

VITAMIN C SOURCES AND LEVELS FOR SHRIMP *Litopenaeus vannamei* IN THE GROWING PHASE

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ABSTRACT

The inclusion of different sources and levels of vitamin C was evaluated on growth, survival, protein retention and health of *Litopenaeus vannamei* with initial weight of 5.90 ± 0.57 g. Five hundred and forty individuals were distributed in 27 cages in a completely randomized design in a 4x2 factorial scheme (four sources and two levels) (n = 3). The sources used were ascorbic acid crystal, coated ascorbic acid, ascorbic acid monophosphate and ascorbic acid PEG (polyethylene glycol) with two levels: 180 and

260 mg kg⁻¹. No influence was observed (P>0.05) from the source or level of vitamin C, nor interaction of these factors on growth, survival, protein retention and parameters of health of the shrimp. Necrosis was observed in the abdomen of all animals. The use of vitamin C in sources and levels estimated for the shrimp *Litopenaeus vannamei* weighing over 5 g shows no improvement in growth, survival, protein retention and health of shrimp.

KEYWORDS: ascorbic acid; nutritional requirement; sanity; shrimp production.

FONTES E NÍVEIS DE VITAMINA C PARA O CAMARÃO *Litopenaeus vannamei* NA FASE DE RECRIA

RESUMO

Avaliou-se a inclusão de diferentes fontes e níveis de vitamina C no crescimento, sobrevivência, retenção de proteína e sanidade de *Litopenaeus vannamei* com peso inicial de 5,90 ± 0,57 g. Quinhentos e quarenta exemplares foram distribuídos em 27 gaiolas em delineamento inteiramente casualizado, esquema fatorial 4x2 (quatro fontes e dois níveis), além do tratamento controle, isento de vitamina C (n=3). As fontes utilizadas foram ácido ascórbico cristal, ácido ascórbico revestido, ácido ascórbico monofosfato e ácido ascórbico PEG

(polietileno glicol) com dois níveis: 180 e 260 mg kg⁻¹. Não foi observada influência (P>0,05) da fonte, nível de vitamina C nem interação desses fatores sobre o crescimento, sobrevivência, retenção de proteína e parâmetros de sanidade dos camarões. Observou-se a presença de necrose no abdômen de todos os animais. A utilização de vitamina C nas fontes e níveis avaliados para o camarão *Litopenaeus vannamei* com peso acima de 5 g não revela melhorias no crescimento, sobrevivência, retenção de proteína e sanidade dos camarões.

PALAVRAS-CHAVE: Ácido ascórbico; carnicultura; exigência nutricional; sanidade.

INTRODUCTION

Shellfish cultivation in Brazil has grown from 7,260 t in 1998 to 60,000 t in 2002 because of *Litopenaeus vannamei* production. The performance of

this shellfish (5458 kg ha⁻¹ year⁻¹) led it to the top of the rank in world productivity (MENDES et al., 2006), and the Brazilian Northeast region accounts for about 97% of national shrimp production (CUNHA et al., 2006).

However, in 2004, the industry suffered a severe crisis triggered mainly by the spread of white spot virus in several shrimp farms, the U.S. *antidumping* action and the dollar instability (ARAÚJO et al., 2009). In 2009, shrimp accounted for 16% of national aquaculture, producing 65,000 t (ROCHA, 2011).

Vitamins represent one of the essential nutrients for shrimp diet. Vitamin C plays an important role in animal health as an antioxidant, inactivating free radicals produced by normal cellular activity, and various stressors (HALVER, 1995). According to HE & LAWRENCE (1993) and NIU et al. (2009), ascorbic acid or vitamin C is an essential nutrient for penaeid shrimp, because it helps in the maintenance and growth of shrimp, and acts in the organism as a cofactor for many biochemical reactions (FUJIMOTO & CARNEIRO, 2001; WANG et al., 2006), besides acting as an antioxidant agent, detoxifying several peroxides originated from metabolism (DARIAS et al., 2011), and as an anti-stressor and immunostimulant agent (LEE & SHIAU, 2002; MAGGIONI et al., 2004).

According to ALMEIDA (2003), dietary supplementation with vitamin C is important for feed efficiency, shrimp health and economic value of rations and breeding. MOREAU & CUZON (1998) and LEE & SHIAU (2002) stated that the use of stable forms of ascorbic acid is a key factor in determining the nutritional requirement for shrimp. The difficulty to apply this micronutrient is due to the lack of information on optimal doses in the diet for this penaeid (HE & LAWRENCE, 1993; LÓPEZ et al., 2003).

LÓPEZ et al. (2003) and MOE et al. (2005) reported that inadequate levels of vitamin C in feed for juvenile shrimp may reduce growth rate, appetite, ability to repair damaged tissues, resistance to stress, molt frequency and also cause incomplete molting. CHEN & CHANG (1994) and MERCHIE et al. (1997) added that diets with low levels of ascorbic acid favors the development of black lesions (black death) in shrimp.

