

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At the same time, it is attractive to the new farmers to interest in studying for the information and preparing to invest more in the aquaculture business. This occupation can make a good income for the farmers. Therefore many people are interested more in freshwater for their vocation. Thus, aquaculture should be developed in order to increase production to consumers in an adequate quantity. As mentioned, the objective is to produce higher products, while the production cost is low and get more business returns. In addition, if the farmer uses high-quality food, which adding the optimal ratio of minerals mixture to the diet, these supplements are still essential for the growth of aquatic life. While, some farmer produces pellet food by themselves for use in farms to reduce production costs, by using the cheap raw materials which easy to find in the local area.

Nomenclature and abbreviation

ADG	Average Daily Weight Gain
FCR	Food Conversion Ratio
CRD	Completely Randomized Design
DO	Dissolved Oxygen
ANOVA	**A**nalysis of Variance
DMRT	Duncan's New Multiple Range Test

In this way, the requirement of minerals addition in the appropriate ratio is necessary. The ratio of minerals supplement-ation is generally around 1-4 %; the research of Hangsapreurke (1992) added 4% for *Lates calcarifer* culture, while Chuapoehuk (1999) reported 1 % for *Channa striata, Oxyleotris mamorata*, and tilapia culture. Because minerals are inorganic substances that life needs to maintain healthy growth, living and metabolism, these minerals were divided into two types as follows: main minerals (macromineral) and secondary minerals (micromineral). Fish generally require minerals for different purposes, such as body composition, maintain of acids and alkalis balance, collaborate and stimulate the respiratory system and stimulate the work of enzymes and hormones (Watanabe, 1988; Wootpanchai, 1993; Chuapoehuk, 1999). However, farmers sometimes tend to neglect to add minerals in fish food.

There were many types of research that report in the same direction; those reports indicated that fish fed with diets without vitamin and mineral supplements or premix showed lower growth and food efficiency (Chimsung and Tantikitti, 2014) as we have known that minerals required by fish are calcium, magnesium, phosphorus and several trace elements such as copper, iodine, iron, manganese, selenium and zinc, the requirement are depended on species. In general, fish do not tend to lack minerals; if the fish lack minerals, the deficiency syndrome will appear as symptoms of poor growth, abnormal skeleton, low feed efficiency and higher in mortality (Chuapoehuk, 1999; Watanabe, 1988). However, there is a few of research which has been done to study the necessity of using mineral supplements in red tilapia, such as Plaipetch (2016) who reported the requirement for minerals which had been studied were phosphorus less than 9000 mg/kg diet, magnesium 590-770 mg/kg diet, zinc 12 mg/kg diet and manganese 12 mg/kg diet. According to this reason, the farmers should mix the minerals mixture into the diets which they produce. This experiment hoped to be a guideline for increasing fish production, maintained fish living as usual, and promoted red tilapia farming also. Therefore, this study focused on the appropriate level or amount of minerals that can be added into the diet for red tilapia, which should be able to enhance the growth of red tilapia that gave higher production with lower cost and to be a guideline for farmers can continue to produce pellet food for their use in the farm.

2. Materials and methods

2.1. Sample Preparation

In the experiment, the 700 fish red tilapia larvae were prepared at an average weight of 3.1 ± 0.48 g. and an average length of 2.5 ± 0.24 cm. in the pond with a capacity of 3 m3 for 2 days acclimatization. After that, fish were randomly taken into experimental ponds with a capacity of 3 m3, each at a water level of 1.5 m. at the density of 30 fish/pond. During the process of environmental acclimatization, fish were fed with food that did not contain minerals

2.2. Experimental Design

This research was a completely randomized design (CRD). The factors that need to be studied are the levels of total mineral mixture supplementation in the diet. There were 3 treatments such as: 0% of mineral premix supplementation as control (diet1), 20 g/kg (diet2). and 40 g/kg (diet3). Each treatment had three replications.

2.3. Experimental Diets Preparation

Prepare 28% protein diet which contained raw materials such as fish meal, soybean meal, fine bran, broken rice, soybean oil, vitamin mix and mineral mix (Table1) with the proximate analysis of the experimental diet nutritional value not less than 28% protein, not less than 4% fat, not more than 12% moisture, not more than 8% residue (Table 2). Then add the mixed minerals to the diet and mix at the ratio of 20 g and 40 g/kg of food. The composition of the minerals and vitamins mix are detailed below table 1.

Table 1
Composition of experimental diets (g/1000g diet).

