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DIETARY ORGANIC MINERAL INFLUENCES THE GROWTH, FEED UTILIZATION, AND VERTEBRAL MINERAL CONTENT OF WILD GOLDEN RABBITFISH, *Siganus guttatus*

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ABSTRACT

The positive effect of organic mineral as a dietary mineral source in aquafeed has been recently reported on several species. Nevertheless, the influence of organic minerals on rabbitfish has not yet been determined. The purpose of the present study was to evaluate the effects of different doses of organic mineral on growth and survival, and mineral content in vertebrae of golden rabbitfish, *Siganus guttatus*. Three diets were formulated containing 0.5%, 1.0%, and 2.0% organic material (OM). A control diet (OM0) did not contain OM. Instead, it was supplemented with an inorganic mineral mixture at a level of 1%. Three hundred fish were randomly selected and distributed in 12 cages to accommodate the four treatments with triplicates. The stocking density was 20 fish per cage. The initial body weight of fish used was 39.2 ± 0.3 g. Fish were fed the test diets twice a day for 150 days. A significant ($P < 0.05$) cubic effect of the treatments was detected on all dependent parameters analysis, excluding feed intake. The influence of dietary OM was not significant for feed intake, indicating that dietary OM did not negatively affect the appetite of rabbitfish. Mineral (Ca, Mg, Zn) content in the vertebrae was significantly improved when dietary OM was included in the diet up to 1% but decreased at the highest inclusion level of 2%. The optimum level of dietary OM to gain the maximum growth rate of rabbitfish was 0.49% as the reflection of the breakpoint of two regressions fitted on specific growth rate (SGR). It is concluded that dietary OM level significantly affected the growth and vertebral mineral content of golden rabbitfish. The study increases our knowledge of the benefit of utilizing dietary OM in the fish diet.

KEYWORDS: organic mineral; growth; vertebral mineral content; rabbitfish

INTRODUCTION

Minerals have several essential functions in fish and other aquatic animals. Calcium and phosphorus are required to form the fish body's skeletal structures (Laining *et al.*, 2012). Sodium, potassium, and chloride, along with phosphates and bicarbonates, maintain homeostasis, and the acid-base balance (Halver, 2002). Certain minerals, such as calcium, magnesium, and manganese, are of particular significance as components of hormones, enzymes, and activators of enzymes, as well as participating in a wide variety of biochemical processes (NRC, 2011). In addition, trace minerals including Cu, Zn, and Mn are also the vital trace elements required for the normal growth, metabolism, and health of aquaculture spe-

cies (Bharadwaj *et al.*, 2014; Muralisankar *et al.*, 2014; Katya *et al.*, 2016).

Although almost all necessary minerals are available in most practical fish diets and fish can absorb minerals from the surrounding water, mineral deficiency still occur in farmed fish. Reduced bioavailability of the mineral is more likely the reason for the deficiency, which may be associated with a dietary imbalance (Lall & Lewis-McCrea, 2007) or with percentages of the element in an unsuitable form (inorganic or organic), or interaction with other dietary ingredients, such as vitamins, phytate and ùbers (Laining *et al.*, 2010).

Generally, fish fulfill their mineral requirement from their diet. Traditionally, supplementation of elements in fish diets is done through the use of inorganic salts, such as sulfate and carbonate. These inorganic mineral premixes are usually commercial products often imported and can be an expensive component of compounded diets in Indonesia

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(Laining & Kristanto, 2015). As an alternative mineral source, organic minerals have been recently applied in feeds (Fodge *et al.*, 2011; Zhao *et al.*, 2014; Laining *et al.*, 2015; Güz *et al.*, 2019), including those used for fish and crustaceans. Our previous study (Laining *et al.*, 2015) demonstrated that dietary organic mineral (OM) significantly increased the weight gain of Pacific white-legged shrimp (*Litopenaeus vannamei*) from 76% (control diet) to 128%. In order to clarify whether OM is also suitable as a mineral source for fish diets, a dose-response feeding trial was carried out with juvenile golden rabbitfish (*Siganus guttatus*). The objective was to evaluate the effects of OM-supplemented diets on the growth, feed utilization, and vertebral mineral content of golden rabbitfish.

