Mem. Fac. Fish., Kagoshima Univ.
Vol. 25, No. 1, pp. 53~57 (1976)

Nutritional Requirements of Prawn–VI

Requirement for Ascorbic Acid*

Maguy GUARY,** Akio KANAZAWA,*** Noriho TANAKA****
and Hubert J. CECCALDI**

Abstract

The ascorbic acid requirement of prawn, *Penaeus japonicus* BATE, was investigated by the feeding trials using the artificial diet. The prawn was fed on the diet containing 0, 20, 200, 1000 or 2000 mg of ascorbic acid per 100 g of dry diet.

From these results, the dietary ascorbic acid was found to be effective for the survival, growth, and molting of prawn. The requirement for ascorbic acid of prawn was about 500–1000 mg per 100 g of dry diet, under the experimental conditions used.

The need for ascorbic acid in the diet of some species of fish has been discussed by McLAREN *et al.* (1947)[1], HALVER (1957)[2], KITAMURA *et al.* (1965[3], 1967[4]), DUPREE (1966)[5], HALVER *et al.* (1969)[6], SAKAGUCHI *et al.* (1969)[7], MANN (1970)[8], PRIMBS and SINNHUBER (1971)[9], ARAI *et al.* (1972)[10], and YONE and FUJII (1974)[11]. However, little is known about the ascorbic acid requirement in crustaceans. COLLIER *et al.* (1956)[12] have reported that the barnacle, *Balanus* sp., immediately initiated copulating activities, when they were exposed to a concentration of 0.014 mg of ascorbic acid per liter of sea water. KITABAYASHI *et al.* (1971)[13] have shown that the growth of the prawn, *Penaeus japonicus,* was accelerated by the diet containing 3.75% of glucose and 0.22% of ascorbic acid. Recently, the authors have demonstrated the inactive ability for ascorbic acid biosynthesis in crustaceans[14]. This result suggests the necessity of an exogenous source of ascorbic acid for normal growth of crustaceans.

In the present paper, an attempt to clarify the requirement of ascorbic acid in the prawn, *P. japonicus,* is realized by the feeding trials using the artificial diet.

---

* This article was presented at the Meeting of the Japanese Society of Scientific Fisheries, Kochi, on October 10th, 1972, and is a part of Doctorate Thesis in Oceanography of M. GUARY (Marseille University, 1974).

** Laboratoire de Biochimie et Ecologie des Invertébrés Marins, EPHE, Station Marine d'Endoume, Marseille, France.

*** Laboratory of Fisheries Chemistry, Faculty of Fisheries, Kagoshima University, Kagoshima, Japan.

**** Present address: Department of Hygiene, Faculty of Medicine, Kagoshima University, Kagoshima, Japan.

Experimental

Prawn. The prawn, *P. japonicus* BATE, 0.2–0.7 g in body weight, spawned in the Kagoshima Prefectural Fisheries Experimental Station was used in this experiment.

Diet. The basal diet was composed of the formula devised by KANAZAWA *et al.* (1970[15], 1971[16], 1976[17]). Each diet containing different amounts of ascorbic acid was tested on two groups of 15–18 animals for 30–37 days. At the end of experiment, the percent gain of body weight, survival, number of molt, and ascorbic acid content in the hepatopancreas of prawn were determined.

The number of molt per prawn is given by the following formula: where x_i and y_i indicate the number of prawns survived and molted at i days, respectively; n shows the experimental period in days.

$$\text{Number of molt per prawn during the } n \text{ day period} = \sum_{i=1}^{n} \frac{y_i}{x_i}$$

The details of the methods for preparation of diet and for feeding were essentially the same as reported by KANAZAWA *et al.* (1970[15], 1971[16], 1976[17]).

