Effects of Sterol Supplements (Cholesterol FG, Cholesterol SF, and Sterols M1M) on Growth and Survival of the Shrimp, *Litopenaeus vannamei* Boone.

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Abstract

The efficacy of three sterol supplements to satisfy the dietary requirement of juvenile *Litopenaeus vannamei* Boone for cholesterol was evaluated in a 57-day growth trial. Sterol supplements were (1) cholesterol SF, which contained ≥91% cholesterol and ≥97% total sterol, (2) cholesterol FG, which contained ≥60% cholesterol and ≥75% total sterol, and (3) sterols M1M, which contained only 21% cholesterol and 63% total sterol. The composition of sterols other than cholesterol in M1M was similar to that in FG. Diets contained five levels of the sterol additives (0.0, 0.05, 0.1, 0.2, and 0.4%). Survival (64-91%) was not affected by either the level or type of sterol supplement. Mean weight gains ranged from 1.6 g for the base diet without sterol supplements to 7.0 g for the diet with 0.4% FG. For sterol levels above 0.05%, growth on both SF and FG was greater than on M1M. Growth increased with sterol level up to 0.4% for FG and M1M, and up to 0.2% for SF with no further increase for 0.4% SF. The growth data suggested that the cholesterol requirement for optimum growth was satisfied by either 0.16% cholesterol SF or 0.25% cholesterol FG, that the dietary requirement for cholesterol by *L. vannamei* was 0.15%, and that sterols other than cholesterol in FG and M1M can partially, but not completely, satisfy the dietary requirement for cholesterol.

Introduction

In shrimp, cholesterol is an essential dietary nutrient. Like other crustaceans, shrimp cannot synthesize cholesterol (Whitney, 1970; Kanazawa, Tanaka, Teshima & Kashiwada, 1971). Dietary cholesterol is necessary for growth and survival. Cholesterol is a necessary constituent of cell membranes and a precursor for steroid hormones.

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Optimum levels depend upon species, feed rate, and the presence of other nutrients. Cholesterol requirements have been reported to be 0.5% for *Feneropenaeus pencillatus* Alcock (Chen & Jenn, 1991), 0.05 to 0.5% for *L. vannamei* (Duerr & Walsh, 1996; Emery, 1987; Gong, Lawrence, Jiang, Castille & Gatlin, 2000), 0.1 to 0.6 for *Macrobachium rosenbergii* de Man (D'Abramo & Daniels, 1994; Teshima, 1998), 0.1 to 2% for *Marsupenaeus japonicus* Bate (Shudo, Nakamura, Ishikawa & Kitabayashi, 1971; Kanazawa *et al*., 1971; Deshamuru & Kuroki, 1974; Teshima, Ishikawa, Koshio & Kanasawa, 1997), and 0.2 to 1% for *Penaeus monodon* Fabricius (Chen, 1993; Sheen, Liu, Chen & Chen, 1994; Paibulkichakul, Piyatiratitivorakul, Kittakoop, Viyakarn, Fast & Menasveta, 1998).

In crustaceans, dietary cholesterol requirements have been primarily determined from growth trials using graded levels of cholesterol, and estimates of requirements can vary with feed rates. Using a factorial method to estimate cholesterol requirements, Teshima (1998) estimated optimum dietary cholesterol levels for *M. japonicus* to be 0.50, 0.40, and 0.29% at daily feed rates of 3, 5, and 7% of body weight.

Dietary phospholipid has been suggested to facilitate utilization of cholesterol by crustaceans. Teshima, Kanazawa, and Kakuta (1986) reported that in *M. japonicus*, dietary phospholipid increased mobilization of cholesterol from the digestive gut to the hepatopancreas, hemolymph, and muscle. In the lobster, *Homarus americanus* H. Milne-Edwards, dietary lecithin increased serum and lipoprotein cholesterol (Baum, Conklin & Chang, 1990). Teshima (1997) suggested that insufficient dietary phospholipid might restrict formation of lipoproteins that transport cholesterol in the hemolymph.

