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Short Paper

Optimun Supplemental Level of L-Ascorbyl-2-Phosphate-Mg to Diet for White Shrimp *Penaeus vannamei*

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A stable form of ascorbic acid, L-ascorbyl-2-phosphate-Mg (APM), was tested to confirm the effect of APM on juvenile shrimp *P. vannamei* and determine the optimun supplemental level to diet. Shrimp, weighing 1.10±0.15 g, were fed 5 diets containing graded levels of supplemental APM (0, 10, 18, 33, and 66 mg/100 g diet) for 18 weeks. Shrimp fed the unsupplemented diet showed deficiency symptoms such as high mortality, molting frequency reduced, and blackened lesions under the exoskeleton, on the abdomen, on the carapace, in the gills and in the foregut. These symptoms were similar to the proposed ascorbic acid deficiency symptoms which is named "black death." The results of the feeding trials indicated that the supplementation of 10 mg APM per 100 g diet was sufficient for a better survival and prevention of clinical signs of vitamin C deficiency symptom in *P. vannamei*.

Key words: *Penaeus vannamei*, requirement, ascorbic acid

The quantitative requirement of ascorbic acid (AA) in *Penaeus vannamei* has been already determined.[1] However, the substantial losses of AA during feed production, storage and leaching to water make it difficult to control the exact amount of AA ingested by shrimp. Whereas, it has been reported that the recent AA derivative such as L-ascorbyl-2-phosphate-Mg (APM) has a high stability and reduced solubility in water, and has shown good vitamin C activity in *Penaeus monodon*[2] and *P. japonicus*.* This study was conducted to confirm the effect of APM on mortality, molting frequency and blacking of juvenile shrimp *P. vannamei* and determine the optimun supplemental level to diet.

The test diets were prepared as shown in Table 1, referring the diet processing and formulation to Camba *et al.*[3] APM was supplemented at the graded levels of 0, 10, 18, 33, and 66 mg/100 g diet. The dry pellets cut into 50 mm length were stored in sealed plastic bags at −28°C until used. After 2-week acclimation of wild juvenile *P. vannamei* under experimental conditions, 30 shrimp with mean body weight of 1.10±0.15 g were held in 2-ton circular tanks and reared for 18 weeks. Triplications were employed for each test diet. All groups were fed the test diets twice daily, according to the feeding rate given by Ralston Purina Co., USA.

Daily mortality and the number of molt were recorded. A photoperiod was maintained at the condition of 12-h light and 12-h darkness. Water temperature and dissolved oxygen ranged between 24-30°C and 6.8-8.6 ppm, respectively. Salinity was approximately 35 ppt during the experimental period.

APM concentration of the diets was measured by partly modifying the method of Shigueno and Itoh.[4] Shrimp with signs of black death were preserved in Davidson's[5] fixative and were later dehydrated, embedded and sectioned using routine histological techniques. Tissue sections were mounted and stained with either hematoxylin and eosin or Mason's trichrome stain. Statistical analyses were performed according to the analyses of variance (ANOVA) and Duncan's multiple range test ($p<0.05$).

Shrimp fed the unsupplemented diet (diet no. 1) had a significantly lower survival (Table 2), than those fed vitamin-supplemented diets. The majority of dead shrimp in this group developed blackened lesions under the exoskeleton, on the abdomen, on the carapace, in the gills and in

Table 1. Composition of experimental diets

Ingredient	g/100 g diet
Shrimp head meal	25.82
Fish meal	10.00
Squid meal	5.00
Wheat gluten	4.00
Wheat bran	5.00
Soybean meal	16.72
Fish oil	4.31
Soy lecithin	1.00
Cholesterol	0.50
Vitamin premix*1	4.50
Mineral premix*2	2.00
Sodium polycrylate (binder)	1.20
Corn starch	19.95-19.88
L-Ascorbyl-2-phosphate Magnesium*3	0-0.066

*1 Vitamin premix without vitamin C (mg/100 g diet): *p*-aminobenzoic acid, 10.00; thiamine-HCl, 12.00; riboflavin, 20.00; pyridoxine-HCl, 12.00; choline chloride, 250.00; nicotinic acid, 75.00; Ca-pantothenate, 50.00; inositol, 200.00; biotin, 0.50; folic acid, 1.50; menadione, 4.00; vitamin B_{12}, 0.03; calciferol, 0.03; alpha-tocopherol, 40.00; beta-carotene, 2.500 I.U = 1.15 e^{-04}; Celullose (as a carrier), 3824.00.

