Comparison of L-ascorbyl-2-polyphosphate with L-ascorbyl-2-sulfate in Meeting Vitamin C Requirements of Juvenile Grass Shrimp *Penaeus monodon*

Tsai-Shen Hsu and Shi-Yen Shiau[†]

Department of Marine Food Science, National Taiwan Ocean University, Keelung, Taiwan 202, ROC

(Received January 28, 1997)

A growth experiment was conducted to compare the relative efficacy of L-ascorbyl-2-polyphosphate (C2PP) and L-ascorbyl-2-sulfate (C2S) in supplying the dietary vitamin C requirement for juvenile grass shrimp *Penaeus monodon*. Purified diets with 6 levels of either supplemental C2PP (0, 120, 200, 800, 2000 and 4000 mg/kg diet) or C2S (0, 30, 50, 200, 500 and 1000 mg/kg diet) were fed to *P. monodon* (mean weight 0.79 ± 0.08 g) for 8 weeks. Each diet was fed to three replicate groups of shrimp. Results indicated that shrimp fed diets containing ≥ 22.81 mg of C2PP/kg or ≥ 72.41 mg of C2S/kg diet had significantly ($p < 0.05$) higher weight gain than shrimp fed the unsupplemented control diet. Feed conversion ratio (FCR) was poor in shrimp fed the diet lacking supplementary vitamin C. Shrimp fed diets supplemented with 126.90 and 292.84 mg of C2PP/kg or ≥ 72.41 mg of C2S/kg diet had a higher survival than shrimp fed the unsupplemented control diet. Ascorbic acid concentration in hepatopancreas was higher in shrimp fed diets supplemented with C2PP and C2S than those fed the unsupplemented control diet, and the concentrations were generally proportional to the level of ascorbic acid supplementation in the diet. Weight gain analyzed by broken-line regression indicated that the dietary level of ascorbic acid from each source for juvenile *P. monodon* is 29.27 mg of ascorbic acid/kg diet for C2PP and 73.83 mg of ascorbic acid/kg diet for C2S suggesting that C2S is about 40% as effective as C2PP in meeting the vitamin C requirements.

Key words: ascorbic acid, shrimp, *Penaeus monodon*, derivatives

L-Ascorbic acid (C1) is the source of vitamin C traditionally used in shrimp diets. The requirement of C1 for optimum growth of *Penaeus japonicus*, *P. californiensis* and *P. monodon* has been reported to be 3,000 mg/kg diet¹⁾ or 10,000 to 20,000 mg/kg diet,²⁾ 2,000 mg/kg diet³⁾ and 2,000 mg/kg diet,⁴⁾ respectively.

C1 is unstable, and ascorbate activity is lost during processing and storage of practical diets due to exposure to high temperature, oxygen and light.⁵⁻⁷⁾ Shiau and Hsu⁸⁾ found that about 75% of the original amount of supplemented C1 in shrimp feed can be lost during processing at ambient temperature. Attempts to minimize loss of vitamin C activity in shrimp feeds have been made through the use of alternative forms of ascorbic acid.

Studies have shown that alternative forms of ascorbic acid can be effectively used to satisfy the requirement of penaeid shrimp. For example, the requirement for ascorbic acid in *P. monodon* decreased from 2,000 mg/kg diet when C1 was the source⁴⁾ to 210 mg/kg diet when L-ascorbyl-2-polyphosphate (C2PP) was the source.⁹⁾ The requirement was additionally reduced to 100-200 mg/kg diet when the phosphorylated Mg salt form (C2P-Mg)¹⁰⁾ was used and to 40 mg/kg with L-ascorbyl-2-monophosphate (C2MP).¹¹⁾ *P. japonicus* requires 215-430 mg of C2PMg/kg diet¹²⁾ and *P. vannamei* requires 90-120 mg of C2PP/kg diet.¹³⁾

Strict comparison of the potency of each source of ascor-

bic acid from these studies is probably misleading, because the experimental conditions such as the duration of the study, the initial weight of the shrimp, the water temperature, etc. were not the same. Furthermore, the data used to quantify the vitamin C requirements vary, some results were based on the supplementation level, whereas others were based on dietary levels that were actually determined. Limited information is available relative to the comparative biopotency of different vitamin C sources for an individual species under the same rearing conditions. Recently, Shiau and Hsu¹¹⁾ have reported that L-ascorbyl-2-sulfate (C2S) is about 25% as effective as C2MP in satisfying the vitamin C requirement of *P. monodon*. The purpose of this study was to compare the biopotency of C2PP to C2S in satisfying the vitamin C requirement of juvenile grass shrimp, *P. monodon*.