Emery (1987) reported an interaction between dietary cholesterol and phospholipid with respect to the growth of *L. vannamei* postlarvae. In a more complete study, Gong, Lawrence, Jiang, Castille & Gatlin (2000) reported that dietary phospholipid decreased the cholesterol requirements for *L. vannamei* fed to excess. Cholesterol levels for optimum growth were 0.35, 0.14, 0.13, and 0.5% at phospholipid levels of 0, 1.5, 3.0, and 5.0%, respectively.

In terms of growth responses, absences of interactions between dietary cholesterol and phospholipid have been reported in *F. pencillatus* (Chen & Jenn, 1991), *M. rosenbergii* (Briggs, Jauncey & Brown, 1988), *M. japonicus* (Teshima, Kanazawa, Sasada & Kawasaki, 1982), and *P. monodon* (Paibulkichiakul *et al.*, 1998; Chen, 1993).

Although fish, shrimp, squid, crab, and other cholesterol containing animal meals can provide a portion of the requirement for cholesterol in shrimp feeds, supplementation with purified cholesterol or other cholesterol containing ingredients is needed to obtain optimum growth (Coutteau, Peters Nur & Kontara, 2002). Cholesterol is a relatively expensive ingredient for use in shrimp feeds, and the costs of supplementing feeds with cholesterol may be significant and even prohibitive (Coutteau, Ceulemans, Nur, Van Halteren & Robles, 2003).

The apparent dietary requirement for cholesterol in crustaceans can potentially be satisfied by sterols other than cholesterol. Conversion of other sterols to cholesterol has been demonstrated for brassicasterol in *Artemia salina* Leach (Teshima & Kanazawa, 1973a), β-sitosterol in *Portunus trituberculatus* Miers (Teshima & Kanazawa, 1972), desmosterol in *Palaemon serratus* Pennant (Teshima, Ceccaldi, Patrois & Kanazawa, 1975) and *M. japonicus* (Teshima & Kanazawa, 1973b), and ergosterol in *A. salina* (Teshima & Kanazawa, 1971) and *P. trituberculatus* (Teshima, 1971). However, the ability of other sterols to satisfy the apparent cholesterol requirement for growth and survival in crustaceans is variable. Replacement of cholesterol with ergosterol reduced growth in *M. rosenbergii* (D'Abramo & Daniels, 1994) and survival in *Artemesia longinaris* Bate (Haran & Fenucci, 2003). Replacement with stigmasterol reduced growth in *M. rosenbergii* (D'Abramo & Daniels, 1994). Replacement of cholesterol with β-sitosterol reduced growth in *M. japonicus* (Kanazawa, Guary, & Ceccaldi, 1976; Teshima, Kanazawa, Koshina, & Lindo, 1989) but not in *M. rosenbergii* (D'Abramo, 1998) and *A. longinaris* (Haran & Fenucci, 2003).

The objective of this study was to compare cholesterol products in growth trials with *L. vannamei*. The cholesterol supplements produced by Solvay Pharmaceuticals, contained different sterol compositions. Cholesterol SF was a purified cholesterol product that contained 91% or more cholesterol and was 97% or more sterols. Cholesterol FG was a less purified product that contained 60% or more cholesterol and was 75% or more sterols. To demonstrate contributions of sterols other than cholesterol, a third sterol mixture, sterols M1M was compared to cholesterol SF and FG. Sterols M1M contained 21% cholesterol and 63% total sterols. The composition of sterols other than cholesterol in sterols M1M was similar to than in cholesterol FG.