*2 U.S.P. XII mixture with trace elements.[6]

*3 APM whose molecular weight is 379.61, contains 46.40% ascorbic acid activity.

* A. Kanazawa *et al.*: Abst. papers of 6th Int. symp. on fish nutrition and feeding, 4th-7th Oct. 1993. Hobart, Australia.

Table 2. Results of feeding trials on the *P. vannamei*[*1]

Diets no.	APM (mg/100 g diet)	Survival (%)[*2]	Body weight (g)		Body Weight Gain[*2] (%)	Biomas (g)[*2,3]	No of molt per shrimp[*2]	Feed conversion ratio[*2]
			Initial	Final				
1	0	66.7[a]	1.11±0.15	14.34±3.05	1189[a]	760.1[a]	6.8[ab]	3.90[a]
2	10	92.2[b]	1.08±0.15	14.73±4.11	1245[a]	1125.4[b]	7.6[b]	2.97[a]
3	18	92.2[b]	1.12±0.15	15.06±2.99	1240[a]	1149.2[b]	7.0[ab]	2.89[a]
4	33	91.1[b]	1.09±0.14	14.85±3.08	1264[a]	1119.6[b]	7.5[ab]	2.94[a]
5	66	87.8[b]	1.09±0.14	15.01±4.17	1284[a]	1087.7[b]	6.6[a]	2.98[a]

[*1] Average value from triplicate tanks with 30 individuals each, reared for 18 weeks at 24-30°C.
[*2] Values with differents superscripts in the same column are significantly different ($p<0.05$).
[*3] Average total body weight in each tank.

the foregut. These symptoms were similar to the proposed AA deficiency symptoms which is named "black death".[6,7] Histological examination of the blackened area in shrimp with "black death" disease showed that the lesions typically were present in loose connective tissues, in subepithelial tissues of cuticular hypodermis and/or in the glandular and epithelial layers of the foregut.

Molting frequency of the shrimp fed the diet 2 was significantly much higher than the other groups. On the other hand, no differences in growth between the treatments were detected. Feeding conversion ratio of the shrimp fed the diet 1 was much higher than the other groups, but not statistically different. Thus, supplementation of 10 mg APM per 100 g diet was sufficient for a better survival and prevention of clinical signs of vitamin C deficiency symptom in *P. vannamei*. An appropiate level of more than 5 mg APM per 100 g diet was also found in *P. japonicus*.[*]

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References

1) H. He and A. L. Lawrence: *Aquaculture*, **114**, 305-316 (1993).
2) S. Shiau and T. Hsu: *Aquaculture*, **122**, 347-357 (1993).
3) E. Camba, A. Pedrazzoli, M. Yaguachi, and T. Akiyama: *Acuicultura Tropical*, **1**, 7-12 (1993).
4) K. Shigueno and S. Itoh: *J. World Aqua. Soc.*, **19**, 168-174 (1988).
5) G. G. Humason: *Animal Tissue Techniques*, 432 (1967).
6) D. Lightner, L. Colvin, C. Brand, and D. Donald: *Proc. World. Maricult. Soc.*, **8**, 611-623 (1977).
7) P. C. Magarelli Jr., B. Hunter, D. V. Lightner, and L. B. Colvin: *Comp. Biochem. Physiol.*, **63A**, 103-108 (1979).
8) J. E. Halver: *J. Nutr.*, **62**, 225-243 (1957).