Determination of ascorbic acid. The amount of ascorbic acid in the hapatopancreas of prawn was determined by the method of FUJITA *et al.* (1969)[18] using thin-layer chromatography (TLC) modified 2,4-dinitrophenylhydrazine method of ROE *et al.* (1948)[19]. The hepatopancreas was homogenized with metaphosphoric acid. After oxidation with 2,6-dichlorophenolindophenol, thiourea and 2,4-dinitrophenylhydrazine were added, and incubated at 37°C for 3 hr. The osazones produced were extracted with ethyl acetate and subjected to TLC using Kiesel gel G with toluene-acetone-5% acetic acid (2: 1: 1). The fraction corresponding to ascorbic acid was dissolved in sulfuric acid and determined photometrically at 530 mμ.

Results

The results of the feeding experiment of prawn (weighing 0.2 g) maintained on the diets containing 0, 20, 200 or 2000 mg of ascorbic acid per 100 g of dry diet are shown in Table 1. After 37 days feeding, the survival and growth of prawn fed on the diet containing 2000 mg of ascorbic acid were distinctly better than those of the other groups. No deficiency symptoms were observed on the prawns used in this experiment.

To decide the requirement of ascorbic acid, further the prawn, weighing 0.7 g, was fed on the diets supplemented with 0, 20, 200, 1000, 2000 or 4000 mg of ascorbic acid per 100 g of dry diet for 30 days. As shown in Table 2, the requirement for ascorbic acid was estimated to be about 1000 mg per 100 g of diet from the body weight gain. The concentration of ascorbic acid in the hepatopancreas of prawn at the

Table 1. Effect of ascorbic acid levels in the diet on the growth and survival of prawn, weighing 0.2 g.

Ascorbic acid added (mg/100 g of dry diet)	Feeding period (days)	No. of prawn at start	Survival (%)	Percent gain
0	37	18	72	205
20	37	18	83	235
200	37	18	83	244
2000	37	18	94	280

Table 2. Effect of ascorbic acid levels in the diet on the growth and molt of prawn, weighing 0.7 g.

Ascorbic acid added (mg/100 g of dry diet)	Feeding period (days)	No. of prawn at start	Percent gain	No. of molt per prawn	Ascorbic acid content in the hepatopancreas (mg/100 g of fresh tissue)
0	30	15	49.2	1.09	2.07
20	30	15	59.2	1.37	2.17
200	30	15	74.2	1.30	2.05
1000	30	15	98.8	1.71	4.37
2000	30	15	93.4	1.85	6.89
4000	30	15	89.4	1.23	5.14

end of the experiment showed the highest level when the prawn was fed on the diet containing 2000 mg of ascorbic acid per 100 g of diet.

Discussion

In the present experiments, the dietary ascorbic acid requirement of prawn, *P. japonicus*, appeared to be about 1000 mg of ascorbic acid per 100 g of diet from the growth, and about 2000 mg from the ascorbic acid content in the hepatopancreas. KITAMURA (1969)[20] has reported that ascorbic acid in the diet of fish was unstable and easily destroyed. VANDERZANT *et al.* (1962)[21] have also shown that 25–90% of ascorbic acid in the diet of insect was lost during a 10-day period at 29°C. As to the artificial diet of prawn, about 40% of ascorbic acid added in the diet was destroyed in preparing of it, especially by heating. However, the decrease of ascorbic acid in the diet during the storage in a refrigerator was very little. From the decrease rate of ascorbic acid in the diet, the net requirement for ascorbic acid of prawn was thought to be about 500 mg per 100 g of diet. The value was slightly high as compared with 220 mg per 100 g of diet in prawn reported previously[13] and 10–200 mg per 100 g of diet in trout[1,3,6], and also similar to 200–400 mg per 100 g of diet in insect[22].

It has been suggested that ascorbic acid may play an important role for molting of insect[23]. In the present study, the increase of the number of molt was also observed in proportion to the addition of ascorbic acid.

There are a number of reports concerning the ascorbic acid content in crustaceans. Recently, the authors have reported on the variations of ascorbic acid during the ovarian development and molting cycle in shrimp, *Palaemon serratus*[24], and the variations of ascorbic acid in the eggs of crab, *Cancer pagurus*, and shrimp, *P. serratus*, during embryogenesis[25]. It is suggested that the dietary requirement for ascorbic acid in prawn may vary at each stage of molting cycle.