Materials and Methods

Diet Preparation

The composition of the experimental diets is given in Table 1. This formulation is similar to that of Shiau and Chou¹⁴⁾ which has been shown to be adequate for *P. monodon*. The vitamin mixture was similar to that used by Shiau and Hsu,⁸⁾ except that it did not contain vitamin C. L-ascorbyl-2-sulfate (C2S, 48% activity, Pfizer Inc., New York, NY, USA) and L-ascorbyl-2-polyphosphate (C2PP,

[†] To whom all correspondence should be addressed

Table 1. Composition of experimental diets for *Penaeus monodon*

Ingredients	Percent dry weight
Casein ^{*1}	45.7
Dextrin	20.0
Amino acid mix ^{*2}	3.0
Glucosamine HCl	0.8
Sodium citrate	0.3
Sodium succinate	0.3
CMC ^{*3}	2.5
Sodium hexametaphosphate	1.0
Cholesterol	0.5
Fish oil	5.0
Mineral mix ^{*4}	8.6
Vitamin mix ^{*5}	2.7
Alpha-cellulose	9.6

^{*1} Vitamin-free, protein content: 85.32%.

^{*2} Amino acid mix: Glycine 0.6%, Glutamic acid 0.6%, Betain 1.2%, Alanine 0.6%.

^{*3} CMC: Carboxymethylcellulose.

^{*4} Mineral mix: according to Shiau and Chou (1991).

^{*5} Vitamin mix: according to Shiau and Hsu (1993).

25% activity, Roche Rovimix Stay-C 25, Switzerland) were each added to the basal diet at the expense of small amounts of cellulose to give concentrations of 0, 30, 50, 200, 500, 1000 mg/kg and 0, 120, 200, 800, 2000, 4000 mg/kg diet, respectively. The diets were prepared by thoroughly mixing the ingredients with oil and then adding water until a stiff dough was produced. The dough was then passed through a mincer fitted with a 1.2 mm die and resulting "spaghetti-like" strings were dried at 25°C using both an air-conditioner and electrical fan. After drying, the diets were broken up, sieved to an appropriate pellet size and stored at -20°C. The actual concentrations of ascorbic acid were determined in the experimental diets three times at 0, 3, 6 wk during the feeding experiment and the average values are presented in Table 2. Levels of dietary C2S were determined as described previously.^{8,11} Dietary C2PP was analyzed according to Schuep and Keck.^{*1} Five grams of diet was weighed into a 100 ml Erlenmeyer flask and 50 ml of extractant (1% metaphosphoric acid containing 0.2% dithiothreitol) was then added. The suspension was stirred for 20 min at room temperature with a magnetic stirrer. An aliquot of the suspension was then centrifuged for 10 min at 3000 × g at 5°C. Two ml of clear su-

pernatant was then mixed with 1 ml of acetate buffer (pH 4.8) containing 0.2% dithiothreitol and 1 ml of acidic phosphatase solution (dissolve 40 mg of acidic phosphatase in 10 ml of acetate buffer-pH 4.8 containing 0.2% dithiothreitol). The mixture was kept at room temperature for 6 h and clarified if necessary, by centrifugation prior to direct quantification by HPLC. The conditions of HPLC analysis were the same as those previously described.¹¹ The diets were prepared and stored as described by Shiau *et al.*¹⁵