MATERIALS AND METHODS

Mineral Content of Organic Mineral and Experimental Diets

Organic mineral (OM) used in this experiment was a local commercial product processed from the lava of the Krakatau volcano combined with natural mines. The OM has a fine powder texture, and according to the product specification, it dominantly contains supra bio-molecular with 21 amino acids and 17 fractions of carbohydrates. In addition, the OM product contains five macro-minerals, including Ca, Mg, Ba, K, and Na; and 67 elements of micro-and trace minerals. Our previous study (Laining *et al.*, 2015) clarified that the OM contained levels of macro-minerals as indicated by Table 1. It also contains several amino acids shown in Table 2, indicating that the OM contains organic material (Laining *et al.*, 2015).

Four experimental diets were formulated to contain different levels of the OM: 0% (OM0), 0.5% (OM0.5), 1% (OM1), and 2% (OM2). Diet OM0 was supplemented with 1% inorganic mineral instead of OM and used as the control diet. The composition of

the test diets is shown in Table 3. All diets were formulated to be iso-nitrogenous, containing 33% crude protein using plant ingredients as the major protein sources, including soybean meal, soy cake meal, and copra cake meal. Fish oil combined with palm oil was supplemented to meet the required lipid level at approximately 10% (Ghanawi *et al.*, 2011). Table 4 lists the proximate composition of the test diets.

Diets were processed by thoroughly mixing all ingredients and oil sources then mixed again until all ingredients were homogenous. Water was further added at 350-400 g/kg of the dry ingredients (Laining *et al.*, 2012). The mixture was then passed through a pelletizer (Hiraga, Co. Ltd, Kobe, Japan) using a 3.1 mm die and dried in an oven until moisture content was below 12%. Dried pellets were then cut to the desired size of 3-5 mm length. All test diets were stored in air-tight polyethylene bags in a freezer (-20°C) until used.

Feeding Trial

The experiment was designed as a completely randomized design. The four test diets were fed to the three replicates groups of wild-caught *S. guttatus* with an initial body weight of 39.2 ± 0.3 g. Rabbitfish were randomly stocked into 12 of 1 m x 1 m x 1.5 m cages with a density of 20 fishes per cage. Treatment groups were fed their respective diets twice a day in the morning at 08:00 and afternoon at 16:00, to satiation at each feeding. Growth and feed consumption were monitored every month by individually weighing the fish in each group. The feeding trial was terminated after 150 days.

Sample Collection and Chemical Analyses

At the end of the feeding trial, five fish from each cage were sampled for biochemical analysis. All fish samples were filleted, dried and kept at -20°C until proximate analysis. From the same fish, vertebrae

Table 1. Mineral composition of commercial mineral premix and the organic mineral (OM) used for this feeding trial. Data from Laining *et al.* (2015)

Mineral	Commercial mineral mix (mg/kg)	OM (mg/kg)
Phosphorus (P)	400	91.97
Calcium (Ca)	30,000	667.78
Magnesium (Mg)	8,600	674.70
Aluminium (Al)	700	4,958.14
Potassium (K)	300	7,863.8
Manganese (Mn)	650	104.36
Sodium (Na)	190	1,245.24
Nickel (Ni)	90	< 0.05
Zink (Zn)	830	81.06

Table 2. Crude protein and amino acid contents of the organic mineral used in the feeding trial