Materials and Methods

Diets were formulated using purified and semi-purified ingredients to contain five different levels of sterol supplements (0.0, 0.05, 0.1, 0.2, and 0.4%) with three different types of sterol supplements (SF, FG, and M1M). In addition, a high quality commercial feed, Rangen 45/10, was used as a control to compare the semi-purified diets to a practical feed. The composition of the base diet without sterols is shown in Table 1. Diets containing sterol supplements were formulated by substituting the sterols for equal amounts of wheat starch. The primary protein sources were purified soy protein, casein, and wheat gluten. Krill meal was added at 2% as an attractant. The diets were extruded at room temperature using carboxymethylcellulose as a binder.

Table 1. Ingredients of base diet without sterol additives.

Ingredient	%	Ingredient	%
Wheat Starch	47.46	Krill meal	2.00
Soy Protein	26.00	Potassium citrate	2.00
Casein	6.00	Magnesium sulfate	1.00
Wheat Gluten	6.00	Sodium chloride	0.90
Carboxymethylcellulose	4.00	Methionine	0.40
Calcium phosphate	4.00	Vitamin/mineral premix	0.40

Fish oil	3.50	Vitamin C	0.30
Soybean oil	2.00	Ferrous sulfate	0.03

Calculated levels of protein, lipid, ash, and fiber levels in the base diet and ranges of analyzed levels in the experimental diets are shown in Table 2. Calculated levels were based on proximate analysis of the ingredients use to make the diet. Proximate analysis was performed by Woodson-Tenent Laboratories, Inc., Memphis, TN.

Table 2. Proximate analysis of feeds

Component	Percentage of dry matter	
	Calculated	Analyzed
Protein	35.5	37.5 - 37.7
Lipid	6.0	6.0 - 6.4
Ash	8.2	8.0 - 8.1
Fiber	4.0	not determined

Cholesterol and total sterol levels in the feeds were calculated for each diet from the level of cholesterol in the base diet, the amount of sterol supplement in the diet, and the amounts of cholesterol and total sterols in the supplements. The amount of cholesterol in the base diet (0.02%) was estimated from the amounts in the ingredients. The amounts of cholesterol and total sterols in the supplements were indicated by the Certificates of Analysis provided by Solvay Pharmaceuticals. In addition, cholesterol levels were analyzed in each feed by Solvay Pharmaceutics to confirm the accuracy of calculated levels. Comparison of calculated and analyzed levels of cholesterol in the experimental feeds is shown in Table 3. Close agreement of calculated and analyzed cholesterol levels indicated that levels of sterol additives in the diets were correct. Calculated levels of total sterols in the experimental diets are shown in Table 4.

Table 3. Comparison of Calculated and Analyzed Levels of Cholesterol in Diets (%).

Level of supplement (%)	Cholesterol SF		Cholesterol FG		Sterol M1M	
	Calculated	Analyzed	Calculated	Analyzed	Calculated	Analyzed
0.00	0.02*	0.02*				

0.05	0.07	0.06	0.05	0.06	0.03	0.03
0.10	0.11	0.1	0.09	0.07	0.04	0.04
0.20	0.22	0.18	0.15	0.12	0.06	0.06
0.40	0.35	0.35	0.29	0.28	0.1	0.09

* base diet with no supplement

Table 4. Calculated Levels of Total Sterols in Diets (%).

Level of supplement (%)	Cholesterol SF	Cholesterol FG	Sterol M1M
0.00	0.02*		
0.05	0.07	0.06	0.05
0.10	0.12	0.11	0.08
0.20	0.22	0.19	0.15
0.40	0.41	0.36	0.27

* base diet with no supplement

A 57-day growth trial was conducted with shrimp reared from postlarvae obtained from Harlingen Shrimp Farm, Harlingen, Texas. Postlarvae were fed Rangen 45/10 feed supplemented with live *Artemia* nauplii. For the growth trial, shrimp with an average weight of 0.21 g were stocked at a density of 44 shrimp / m^2 (4 shrimp / tank).