Experimental Procedure

Juvenile *P. monodon* were supplied from the TungKang Marine Laboratory (TungKang, Pingtung, Taiwan). Upon arrival, they were acclimated to laboratory conditions for 3 weeks in a plastic tank (74W × 95L × 45H cm) and fed an ascorbic acid-free basal diet to acclimatize them to the experimental diet and to deplete the body stores of ascorbic acid. At the beginning of the experiment, ten shrimp with an average wet weight of 0.79 ± 0.08 g were stocked in each experimental aquaria (60W × 60L × 45H cm). Each experimental diet was fed to three aquaria of shrimp. Each aquarium was provided with continuous aeration. Seventy-five percent of the water in each aquarium was exchanged weekly to remove impurities and maintain water quality. Dissolved oxygen was monitored weekly and was > 7.5 ppm throughout the experimental period. Water temperature ranged from 27 to 29°C, pH from 6.3 to 6.5 and salinity from 19 to 21 ppt. A photoperiod of 12 h light, 12 h dark (08:00 to 20:00) was used. Shrimp were fed their respective diet at a rate of 8% of body weight per day. This daily ration was subdivided into two equal feedings at 08:00 and 17:30 h. Shrimp were weighed once every two weeks and the daily ration adjusted accordingly. The duration of the study was 8 weeks. At the end of the feeding trial, the shrimp were weighed. Growth (as measured by the percentage of body weight gain) and feed conversion ratio (FCR) were calculated as described previously.¹⁶ After the final weighing, the hepatopancreas was removed and pooled for ascorbic acid concentration determination as described previously¹¹ and analyzed by the same method described here for dietary analysis.

Statistical Analysis

Data were analyzed by one-way ANOVA (SAS/PC pro-

Table 2. Ascorbic acid concentration (mg/kg diet) in diets containing different ascorbic acid sources

	0	120	200	800	2000	4000
C2PP						
C2PP added	0	120	200	800	2000	4000
Free ascorbic acid added*	—	30	50	200	500	1000
Analyzed C2PP in finished diet (ascorbic acid equivalent)	—	16.59	22.81	126.90	292.84	595.56
C2S						
C2S added	0	30	50	200	500	1000
Free ascorbic acid added*	—	14.4	24.0	96.0	240.0	480.0
Analyzed C2S in finished diet (ascorbic acid equivalent)	—	10.82	17.68	72.41	191.12	363.01

* Calculated ascorbic acid equivalency of ascorbyl-ester based on manufacturers' declarations of the source materials.

^{*1} W. Schuep and E. Keck: The analysis of ascorbic acid derivatives, in: Proc. Smp. Ascorbic acid in Domestic Animals, 9-12 Oct. 1990, Ittingen, Switzerland, Basel, Switzerland: F. Hoffmann-La Roche Ltd.

gram) to test for difference among treatments. When ANOVA identified significant differences among groups, multiple comparison among means were made with Duncan's new multiple range test. Statistical significance was determined by setting the aggregate type I error at 5% ($p < 0.05$) for each set of comparisons. Broken-line analysis was used to estimate the level of C2PP and C2S needed for maximal growth.¹⁷⁾

Results

Percent weight gain, FCR and survival are summarized in Table 3. In the C2PP group, the weight gains were the highest for shrimp fed diets supplemented with 292.84 mg ascorbic acid/kg, followed by 126.90, 595.56, 22.81 and 16.59 mg/kg, then by the unsupplemented control group. The differences between ≥ 22.81 mg/kg and the unsupplemented control group were significant ($p < 0.05$). In the C2S group, the weight gains were the highest for shrimp fed diets supplemented with 191.12 mg ascorbic acid/kg, followed by 363.01, 72.41, and intermediate for those fed diets supplemented with 17.68 and 10.82 mg/kg, and the lowest for shrimp fed the diet without ascorbic acid supplementation. The differences between ≥ 72.41 mg/kg and unsupplemented control group were significant. FCR values generally followed the weight gain data.

Shrimp fed the diets supplemented with ascorbic acid generally had higher survival than those fed the unsupplemented control diet. In the C2PP group, shrimp fed 126.90 mg ascorbic acid/kg had the highest survival, followed by 292.84 mg/kg, then 595.56, 22.81 mg/kg, and 16.59 mg/kg. The unsupplemented control group showed the lowest survival. The differences between 126.90 and

16.59 mg/kg, the unsupplemented control diet as well as those between 292.84 mg/kg and unsupplemented control diet were significant. In the C2S group, the highest survival was observed in shrimp fed diet supplemented with 191.12 mg ascorbic acid/kg, followed by 363.01 and 72.41 mg/kg, then 17.68 mg/kg and 10.82 mg/kg. The lowest survival was obtained in shrimp fed the unsupplemented control diet. The differences between 191.12 mg ascorbic acid/kg and 10.82 mg/kg, the unsupplemented control diet as well as those between 363.01, 72.41 mg/kg and the unsupplemented control diet were significant.