Nutrient	mg/kg
Crude protein	8,900 ± 49.97
Amino acids	
Aspartic acid	54.30 ± 0.14
Glutamic acid	69.62 ± 1.15
Serine	92.16 ± 0.25
Histidine	23.08 ± 0.74
Glycine	76.42 ± 1.33
Threonine	27.28 ± 0.77
Arginine	26.55 ± 0.25
Alanine	51.91 ± 0.16
Tyrosine	24.52 ± 0.12
Methionine	19.11 ± 0.30
Valine	43.48 ± 0.21
Phenylalanine	95.11 ± 0.05
Isoleucine	125.44 ± 0.25
Leucine	280.14 ± 0.42
Lysine	306.75 ± 0.08

Source: Laining *et al.* (2015)

Table 3. Formulation of experimental diets (g/100g diet)

Ingredients	Diet			
	OM0	OM0.5	OM1	OM2
Soybean meal	28	28	28	28
Casein	12	12	12	12
Tofu by-product (soy cake)	18	18	18	18
Copra cake meal	18	18	18	18
Tapioca	4.7	5.2	4.7	3.7
Sorghum	14.5	14.5	14.5	14.5
Fish oil	0.5	0.5	0.5	0.5
Palm oil	1	1	1	1
Vitamin premix	2	2	2	2
Stay-C	0.1	0.1	0.1	0.1
Green-SP	0.2	0.2	0.2	0.2
Mineral mixture	1	0	0	0
Organic mineral	0	0.5	1	2
Total	100.0	100.0	100.0	100.0

Table 4. Proximate composition of experimental diets (g/100 g diet, dry basis)

Nutrients	OM dosages			
	OM0	OM0.5	OM1	OM2
Crude protein	33.3	33.3	32.1	31.7
Crude lipid	10.2	10.7	9.9	10.0
Ash	5.4	6.1	6.3	6.7
Fibre	7.4	6.9	8.2	7.8

were removed for mineral content analysis. The vertebrae were heated for three min using a microwave oven, cleaned of connective tissues and finally washed with deionized water (Laining *et al.*, 2011). The vertebrae were defatted by chloroform-methanol (2:1) extraction, according to Folch *et al.* (1957). Defatted vertebrae were dried and pulverized with a mortar and pestle and stored at -20°C until analysis.

Proximate analysis of feed ingredients, test diets, and fish muscle tissue were analyzed according to AOAC (2007). Crude protein was determined according to the micro-Kjeldahl procedure. Lipid was extracted by using the Soxtherm apparatus with petroleum benzene. Ash was analyzed using a muffle furnace set at 550°C (Barnstead, Thermolyne, CA, USA). The mineral content of the vertebrae was determined using an atomic absorption spectrophotometer (ASS, Shimadzu, Tokyo, Japan).

Calculations and Statistical Analyses

Biological parameters calculated were weight gain (WG), specific growth rate (SGR), and survival rate (SR). Feed utilization was indicated by calculating feed given (FG) and feed conversion ratio (FCR). All data collected were statistically analyzed using software SPSS version 25. A nonlinear regression method was applied for biological performances to explain the relationship between the treatments and the observed parameters. The appropriate regression model was selected based on the significant model and r-squared value (Bhujel, 2008; Yossa & Verdegem, 2015). The estimated optimal inclusion level of OM for maximum growth rate was determined by a significant polynomial regression followed by a broken-line regression procedure following the methods of Yossa & Verdegem (2015) and Laining *et al.* (2017). Biochemical responses among the treatment groups were analyzed by one-way ANOVA with multiple comparisons evaluated using Tukey's test to compare the treatment effects.

RESULTS AND DISCUSSIONS

Growth, Survival Rate, and Feed Utilization

Weight gain (WG), specific growth rate (SGR), and survival rate of fish after 150 days are presented in Table 5. These three variables observed had a higher r-squared for cubic regression than quadratic regression (Table 5) indicates that the cubic regression is the appropriate model for the statistical analysis. Both WG and SGR were significantly ($P < 0.05$) affected by dietary OM where the highest was observed in fish fed OM0.5 diet (Table 5). The results for survival could not be analyzed because three fish from OM0 group jumped out of cages during the sampling.