Ingredients	diet 1	diet 2	diet 3
Fish meal	250	250	250
Soybean meal	250	250	250
Fine rice bran	250	250	250
Broken rice	180	160	140
Soybean oil	30	30	30
Cod liver oil	30	30	30
Minerals mix[1]	0	20	40
Vitamins mix[2]	10	10	10

[1]Mineral mix (g/diet 1 kg) : NaCl 1.73; $MgSO_4.7H_2O$ 5.45;

$NaH_2PO_4.2H_2O$ 3.47; KH_2PO_4 9.54; $Ca(H_2PO_4)_2.H_2O$ 5.4; Fe-citrate 1.18; Ca-lactate 13.01; KI 5.97(mg); AlCl 5.97(mg); CuCl 3.98(mg) $MnSO_4$ 31.84(mg); $CoCl_2$ 39.79(mg); $ZnSO_4$ 119.40(mg). [2]Vitamin mix (g/kg) : Thiamine 1.2500: Riboflavin 5.000; Pyridoxine 1.250; Nicotinic acid 18.7500; Inositol 50.0000; Biotin 0.1250; Folic acid 0.3750 Cobalamin 0.0025;Menadione 1.0000; Tocopheral acetate 10.0000; Vitamin AD3 0.500; BHT 0.2500; Dextrose monohydrate 196 Choline chloride 125.000; Ascorbic acid 500 (Hangsapreurke, 1992).

2.4. Experimental Set-up

After acclimatization, the experiment began by feeding diet that was prepared in the different levels of minerals mixture as diet1, diet2 and diet3. The fish were fed twice a day around 08.00 a.m. and 04.00 p.m., sediment suction daily at 06.00 p.m.and 50% water exchange every three days. Furthermore, water quality parameters such as water temperature, pH, dissolved oxygen had been analyzed every three days. Ammonia and nitrite were tested every 5 days by a field test kit. Growth monitoring, the fish were randomly from each replication in the amount of 7 fish for weighing individually and weighed for the total weight of the fish in each replication every 10 days. The survival rate was monitored by counting the remaining fish at the end of the experiment (80 days).

Table 2
Proximate analysis (dry weight basis) of the experimental diet

Diet	Protein[1] (%)	Lipid[1] (%)	Moisture[1] (%)	Ash[1] (%)	Fiber[1] (%)
1	28.47±0.15[a]	6.93±0.03[a]	8.10±0.11[a]	10.24±0.08[a]	6.67±0.17[a]
2	28.50±0.25[a]	6.87±0.05[a]	7.98±0.28[a]	10.25±0.12[a]	6.18±0.02[a]
3	28.44±0.20[a]	6.95±0.07[a]	8.09±0.16[a]	10.28±0.98[a]	6.11±0.11[a]

Data represented as Mean±S.E. Value in the same column with different superscripts are significantly different (p<0.05)

2.5. Calculation and analysis of statistical data

The experiment was the termination period the data of fish weight, a number of fish remaining and food quantities that fish fed were computed for the average weight, weight gain, average daily growth (ADG), feed conversion ratio (FCR) and survival rate. All data were computed for statistical significance by analysis of variance (ANOVA) to compare the difference of the mean between the experiment groups by Duncan's New Multiple Range Test (DMRT) at the 95 percent confidence level.

3. Results and discussion

3.1. Growth performances and Survival rates

From the experiment, the fish fed with two different levels of mineral supplements, 20 and 40 g/kg diet, from the initial size of weight 3.1+ 0.48 g weight during 80 days. The result showed that the initial weight of red tilapia larvae in all experiments was the non-statistical difference (p> 0.05). At the end of the experiment, the fish fed with diet3 showed better in the final weight, ADG and FCR than others significantly (p<0.05). When the experiment was terminated, the survival rate of the red tilapia larvae was quite high all, and the highest in the group which received 40 g/kg diet but no significant difference (p> 0.05) between the treatments (Table 3).

Table 3
Growth performances, survival rate and feed utilization of red tilapia fingerlings culture with 3 diets which contained the different levels of minerals supplementation for 80 days

Growth performances parameter	Control	Mineral supplement 20g/kg	Mineral supplement 40g/k
Initial weight (g)	3.12±0.21[a]	3.11±0.24[a]	3.10±0.20[a]
Final weight(g)	53.52±1.19 [a]	51.91±1.17 [a]	59.10±1.20 [b]
ADG (g/fish/day)	0.63±0.03 [a]	0.61±0.03 [a]	0.70.02 [b]
Survival rate (%)	91.7±1.9 [a]	91.7±5.8 [a]	93.3±2.7 [a]
FCR	2.07±0.02 [a]	2.09±0.04 [a]	1.72±0.02 [b]

Data represented as Mean±S.E. Value in the same row with different superscripts are significantly different (p<0.05).