Ascorbic acid concentrations in hepatopancreas of *P. monodon* fed diets containing various levels of C2PP and C2S are presented in Table 4. Generally speaking, the hepatopancreatic ascorbic acid concentrations of the shrimp increased as the dietary ascorbic acid supplementation level increased in C2PP and C2S.

When broken-line analysis was used for estimating the adequate ascorbic acid level of C2S and C2PP for maximal growth, as shown in Fig. 1, the regression equations used were $Y = 103.7568 + 1.1987X$ and $Y = 191.8962 - 0.0050X$ for C2S and $Y = 73.4162 + 3.7346X$ and $Y = 182.1943 + 0.0188X$ for C2PP. Because the breakpoints were at 73.83 mg/kg diet and 29.27 mg/kg diet gave the least mean square error, these values are estimates of the amount of each source of ascorbic acid to provide maximal growth of *P. monodon* juveniles.

Discussion

The present study permits a comparison of the biopotency of C2S and C2PP and an estimation of the dietary C2S

Table 3. Weight gain, feed conversion ratio (FCR) and survival of shrimp fed different diets for 8 weeks^{*1,2}

C2PP group	Analyzed ascorbic acid concentrations (mg/kg diet)					
	0	16.59	22.81	126.90	292.84	595.56
Weight gain (%)	76.58 ± 19.32 ^a	123.77 ± 12.31 ^{ab}	167.04 ± 51.20 ^b	195.93 ± 10.11 ^b	199.62 ± 55.25 ^b	185.72 ± 53.30 ^b
FCR ^{*3}	3.57 ± 0.24 ^b	2.86 ± 0.30 ^a	2.56 ± 0.20 ^a	2.33 ± 0.27 ^a	2.27 ± 0.24 ^a	2.50 ± 0.30 ^a
Survival (%)	33.33 ± 11.11 ^a	40.34 ± 12.83 ^{ab}	48.15 ± 6.42 ^{abc}	55.56 ± 0.00 ^c	51.85 ± 6.42 ^{bc}	48.15 ± 6.42 ^{abc}
C2S group	Analyzed ascorbic acid concentrations (mg/kg diet)					
	0	10.82	17.68	72.41	191.12	363.01
Weight gain (%)	76.58 ± 19.32 ^a	128.48 ± 49.07 ^{ab}	148.68 ± 54.11 ^{ab}	183.25 ± 58.52 ^b	204.93 ± 18.10 ^b	184.36 ± 56.31 ^b
FCR ^{*3}	3.57 ± 0.24 ^c	2.86 ± 0.29 ^b	2.63 ± 0.27 ^{ab}	2.33 ± 0.30 ^a	2.13 ± 0.21 ^a	2.38 ± 0.22 ^a
Survival (%)	33.33 ± 11.11 ^a	40.34 ± 6.41 ^{ab}	44.44 ± 0.00 ^{abc}	48.15 ± 6.42 ^{bc}	51.85 ± 6.42 ^c	48.15 ± 6.42 ^{bc}

^{*1} Initial body weight of shrimp was 0.79 ± 0.08 g.

^{*2} Values are means of 3 groups of shrimp, with 10 shrimp per group (n=3). Values in a row with different superscripts are significantly different ($p < 0.05$).

^{*3} FCR = Total feed intake (g) / (Final weight (g) - initial weight (g)).

Table 4. Ascorbic acid concentrations in hepatopancreas of *P. monodon* after fed different diets for 8 weeks^{*1,2}

C2PP group	Analyzed ascorbic acid concentrations (mg/kg diet)					
	0	16.59	22.81	126.90	292.84	595.56
Hepatopancreas (C1 µg/g)	8.06 ± 1.51 ^a	25.07 ± 3.19 ^b	39.64 ± 9.84 ^c	54.85 ± 5.49 ^d	63.16 ± 7.36 ^{de}	72.53 ± 9.22 ^e
C2S group	Analyzed ascorbic acid concentrations (mg/kg diet)					
	0	10.82	17.68	72.41	191.12	363.01
Hepatopancreas (C1 µg/g)	8.06 ± 1.51 ^a	13.26 ± 3.38 ^b	21.50 ± 4.21 ^c	29.78 ± 4.37 ^d	38.03 ± 6.30 ^e	43.76 ± 3.66 ^e

^{*1} Initial body weight of shrimp was 0.79 ± 0.08 g.