The cubic regression for the inclusion level of OM on SGR was significant (Figure 1). Therefore, the breakpoint of two regressions fitted to SGR was applied to estimate the optimum level of dietary OM to gain the maximum growth of rabbitfish. For this model, the calculated optimal level of the OM for rabbitfish was 0.49% (Figure 2).

The low dose of dietary OM required by rabbitfish demonstrated by the present study was similar to the required dose of tilapia reported by Fodge *et al.* (2011). Tilapia with a size of 20 g grew significantly larger than the control on a diet supplemented with dietary OM at inclusion rates between 0.25 to 0.75. In the case of *L. vannamei*, an OM dose of 1% produced significantly higher SGR (1.1%/d) than shrimp fed the control diet containing inorganic mineral (SGR 0.8%/d). Furthermore, increasing inclusion levels of OM from 1%-4% did not improve shrimp growth but negatively affect the survival rate (Laining *et al.*, 2015).

The optimal level of dietary OM found in the present study indicated that the dietary OM is generally required at low levels in rabbitfish. Rabbitfish cannot accept the dietary OM at higher inclusion levels probably because certain elements in the mineral have an adverse effect on the taste of the diets. Elements such as calcium are related to bitterness (Tordoff & Sandell, 2009) and have shown a negative effect on the growth and feed intake of tiger puffer (*Takifugu rubripes*) when fed at a high inclusion level of 24g/kg diet (Laining *et al.*, 2011).

The amount of feed given did not significantly differ among dietary OM treatments indicating that the OM used in the diets did not negatively affect the appetite of fish (Table 5). It is argued that a more extended feeding trial will possibly show more clearly the effect of a higher inclusion level of the dietary OM on feed intake. The FCRs were significantly ($P < 0.05$) different among groups where the OM0 diet (control) had the smallest FCR of 2.2 and further increased with the increased level of the OM in the diets.

Vertebral Mineral Content

Content of three selected minerals in fish vertebrae demonstrated significant ($P < 0.05$) differences between treatments (Table 6). Vertebral Ca and Mg content increased with increasing dietary OM in the diet up to 1%. In comparison, vertebral Zn content significantly increased when the diet contained 0.5% OM, but further decreased with increasing OM levels (Table 6).

An increase in vertebral content of the minerals Ca, Mg, and Zn at dietary inclusion levels of 0.5%-1% demonstrated that the juvenile rabbitfish were able

Table 5. Growth performance, survival rate, and feed utilization of rabbitfish fed dietary OM at different levels and the cubic regression (polynomial contrast) of each parameter (n=3)

Variable	OM0	OM0.5	OM1	OM2	Cubic regression equation*
Initial weight (g)	38.7 ± 0.5	39.3 ± 0.8	39.4 ± 0.9	39.8 ± 0.9	$y = -0.45x^3 + 1.19x^2 + 0.01x + 38.69$ ($R^2 = 0.374$)
Final weight (g)	172.1 ± 3.7	176.6 ± 16.6	153.0 ± 11.9	147.9 ± 27.6	$y = 48.88x^3 - 143.08x^2 + 81.94x + 165.25$ ($R^2 = 0.745$)
Weight gain (%)	344.6 ± 4.4	353.6 ± 28.3	288.1 ± 31.2	271.0 ± 10.1	$y = 77.7x^3 - 200.5x^2 + 40.4x + 370.5$ ($R^2 = 0.722$)
SGR ¹ (%/day)	0.99 ± 0.01	1.01 ± 0.08	0.90 ± 0.05	0.87 ± 0.12	$y = 0.18x^3 - 0.50x^2 + 0.23x + 0.99$ ($R^2 = 0.800$)
Survival rate (%)	90.0 ± 5.0	98.3 ± 2.9	100 ± 0.0	98.3 ± 2.9	$y = 5.0x^3 - 20.8x^2 - 25.8x + 90$ ($R^2 = 0.688$)
Feed given (g/fish)	282.5 ± 39.0	324.8 ± 25.5	308.5 ± 26.6	292.1 ± 18.2	$y = 51.3x^3 - 168.7x^2 - 130x - 295.2$ ($R^2 = 0.287$)
FCR ² (g/g)	2.2 ± 0.3	2.4 ± 0.2	2.7 ± 0.2	2.7 ± 0.2	$y = -0.18x^3 + 0.2x^2 + 0.611x + 2.1$ ($R^2 = 0.675$)