The results of this experiment showed that minerals supplementation in pellet food for red tilapia larvae was essential for growth. The red tilapia larvae that received different levels of mineral supplements showed mean that the average final weight and the ADG higher than the fish that did not receive mineral supplements. By supplementing the total minerals in the diet pill at the level of 40 g/kg diet) with calcium 3.19 g/kg mixed in diet and magnesium 0.54 g / kg diet) showed the best growth rate, including FCR than control fish. The concentration of 3.19 g / kg of calcium of diet3 conformed to the research of Chimsung and Tantikitti ((2014, which experimented on transgenic red tilapia, raising the initial weight of 0.34 ± 6.55g in net cages fed with 2 formula diets which consist of the same protein and fat levels. Their experimental, they fed the fish with 2 diets, diet formula1 which adding vitamins and minerals premix and diet food formula ,2 which non-addition with vitamins and minerals premix during 9 weeks; they found that fish fed with formula 2 which no additive of vitamins and minerals premix showed lower growth and feed efficiency. Likewise, Shim and Ho ((1988 reported that Nile tilapia (*O. niloticus*) generally requires calcium supplements in the diet of 2.7g / kg of food. While there was a report on calcium requirement in tilapia (*O. niloticus*) showed good growth and FCR and did not show deficiency symptoms when receiving calcium at the levels of %0.17and %0.65in the diet (Robinson et al., (1984.

This report showed that increasing calcium intake leads to growth and the FCR increased. Nevertheless, there are still quite different needs for magnesium, and there was the experiment in guppy shown that the optimal requirement of magnesium (*Peocilia reticulata*) was 0.54 for the healthy growth of guppy. In addition, there was some report about magnesium supplementation in tilapia (*O. niloticus*), which found that %0.56and %0.62magnesium supplementation resulted in higher growth (Shim and Ng, 1988; Dabrowska et al. (1989. Therefore, It showed that the higher intake of calcium and magnesium causes better growth and FCR. As we already understood that calcium and magnesium play a role in bone formation and the body's metabolism stimulates the work of many enzymes. Thus, a calcium and magnesium supplementation diet may help the farmer to increase the food efficiency.

This experiment found that the use of phosphorus mixed in the diet at the level of 3.60 g/kg diet (mineral supplement at the level of 40g/kg diet (shown the better growth rate and FCR than fish which the concentration of phosphorus at 18 g/kg (20 g/kg supplementation). When compared to the relevant research, it found that fish generally require the amount of phosphorus supplemented in the diet 0.7-0.6 %, and in chum salmon, the fish can be better-utilized diet when supplementing phosphorus with the food between 0.7-0.6g/kg. In addition, the report of Rowena et al. ((1996found that in the tilapia (*O. niloticus*) supplemented with phosphorus was the vital factor to increase weight and food conversion ratio. It was the same as the report of Hepher et al. ((1984, who found that phosphorus supplementation in tilapia fish (*Sarotherodon aureus*) at /%1kg resulting in the growth rate and weight gain, while compared to the experiment without mineral supplement, so it can be seen that increasing levels of phosphorus were necessary for growth. According to the research report, the demand for phosphorus in the diet of tilapia fish was not constant from) %0.9Watanabe et al., (1980,) %0.6-0.45Viola and Arieli, (1983,) %0.5-0.3Robinson et al., (1984,) %0.46Haylor et al., .(1988It is understood that phosphorus plays a role in important metabolic processes and for the body and is a component of the enzyme that necessary in the body (Wootpanchai, 1993). It is possible that when the tilapia fish received phosphorus at the level of 3.6 g/kg in diet3 show a better growth rate of the tilapia.

From the results, it was found that sodium and potassium supplementation Into diet3 that have a concentration of 1.19 and 4.26 g / kg food (Mineral supplement at the level of 40 g/kg) resulted in the red tilapia raised with specific growth rates, FCR better than fish in control, due to sodium and potassium is considered the most electrolyte or buffer substance in the body. The related functions are helping to control the osmosis system of the body to be stable and maintain the balance of acids and alkalis, liquid within the body. Currently, the study of sodium and potassium requirement in fish is still minimal, as these minerals are abundant in water because fish can easily use without mineral deficiency symptoms. However, even though fish and sodium requirement is not known. The study in Nile tilapia by Rowena et al. ((1996 reported that sodium and potassium did not affect growth and FCR of the tilapia because fish can absorb enough from water as their needs.

In practice, fish nutritionists prepare a diet for aquaculture, and it is popular to mix these minerals into the diet to ensure that the fish will grow naturally. It is not known what the exact requirement is, but some people have reported that most fish require sodium and potassium from foods in the range of %0.3-0.1 and %0.5-0.1, respectively (Wootpanchai, .(1993 From the experimental results, the use of manganese that mixed in the diet at a concentration of 11.6 mg/kg diet (40 g/kg mineral supplement) showed the best growth, including FCR were higher than the control. Furthermore, it was found that common carp and rainbow trout will normally grow if they get manganese from food 13-12 mg/kg food, while channel catfish need 2.4mg/kg food (Watanabe, 1988). If they get lower than the requirement, their growth rate will be decreased and their caudal fin and spine will abnormally develop (Wootpanchai, 1993).