^{*2} Values are means of 3 groups of shrimp, with 10 shrimp per group (n=3). Values in a row with different superscripts are significantly different ($p < 0.05$).

Fig. 1. Effect of L-ascorbyl-2-sulfate (C2S) and L-ascorbyl-2-polyphosphate (C2PP) supplementation on the increase in weight gain per shrimp.

Each point represents the mean of three groups of shrimp, with 10 shrimp per group ($n=3$). Requirements derived with the broken-line method for weight gain percentage are 73.83 mg/kg diet of C2S and 29.27 mg/kg diet of C2PP.

and C2PP requirement of juvenile *P. monodon* for maximum weight gain. Our results demonstrate that the requirement dietary levels of the two different sources of ascorbic acid for *P. monodon* were C2PP (29.27 mg/kg) < C2S (73.83 mg/kg). Chen and Chang⁹ have reported that the adequate dietary ascorbic acid from C2PP for juvenile *P. monodon* was about 210 mg/kg diet. One should note that the requirement obtained from Chen and Chang⁹ was based on the level of supplementation of C2PP. When the analyzed value of the present study is converted to the supplementation level, the analyzed value of 22.81 mg/kg refers to 200 mg/kg of supplementation level (data not shown) indicating that the results of the two studies are comparable.

A comparison between the different ascorbic acid derivatives for an individual aquatic species would be useful. In the present study, the maximum attainable response of the two forms of ascorbic acid with respect to the growth response of *P. monodon* were similar. When the average weight gains of the shrimp fed diets supplemented with different sources of ascorbic acid in reaching the plateau were calculated, values of 193.8 and 190.0% with C2PP and C2S, respectively, were obtained (Fig. 1). The requirement of *P. monodon* for C2PP and C2S were 29.27 and 73.83 mg/kg diet based on mg equivalent of ascorbic acid/kg for C2PP and C2S, respectively, indicating that C2S was about 40% as effective as C2PP in meeting the vitamin C requirements.

When the hepatopancreas ascorbic acid concentration was plotted against the analyzed ascorbic acid concentration (figure not shown), the hepatopancreas ascorbic acid concentrations were higher in shrimp fed C2PP than shrimp fed C2S. The results seem to support the hypothesis of poor absorption of C2S resulting in the low activity. Meier and Gabaudan² found negligible amounts of ascorbic acid in blood of rainbow trout following stomach gavage of C2S. Dabrowski and Kock¹⁸ reported that absorption of ascorbic acid from C2S is negligible because of the absence of sulfatase in the intestinal epithelium. Murai *et al.*¹⁹ had also suggested that the rate of enzymatic hydrolysis of C2S to C1 or rapid excretion of C2S may have been the limiting factor.

The low survival (40–55%) of shrimp fed supplemented vitamin C is not unusual for shrimp raised indoors under these conditions and subjected to repeated weighing over the 8-week study. Most of the deaths that occurred in the unsupplemented group seemed to be related to incomplete molting. Cannibalism may also contribute to the mortality. However, since the level of cannibalism was similar in all dietary treatment groups in the present study, the differences in weight gain and survival among the dietary groups were not due to cannibalism.

In a previous study, we demonstrated that C2S is only about 25% as effective as C2MP in meeting the vitamin C requirement for *P. monodon*.¹¹ In that study, the requirement for maximum growth of juvenile *P. monodon* of C2S was quantified as 156.97 mg/kg diet, equivalent to 74.8 mg ascorbic acid/kg diet. This value is comparable to the result of the present study (equivalent of 73.83 mg ascorbic acid/kg diet). Thus, it is reasonable to have the effectiveness of the three sources of ascorbic acid compared together and that their biopotency for the rapid growing *P. monodon* is C2MP (1) > C2PP (64%) > C2S (25%).

Acknowledgments This work was partly supported by a grant from the National Science Council of the Republic of China, grant number NSC 86-2313-B-019-030.