Therefore, the objective of this study was to evaluate different sources and levels of vitamin C in the diet for the marine shrimp *Litopenaeus vannamei* in the fattening stage, by the analysis of health, production performance, and nutrient retention in the carcass.

MATERIAL AND METHODS

The experiment was conducted in a private property, Maricanes Farm, located on the Highway BA-001, km 18, in the municipality of Canavieiras, BA (15°40'30"S and 38°56'50"W), during 62 days. We utilized an earth pond, with area of 3,400 m² and mean

depth of 1.2 m, with control supply by pumping and water drainage by sluices. We used 27 cages (1 x 1 x 1 m) made of steel rebar frames (1 cm diameter) and coated with PVC meshes (6 mm mesh), which were distributed in the nursery.

We used 540 *Litopenaeus vannamei* juvenile shrimps with an initial weight of 5.90 ± 0.57 g, randomly divided in experimental cultivation units at a density of 20 individuals m⁻², the same density used in the farm production area. The experimental design was completely randomized in a 4 x 2 factorial arrangement (four sources and two levels of vitamin C), besides the control treatment, free of vitamin C (n = 3).

Control diet (Table 1) was formulated without vitamin C, and from that, nine experimental diets were formulated, differing only as for sources and levels of vitamin C. We assessed four vitamin C sources: ascorbic acid crystal, coated ascorbic acid, ascorbic acid monophosphate (2-monophosphate calcium salt of L-ascorbic acid) and ascorbic acid PEG (polyethylene glycol); and two levels of these sources: 180 and 260 mg kg⁻¹.

Table 1 - Percentage composition of control diet for *Litopenaeus vannamei*

Ingredient	Basal diet (%)
Fish meal 60%	15.00
Corn gluten 60%	9.35
Soybean Meal 45%	25.96
Wheat meal	27.03
Corn grain	11.50
Salmon oil	3.63
Supplement (vitamin and mineral) ¹	1.50
Vitamin C	0.00
Cellulose	6.00
Antioxidant BHT	0.03

¹Warranty levels per kg: vit. A – 2200 UI; vit. E – 17.00 UI; vit. D3 – 1600 UI; vit. B6 – 2.00 mg; vit. B1 – 2.50 mg; vit. B2 – 4.00 mg; vit. K – 2.5 mg; vit. B12 – 30.00 mcg; folic ac. – 1.00 mg; pantothenic ac. – 15.00 mg; biotin – 0.10 mg; choline – 4.50 mg; niacin – 50.00 mg; Co – 0.03 mg; Cu – 7.50 mg; Fe – 50.00 mg; I – 2.00 mg; Mn – 50.00 mg; Se – 0.07 mg; Zn – 80.00 mg

The experimental diets were formulated using the Super Crac® 4.0. *software*. All ingredients were supplied by the company Agroceres Nutrição Animal, including the vitamin C sources. The experimental diets were prepared at the Aquaculture Laboratory, University of São Paulo (USP), campus of the College of Agriculture Luiz de Queiroz (ESALQ).

The ingredients, except the salmon oil and the vitamin and mineral mixtures, were sieved, weighed on a precision scale (0.01 g), and

subsequently mixed in an industrial mixer. Vitamin C sources and vitamin and mineral mixtures were weighed on a precision balance (0.001 g), and added to the other ingredients. Afterward, the mixture was homogenized for five minutes, and the salmon oil was added.

For pelleting the diet, 800 mL of water was added and the mixture passed through pelletizer with a 4 mm matrix, and then it was taken to an oven with forced air circulation at 45 °C for 24 hours. After being cooled at room temperature, the diets were properly packaged, identified and stored in a freezer

(-10 °C) until the supply to the shrimp. Sources of vitamin C were incorporated into the experimental diets at the time of mixing the ingredients.

Chemical analyzes of dry matter, crude protein, ether extract and ash were carried out according to SILVA & QUEIROZ (2006), in the Animal Nutrition Laboratory, State University of Santa Cruz (UESC). A bomb calorimeter was used to determine gross energy. Analyses of crude fiber, calcium, phosphorus and vitamin C were performed by the company Agroceres Nutrição Animal (Table 2).