It was understood that manganese is very important for the metabolism of fats and carbohydrates because it stimulates many enzymes and also plays a role in bone formation and the function of the nervous system. Also, the reports have been found that minerals are elements of coenzyme-related body growth and fish require in small amounts of minerals but must be sufficient; otherwise, fish will show abnormal symptoms such as lack of Ca, P, Mg, and poor growth. The food efficiency will also decrease and low ash in bone and scales (Chuapoehuk,1999). Therefore, it is possible that manganese at 11.6 mg/kg (diet3) diet showed the result of higher specific growth rates and food efficiency than the control.

3.2. Feed and Production Cost

From the cost of food calculation, the cost of raw materials for aquatic animal feed that is used as ingredients in all diet formulas, the experiment found that diet1, which was a non-mineral premix supplement, had a lower cost than those in diet2 and diet3, which supplement 20 and 40 g/kg diet respectively. However, when we calculated feeding cost and food intake per weight gain for fish production, the cost showed that the fish fed diet 3 had the lowest fish production cost by 39.10 baht/kg than those in diet1 and diet 2 (Table 4).

Although vitamins and minerals premix is expensive food ingredients, for reducing food costs by vitamin and mineral premix supplement in fish food, the experiment indicates that when we considered the cost of production, the mineral supplementation in the diet had produced a cost of 1 kg of fish production reduced to 39.10 Thai Baht. Likewise, the Chimsung and Tantikitti (2014) experiment calculated the prices of both formulas based on the price of food ingredients, and they found that formulas with added vitamins and minerals premix cost 17.00 Thai Baht/kg compare to non-added formulas cost 14.80 Thai Bath/kg.

Table 4
Feed cost and feed production cost[1].

Diet	Diet feed cost[2](Baht/kg)	Fish production cost[3] (Baht/kg)
1	22.50	46.58±0.67[a]
2	22.75	47.55±0.22[a]
3	23.00	39.10±0.18[b]

Note: Data represented as Mean±S.E. Value in the same column with different superscripts are significantly different (p<0.05). The sum of feed ingredient cost as per kilogram of diet ,calculation of total feed intake (kg) x feed cost (Baht/kg)/total fish production (kg).

However, when calculating the cost of fish production considering the production cost, it was found that both formulas were not different. Although in this experiment, cost of the diet1 and diet2 were cheaper than diet3, the use of diet1 and diet2 could not reduce the cost of fish production because the fish fed with diet2 had a lower growth rate than the fish fed with the diet3 significantly (p <0.05) and diet3 shown the better FCR. It is generally known that food is the highest cost for aquaculture (Chuapoehuk,1999; Watanabe, 1988; Lovell, 1989). Therefore the quality of food will lead the farmers to success in their productions. According to the statistical data from the Department of Fisheries of Thailand which reported for the price of tilapia for sale at their farm was 39.82 Baht/kg at the size of 3-4 fish/kg (Department of Fisheries, 2019) if the fish production cost can be reduced by good quality food or minerals supplementation, it should be done. In this experiment, the fish production cost of diet3 was 39.10 Baht that lower than the others and lower than the market price. Therefore, it can be concluded that It is necessary to add vitamins and minerals in premixes that are produced for fish farming.

3.3. Water Quality

Water quality during the experiment, all parameters in each experiment set were not significantly different (P> 0.05). From the monitoring showed, the pH was 7.8 - 8.5, the dissolved oxygen (DO) in water was 5.5 - 7.0 ppm, the water temperature was in the range of 27.8^{o}-31.0^{o} C, the ammonia and nitrite values less than 0.02 mg/L. All parameters were not hazardous for the fish in this experiment (Boyd,1990).

4. Conclusions

Supplementation of the total minerals in the pellet food, which were used to feed red tilapia, is essential. The food with the optimal ratio of minerals mix caused better growth, FCR and survival rate of red tilapia larvae, while the fish production cost was reduced. Although multivitamins and minerals are expensive food ingredients, the reduction of the cost of fish feed by not supplement total minerals does not reduce the cost of fish production in any way. Also, it negatively affects the growth and food efficiency of fish. However, to conclude that mineral in different levels in the form of combined minerals is just indicated that it is better to supplement minerals at 40g/kg diet. It may be the result of any type of minerals or combined. Therefore, in order to understand the role of each mineral, it should be a further study that examines each type of mineral and what concentration is suitable for this fish.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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