The trial was conducted in two culture systems, each containing 100 tanks. Each tank had a bottom area of 0.1 m^2, a depth of 0.3 m, and contained 30 l of seawater. Seawater was recirculated through the tanks at a rate of 1440% per day to remove uneaten feed and insure equal and high water quality in all tanks. For each culture system, recirculating seawater was passed through a pressurized sand filter, trickle biofilter, inline heater, U.V. sterilizer, and 50 µ cartridge filter before returning to the culture tanks. Approximately 9-12% of the recirculating seawater was replaced with new filtered seawater each day. Salinity was adjusted by the addition of fresh water to maintain salinity between

25 and 26 ppt. Temperature was maintained at 30-32°C. Shrimp were fed 15 times daily with automatic feeders at a rate in excess of consumption. The daily feed rate was 2 times the predicted maximum daily growth of the shrimp. During the growth trials, temperature, dissolved oxygen, and salinity were measured daily. Ammonia, nitrite, nitrate, and pH were measured weekly. At the end of the growth trial, the surviving shrimp in each tank were counted and weighed. Survival was expressed as the percentage of shrimp that remained. Growth was expressed as mean weight gain per shrimp.

Growth and survival were statistically analyzed by two-way analysis of variance (ANOVA) with level and type of sterol supplement as independent variables. Culture system was treated as a blocking factor (6 replicate culture tanks for each dietary treatment in each culture system). For ANOVA, the Arc Sin transformation was used for survival and the Natural Log transformation for growth. The SAS System for Windows, release 6.12, software was used for statistical analysis. However, since level of sterol was a quantitative factor with zero level, the analysis required multiple SAS passes and manual construction of the ANOVA tables (Gates, 1991).

Results and Discussion

Water quality parameters during the trial were adequate for good growth and survival (Table 5).

Table 5. Summary of Water Quality Parameters for 57-Day Growth Trial

Parameter	Mean ± Standard deviation (Number of observations)
Temperature (°C)	30.1 ± 0.5 (112)
Salinity (ppt)	25.4 ± 1.0 (112)
Dissolved oxygen (ppm)	5.8 ± 0.2 (112)
Ammonia (mg NH_4-N/l)	0.12 ± 0.05 (16)
Nitrite (mg NO_2-N/l)	0.17 ± 0.09 (16)
Nitrate (mg NO_3-N/l)	1.94 ± 1.93 (16)

pH	8.1 ± 0.1 (16)
Exchange (%)	11.57 ± 10.92 (56)

Effects on type (SF, FS, and M1M) and level (0-0.4%) of sterol on survival are summarized in Figure 1. Survival after 57 days ranged from 64% for the diet without supplementation with sterols to 94% for shrimp on the Rangen 45/10 diet. Two-way ANOVA of survival indicated that interactions between type and level of sterol were not significant (P=0.1741). Differences in survival due to both type and level of sterol supplement were also not significant (P=0.4324 and P=0.0806, respectively).