Table 2 – Analyzed composition of nutrients in the experimental diets for *Litopenaeus vannamei*

Item	Ration ¹								
	C 0	F1 180	F1 260	F2 180	F2 260	F3 180	F3 260	F4 180	F4 260
Moisture (%)	7.70	6.90	6.10	6.60	4.60	6.40	5.80	6.70	6.00
Crude protein (%)	35.56	34.98	36.17	35.96	36.21	36.29	36.53	35.84	36.01
Gross energy (kcal kg ⁻¹)	4488	4528	4337	4511	4642	4619	4609	4536	4545
Ether extract (%)	5.51	6.83	5.94	6.23	6.36	5.93	5.90	6.19	6.24
Crude fiber (%)	4.49	6.56	4.78	5.29	5.63	4.29	6.65	4.04	5.77
Ash (%)	5.95	6.00	6.14	5.96	6.09	6.15	6.13	6.02	6.04
Calcium (%)	0.61	0.60	0.57	0.57	0.62	0.55	0.63	0.60	0.65
Phosphorus (%)	0.90	0.89	0.92	0.86	0.90	0.89	0.88	0.90	0.90
Vitamin C (mg kg ⁻¹)	0.00	186	268	177	239	175	289	179	240

¹C, control ration (without vitamin C); F1, ascorbic acid crystal (vit.C - 6%); F2, coated ascorbic acid (Vit. C - 97%); F3, ascorbic acid monophosphate (calcium salt 2 - monophosphate L-ascorbic acid) (Vit. C - 35%); F4, Ascorbic Acid PEG (Polyethylene glycol) (vit.C - 50%).

The diets were supplied to the shrimp in trays, twice a day, at 9 a.m. and 2 p.m. Physicochemical parameters of water were monitored daily, by the use of a portable digital equipment, obtaining the following results: 5.4 ± 1.0 for dissolved oxygen (mg L⁻¹); 29.5 ± 1.6 for temperature (°C) 21.7 ± 3.1 for salinity (ppm), and 8.04 ± 0.1 for pH, and the results were within the recommended range for the species (LOWE-MCCONNELL, 1975; NEILL & BRYAN, 1991; BOYD, 2001; NUNES & MARTINS, 2002; KUBITZA, 2003).

At the beginning of the experiment, all animals of each repetition were weighed and 20 animals were collected and euthanized for body composition analyzes.

At the end of the experimental period, all animals were weighed and euthanized with an overdose of the anesthetic Benzocaine 120 mg L⁻¹ for determining performance parameters and nutrient retention. The following variables were determined for the evaluation of the different diets: feed intake; weight gain [(final biomass - initial biomass) /

number of animals]; feed conversion [feed intake / weight gain]; specific growth rate [Ln (final weight) - Ln (initial weight) / experimental period x 100]; survival rate [(number of dead animals / number of animals) x 100]; protein efficiency ratio [weight gain / protein intake], and retention coefficient of crude protein [100 x (x final body protein x final weight) - (initial body protein x initial weight) / protein consumed x 100].

To assess the health of animals, we observed body shape and identified possible pathological symptoms (absence / presence of necrosis). At the end of the experiment, all animals of the different treatments underwent macroscopic analysis.

Data were submitted to analysis of variance (ANOVA), with two factors, using SAS 9.0 software (SAS Inc., Cary, NC, USA).

RESULTS AND DISCUSSION

The factorial analysis of variance showed no effect (P > 0.05) of the interaction between source and level of vitamin C used in the diet on growth,

survival and crude protein retention rates as well as on sanity parameters in shrimps (Table 3).

Table 3 – Growth, survival and crude protein retention of *Litopenaeus vannamei* according to the source and level of inclusion of vitamin C in feed¹

Treatment	Variable ²						
	FI	WG	AFC	SGR	SR	PER	CPR
Ascorbic acid crystal	9.53	6.86	1.41	1.28	81.44	2.02	91.58
Coated ascorbic acid	9.52	6.46	1.50	1.18	79.56	1.89	85.65
Ascorbic acid monophosphate	9.50	6.80	1.42	1.24	81.89	1.98	91.92
Ascorbic acid PEG	9.40	6.84	1.40	1.26	82.00	2.04	93.54
0 mg kg ⁻¹	9.49	6.63	1.47	1.23	79.00	1.96	88.22
180 mg kg ⁻¹	9.48	6.74	1.42	1.26	82.33	1.99	90.58
260 mg kg ⁻¹	9.49	6.85	1.40	1.23	82.33	1.99	93.22
CV(%)	2.65	15.72	14.99	15.27	7.76	15.38	15.44
P Value							
Source	0.6592	0.8793	0.7524	0.6687	0.8300	0.7410	0.6542
Level	0.9873	0.8375	0.7011	0.9303	0.3428	0.9543	0.6854
Source x Level	0.3294	0.9470	0.8734	0.8961	0.9946	0.9084	0.7567

¹No significant difference at 5% probability by Tukey test.