References

- 1) O. Deshimaru and K. Kuroki: Studies on a purified diet for prawn-VII. Adequate dietary levels of ascorbic acid and inositol. *Nippon Suisan Gakkaishi*, **42**, 571–576 (1976).
- 2) M. Guary, A. Kanazawa, N. Tanaka, and H. J. Ceccaldi: Nutritional requirements of prawn. VI. Requirement for ascorbic acid. *Mem. Fac. Fish. Kagoshima Univ.*, **25**, 53–57 (1976).
- 3) D. V. Lightner, B. Hunter, P. C. Magarelli, Jr., and L. B. Colvin: Ascorbic acid: Nutrition requirement and role in wound repair in penaeid shrimp. *Proc. World Maricult. Soc.*, **8**, 611–623 (1979).
- 4) S. Y. Shiau and F. L. Jan: Ascorbic acid requirements of grass shrimp *Penaeus monodon*. *Nippon Suisan Gakkaishi*, **58**, 363 (1992).
- 5) J. W. Hilton, C. Y. Cho, and S. J. Slinger: Factors affecting the stability of supplemental ascorbic acid in practical trout diet. *J. Fish Res. Board Can.*, **34**, 683–687 (1977).
- 6) R. T. Lovell and C. Lim: Vitamin C in pond diets for channel catfish. *Trans. Am. Fish. Soc.*, **107**, 321–325 (1978).
- 7) A. K. Solimam, K. Jauncey, and R. J. Roberts: Stability of L-ascorbic acid (vitamin C) and its forms in fish feeds during processing,

² W. Meier and J. Gabaudan: Influence of the dietary source and dosage of vitamin C in rainbow trout (*Oncorhynchus mykiss*): histology, metabolism and immunity. Paper presented at: Symposium on Ascorbic acid in Domestic Animals, 5–9 October 1990, Zurich, Switzerland.

- storage and leaching. *Aquaculture*, **60**, 73–83 (1987).
- 8) S. Y. Shiau and T. S. Hsu: Stability of ascorbic acid in shrimp feed during analysis. *Nippon Suisan Gakkaishi*, **59**, 1535–1537 (1993).
 - 9) H. Y. Chen and C. F. Chang: Quantification of vitamin C requirements for juvenile shrimp (*Penaeus monodon*) using polyphosphorylate L-ascorbic acid. *J. Nutr.*, **124**, 2033–2038 (1994).
 - 10) M. R. Catacutan and C. R. Lavilla-Pitogo: L-Ascorbyl-2-phosphate-Mg as a source of vitamin C for juvenile *Penaeus monodon*. *Isr. J. Aquacult.-Bamidgeh*, **43**, 35–41 (1994).
 - 11) S. Y. Shiau and T. S. Hsu: Vitamin C requirement of grass shrimp, *Penaeus monodon*, as determined with L-ascorbyl-2-monophosphate. *Aquaculture*, **122**, 347–357 (1994).
 - 12) K. Shigueno and S. Itoh: Use of Mg-L-ascorbyl-2-phosphate as a vitamin C source in shrimp diets. *J. World Aquacult. Soc.*, **19**, 168–174 (1988).
 - 13) H. He and A. L. Lawrence: Vitamin C requirements of the shrimp *Penaeus vannamei*. *Aquaculture*, **114**, 305–316 (1993).
 - 14) S. Y. Shiau and B. S. Chou: Effects of dietary protein and energy on growth performance of tiger shrimp *Penaeus monodon* reared in seawater. *Nippon Suisan Gakkaishi*, **57**, 2271–2276 (1991).
 - 15) S. Y. Shiau, J. L. Chung, and C. L. Sun: Inclusion of soybean meal in tilapia (*Oreochromis niloticus* x *O. aureus*) diets at two protein levels. *Aquaculture*, **65**, 251–261 (1987).
 - 16) S. Y. Shiau, C. C. Kwok, and B. S. Chou: Optimal dietary protein level of *Penaeus monodon* reared in seawater and brackish water. *Nippon Suisan Gakkaishi*, **57**, 711–716 (1991).
 - 17) K. R. Robbins, H. W. Norton, and D. H. Baker: Estimation of nutrient requirements from growth data. *J. Nutr.*, **109**, 1710–1714 (1979).
 - 18) K. Dabrowski and G. Kock: Absorption of ascorbic acid and ascorbic sulfate and their interaction with minerals in the digestive tract of rainbow trout (*Oncorhynchus mykiss*). *Can. J. Fish. Aquat. Sci.*, **46**, 1952–1957 (1989).
 - 19) Y. Murai, J. W. Andrews, and J. C. Bauerfeind: Use of L-ascorbic acid, ethocel coated ascorbic acid and ascorbate-2-sulfate in diets for channel catfish (*Ictalurus punctatus*). *J. Nutr.*, **108**, 1761–1766 (1978).