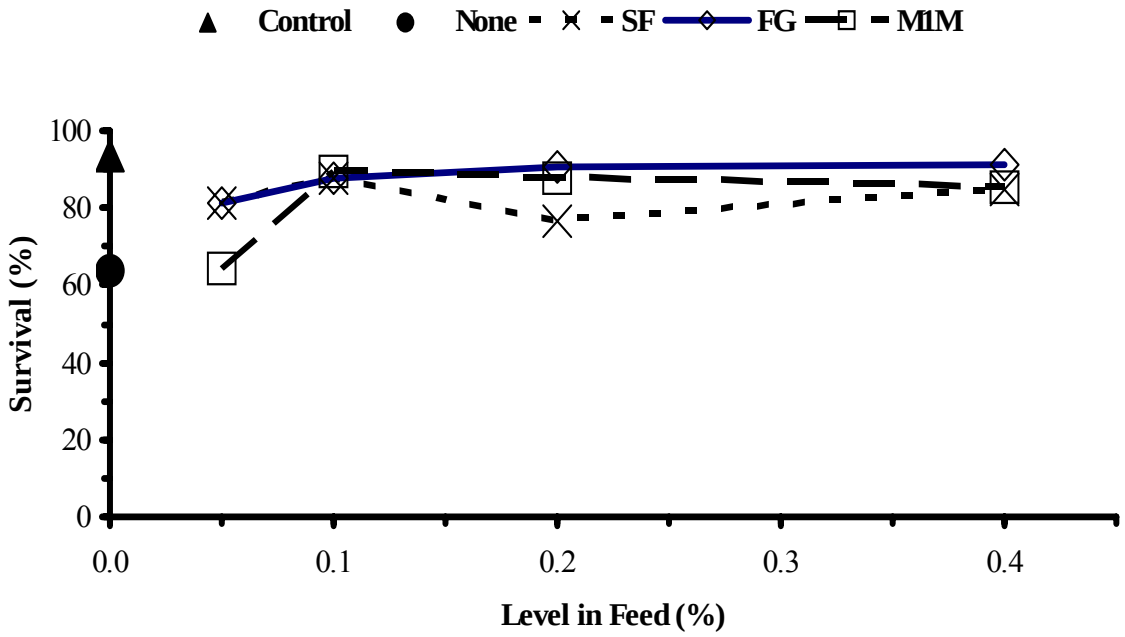

Figure 1. Effects of Sterol Supplements on Survival

Effects of type and level of sterol supplement on growth are summarized in terms of weight gain in Figure 2. With the semi-purified, experimental diets, weight gains ranged from 1.6 g for the diet without sterol supplements to 7.0 g for the diet with 0.4% cholesterol FG. Weight gain on the

Rangen 45/10 control was 9.7 g. Two-way ANOVA of weight gain indicated that there was a significant ($P<0.00001$) interaction between type and level of sterol. Comparison of least square means and one-way ANOVA of effects of sterol type at each level, was used to compare the effects of sterol type. For the 0.05% level, growth on FG was greater than on M1M. For levels from 0.1 to 0.4%, growth on both SF and FG was greater than on M1M. The results indicated that growth on SF and FG was greater than on M1M, and that differences between SF and FG were small. Comparison of least square means and one-way ANOVA of effects of sterol level for each type were used to compare the effects of sterol level. In general, growth increased with level of sterol. However, differences were not statistically significant between 0.2 and 0.4% for SF, and below 0.1% for FG and M1M. The results suggested that the optimum level for SF was between 0.1 and 0.2%. Broken line analysis indicated that the requirement for cholesterol SF to give optimum growth was 0.16% and that for cholesterol FG was 0.25%.