²FI, feed intake; WG, weight gain; AFC, apparent feed conversion; SGR, specific growth rate; SR, survival rate; PER, protein efficiency rate; CPR, crude protein retention coefficient; CV, coefficient of variation

There was no effect ($P > 0.05$) of the vitamin C source used in the feed, i.e., growth, survival, crude protein retention and health parameters of shrimp fed diets with crystal, coated, monophosphate and PEG ascorbic acid were equivalent. Similarly, the level of inclusion of vitamin C in the diet did not affect ($P > 0.05$) growth, survival, crude protein retention and health parameters in shrimp.

The average results for feed intake, weight gain, feed conversion ratio and specific growth rate were, respectively, 9.48 g; 6.78 g; 1.42:1, and 1.24%. These results are similar to those obtained by other authors (BARBIERI JR. & OSTRENSKY NETO, 2002; MARTINEZ-CORDOVA et al., 2002; GOMEZ-JIMENEZ et al., 2005), who used shrimp fed with the same level of crude protein used in this experiment

These results contrast with those obtained by LEE & SHIAU (2002), who worked with different sources and levels of vitamin C in the diet for juvenile *Penaeus monodon* (0.37 g), and observed that the shrimp fed with any of the sources and levels performed significantly better than the shrimp fed diets free from vitamin C.

The mean value observed for survival rate was 81.96%, which is comparable to those obtained by SHIAU & HSUB (1994) and by GOMEZ-JIMENEZ et al. (2005). Contrary to these results, HE & LAWRENCE (1993), evaluating different levels of vitamin C (L-ascorbyl-polyphosphate) in diets for *L. vannamei* (0.1 g), observed that the survival of

animals fed diets free from vitamin C was significantly lower than those fed diets with inclusion of vitamin C.

The differences observed for both growth and survival parameters may be related to the respective development stages of shrimp. According to HE & LAWRENCE (1993) and NIU et al. (2009), the requirement of vitamin C in the diet for shrimps decreases as these organisms grow. Also, according to these authors, the animal in the early growth phase has the highest percentage of change in weight per time unit, thus, it requires more vitamin C to meet their metabolic needs.

The protein efficiency rate and the crude protein retention coefficient were, on average, 2 and 91.5%, respectively. The similarity between the results was expected, since the experimental diets were isocaloric and isoproteic and the shrimps showed similar performance.

Macroscopically, there were no deformations in the body of the shrimps in the different treatments; however, the presence of necrosis in animals' carapace (cephalothorax and abdomen) was observed in all treatments (Figures 1 and 2).

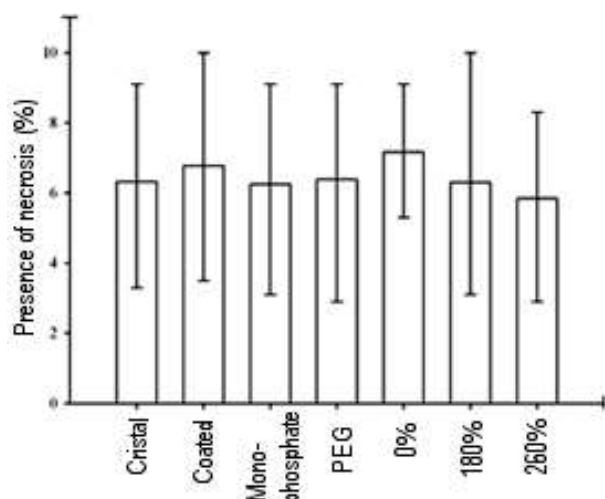


Figure 1 - Presence of necrosis (mean \pm standard deviation) in *Litopenaeus vannamei* according to the source and level of vitamin C.



Figure 2 – Necrosis points in the abdomen of *Litopenaeus vannamei*.

In addition to reduced growth, deficiency of vitamin C may be responsible for physical abnormalities and deformities of the skeleton in shrimps (DESHIMARU & KUROKI, 1976; SHIGUENO & ITOH, 1988; HE & LAWRENCE, 1993), which were not observed in any specimen regardless of the treatment. However, the presence of necrosis, which is a result of enzymatic reaction due to stress situation (ABCC, 2005), was observed in all specimens, which may be related to the restriction of movement of the shrimps when kept in 1 m³ cages, and suggests that the highest concentration of vitamin C used was not sufficient to alleviate this stress factor.

According to the NRC (2011), the requirement of vitamin C by *L. vannamei* ranges from 90 to 190 mg / kg of diet. Even for animals fed

diets free from vitamin C, the requirement of this nutrient may have been met by the intake, although restricted, of natural food present in the nursery.

As there was no effect of different sources and levels of vitamin C on growth, survival and health of shrimp in all treatments, it is suggested that the inclusion of vitamin C in concentrations above the assessed can reduce or eliminate the possible stress on shrimp above 5 g caused by space restriction

CONCLUSIONS

The use of vitamin C to the level of 260 mg/ shows no improvement in performance and health of *Litopenaeus vannamei* shrimp in the growing phase.

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