Note: * All values are significant (P<0.05) except for initial weight and feed given:

- ¹ SGR = specific growth rate
- ² FCR = feed conversion ratio

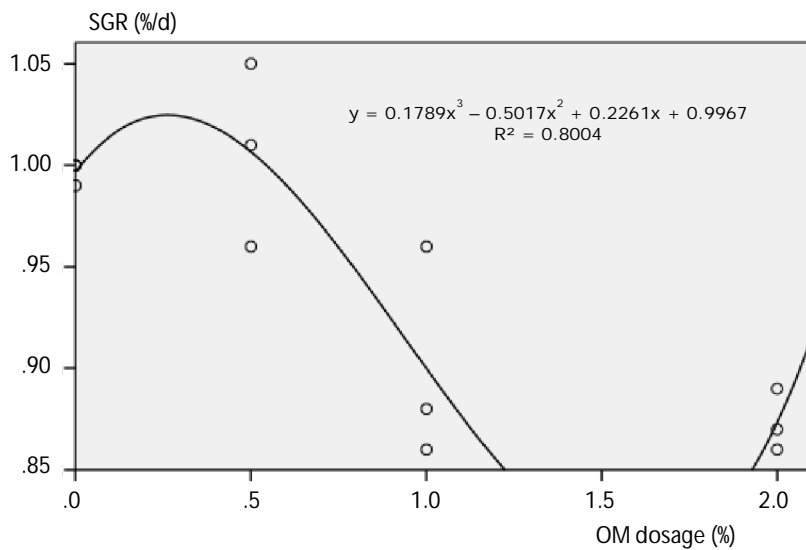


Figure 1. The significant (P<0.05) relationship between SGR of rabbitfish and dietary OM inclusion levels.

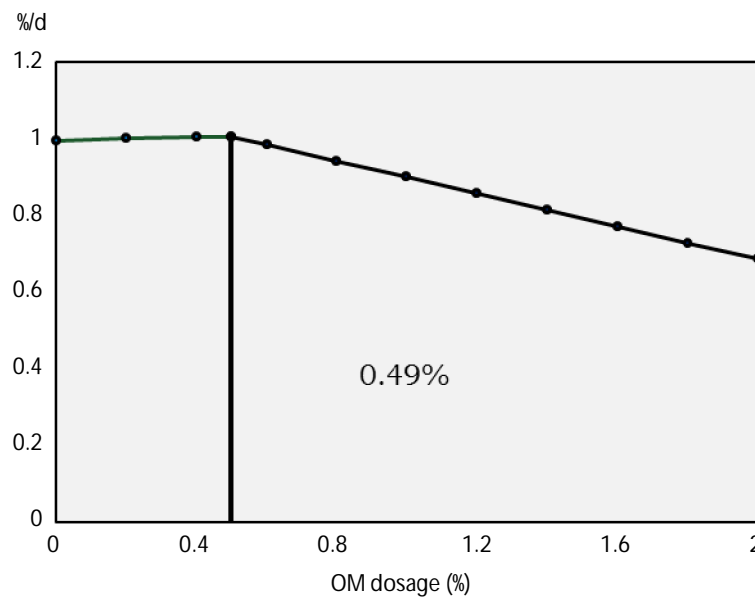


Figure 2. The breakpoint broken-line model fitted to estimate the optimum OM inclusion level for the maximum SGR response of rabbitfish (0.49%).