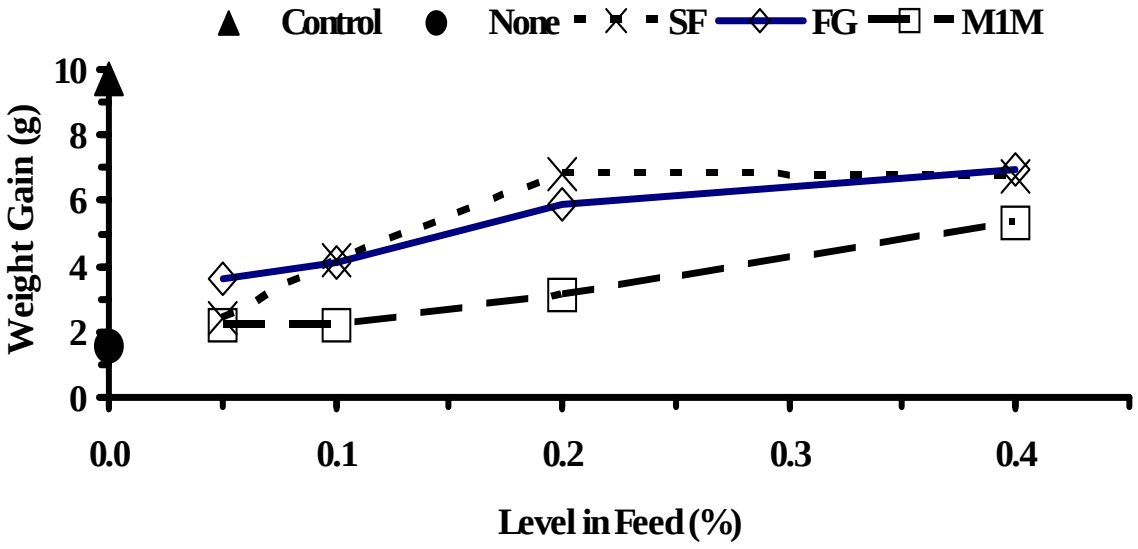

Figure 2. Effects of Sterol Supplements on Weight Gain

The effect of cholesterol in the diets on growth is shown in Figure 3. Cholesterol was calculated as the amount in the sterol additive plus the amount in the base diet rather than the analyzed amounts.

Broken line analysis of the cholesterol SF data indicated that the requirement for cholesterol was 0.15% under the conditions of this trial. Even though culture conditions, diets, and growth in this study were similar to that reported by Gong *et al*. (2000), the cholesterol requirement for optimum growth in this study was lower than the value of 0.35% reported by Gong *et al*. for diets without supplemental phospholipid. In Figure 3, the growth responses for cholesterol FG and sterols M1M lie above the line generated by cholesterol SF. This observation suggested that growth was greater than would have been expected due to cholesterol alone, and that the sterols other than cholesterol in cholesterol FG and sterols M1M contributed to the increased growth.