Acknowledgements

The authors wish to thank Dr. J.-C. GUARY, Laboratoire de l'Ecole Pratique des Hautes Etudes, Station Marine d'Endoume, Marseille, and Dr. S. TESHIMA, Laboratory of Fisheries Chemistry, Faculty of Fisheries, Kagoshima University for their helpful advices. The authors are also indebted to the Kagoshima Prefectural Fisheries Experimental Station for supplying the prawn.

References

1) MCLAREN, B.A., E. KELLER, D. J. O'DONELL, and C. A. ELVEHJEM (1947): *Arch. Biochem.*, **15**, 167–178.
2) HALVER, J. E. (1957): *J. Nutrition*, **62**, 225–243.
3) KITAMURA, S., S. OHARA, T. SUWA, and K. NAKAGAWA (1965): *Bull. Jap. Soc. Sci. Fish.*, **31**, 818–824.
4) KITAMURA, S., T. SUWA, S. OHARA, and K. NAKAGAWA (1967): *ibid.*, **33**, 1120–1125.
5) DUPREE, H. K. (1966): *Technical Papers of the Bureau of Sport Fisheries and Wildlife*, No. 7, 1–12.
6) HALVER, J. E., L. M. ASHLEY, and R. R. SMITH (1969): *Trans. Amer. Fish. Soc.*, **98**, 762–771.
7) SAKAGUCHI, H., F. TAKEDA, and K. TANGE (1969): *Bull. Jap. Soc. Sci. Fish.*, **35**, 1201–1206.
8) MANN, H. (1970): *Fette, Seifen, Anstrichm.*, **72**, 1079–1083.
9) PRIMBS, E. R. J. and R. O. SINNHUBER (1971): *Prog. Fish-Cult.*, **33**, 141–149.
10) ARAI, S., T. NOSE, and Y. HASHIMOTO (1972): *Bull. Freshwater Fish. Res. Lab.*, **22**, 69–83.
11) YONE, Y. and M. FUJII (1974): *Report Fish. Res. Lab., Kyushu Univ.*, No. 2, 25–32.
12) COLLIER, A., S. RAY, and W. B. WILSON (1956): *Science*, **124**, 200.
13) KITABAYASHI, K., K. SHUDO, K. NAKAMURA, and S. ISHIKAWA (1971): *Bull. Tokai Reg. Fish. Res. Lab.*, No. 65, 109–118.
14) KANAZAWA, A., M. GUARY, and N. TANAKA: *Comp. Biochem. physiol.*, in press.
15) KANAZAWA, A., M. SHIMAYA, M. KAWASAKI, and K. KASHIWADA (1970): *Bull. Jap. Soc. Sci. Fish.*, **36**, 949–954.
16) KANAZAWA, A., N. TANAKA, S. TESHIMA, and K. KASHIWADA (1971): *ibid.*, **37**, 211–215.
17) KANAZAWA, A., S. TESHIMA, and N. TANAKA (1976): *Mem. Fac. Fish., Kagoshima Univ.*, **25**, 47–51.
18) FUJITA, A., F. HIROSE, and Y. UCHIYAMA (1969): *Vitamins* (Japan), **40**, 17–26.
19) ROE, J. H., M. B. MILLS, M. J. OESTERLING, and C. M. DAMRON (1948): *J. Biol. Chem.*, **174**, 201–208.
20) KITAMURA, S. (1969): *Gyobyō Kenkyū*, **3**, 73–92.

21) VANDERZANT, E. S., M. C. POOL, and C. D. RICHARDSON (1962): *J. Insect Physiol.*, **8**, 287–297.
22) KAMANO, S. (1964): *Japanese Jour. Appl. Ent. Zool.*, **8**, 101–105.
23) SATO, Y. (1970): *Vitamins* (Japan), **42**, 50.
24) GUARY, M., H. J. CECCALDI, and A. KANAZAWA (1975): *Marine Biology*, **32**, 349–355.
25) GUARY, M. and J.-C. GUARY (1975): *ibid.*, 32, 357–363.