Table 6. Vertebral mineral content of rabbitfish fed different levels of dietary OM and the cubic regression for each parameter (n=2)

Variable	Control	OM0.5	OM1	OM2	Cubic regression equation*
Vertebral Ca	1.368 ± 0.71	1.285 ± 0.10	1.818 ± 0.03	1.610 ± 0.26	$y = -1.09x^3 + 2.87x^2 - 1.33x + 1.37$ ($R^2 = 0.897$)
Vertebral Mg	0.092 ± 0.71	0.193 ± 0.03	0.210 ± 0.01	0.189 ± 0.00	$y = 0.07x^3 - 0.27x^2 + 0.32x + 0.09$ ($R^2 = 0.835$)
Vertebral Zn	0.077 ± 0.71	0.114 ± 0.01	0.100 ± 0.01	0.069 ± 0.02	$y = 0.05x^3 - 0.18x^2 + 0.15x + 0.08$ ($R^2 = 0.862$)

Note: * All values are significant (P < 0.05)

to utilize these elements in the form of the dietary organic mineral. However, this study found that the concentration of these elements in vertebrae significantly decreased at the higher dietary inclusion level of 2%. In the case of Ca element, several studies reported that organic Ca in the form of tricalcium phosphate (TCP) in fishmeal is also considered to be available to several fish species, including scorpion fish (Hossain & Furuichi, 2000) and tiger puffer (Laining *et al.*, 2011).

Fillet Proximate Composition

Generally, the crude protein of the fillet from all treatments was relatively high at over 75% dry matter (Table 7). There were no significant differences for protein, lipid, fiber, and ash content of fillet among treatments, indicating that levels of dietary OM did not affect the proximate content of the rabbitfish fillet. A similar result was found for *L. vannamei* fed with different dietary levels of OM (Laining *et al.*, 2015).

The positive effects of adding organic minerals in the diet of rabbitfish, as found in the present study, demonstrated that minerals bound to organic molecules were available to the fish. Similar studies on utilizing organic minerals are scarcely reported. However, chelated trace mineral using amino acid, glycine or other organic molecules have been viewed as an alternative to inorganic trace minerals (Bharadwaj *et al.*, 2014; Buentello *et al.*, 2009; Katya *et al.*, 2016; Lin *et al.*, 2013; Prabhu *et al.*, 2019). Typically, organic chelated trace minerals are more stable in the digestive tract and less prone to interactions and antago-

nisms as they are bound to organic molecules and less available to interaction and binding, potentially improving their bioavailability (Silva *et al.*, 2019; Nie *et al.*, 2016).

CONCLUSION

In conclusion, this study successfully demonstrates that dietary OM level significantly affected the growth and vertebral mineral content of golden rabbitfish. The optimum level of the OM used in this experiment which provided the maximum growth rate of golden rabbitfish *S. guttatus* reared in floating net cages is 0.49%. Vertebral Ca, Mg, and Zn content can be increased by including dietary OM up to 1.0%. These results suggest that dietary OM can be utilized as a mineral source to substitute for inorganic minerals in aquafeeds. Using dietary OM could reduce utilization of imported mineral premix that would further reduce the price of diet produced.

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Table 7. Proximate content of fillet of rabbitfish fed different levels of dietary OM (% dry matter)

Nutrient	OM dosages			
	OM0	OM0.5	OM1	OM2
Crude protein	79.9 ± 2.6 ^a	79.3 ± 0.85 ^a	78.1 ± 0.95 ^a	79.5 ± 0.51 ^a
Crude lipid	9.1 ± 0.9 ^a	9.6 ± 0.21 ^a	9.9 ± 0.26 ^a	9.8 ± 0.15 ^a
Ash	5.5 ± 0.0 ^a	5.1 ± 0.96 ^a	5.2 ± 0.09 ^a	5.3 ± 0.43 ^a
Fibre	0.2 ± 0.2 ^a	0.2 ± 0.15 ^a	0.6 ± 0.17 ^a	0.6 ± 0.06 ^a

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