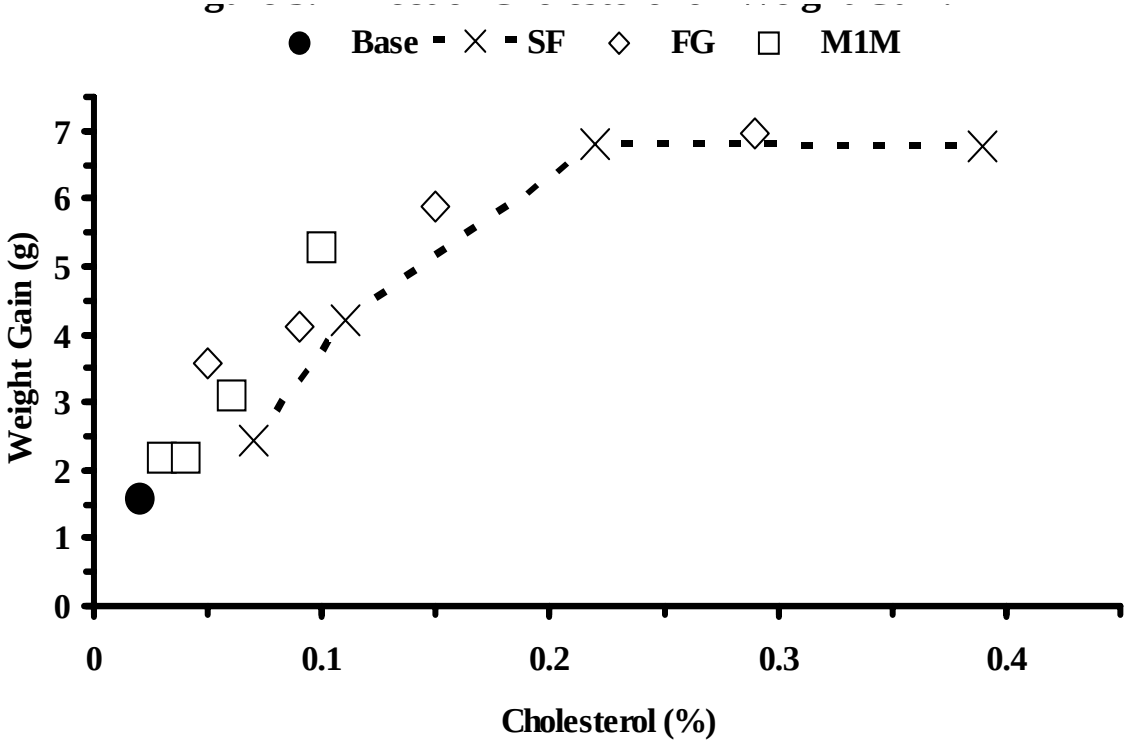

Figure 3. Effect of Cholesterol on Weight Gain

The effect of total sterols in the diets on growth is shown in Figure 4. In contrast to the effect of cholesterol on growth in the preceding figure, the growth response for sterols M1M lies below the

line generated by cholesterol SF, which suggested that the sterols in M1M were not as effective as the predominately cholesterol sterols in cholesterol SG.

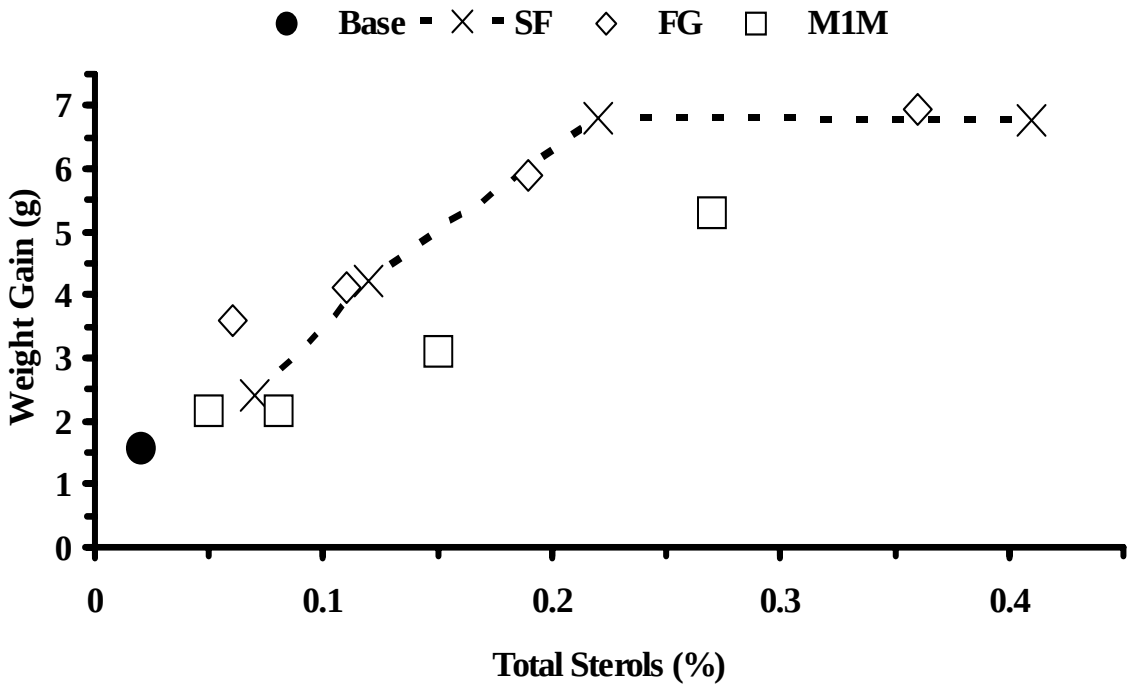

Figure 4. Effect of Total Sterol on Weight Gain

In summary, the growth data suggested (1) that the cholesterol requirement for optimal growth was satisfied by either 0.16% cholesterol SF or 0.25% cholesterol FG, (2) that under the conditions of the growth trial, the dietary requirement for cholesterol by *L. vannamei* was 0.15%, and (3) that sterols other than cholesterol in cholesterol FG and sterols M1M can partially, but not completely, satisfy the dietary requirement for cholesterol.

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