Acta Scientiarum. Animal Sciences
ISSN: 1806-2636
eduem@uem.br
Universidade Estadual de Maringá
Brasil

Damasceno Pinheiro, Paloma; de Holanda Cavalcante, Davi; Gazzineo de Moraes, Manuella; do Carmo e Sá, Marcelo Vinícius
Calcium-free and low-calcium water production for aquaculture research
Acta Scientiarum. Animal Sciences, vol. 33, núm. 1, 2011, pp. 47-49
Universidade Estadual de Maringá
.png, Brasil

DOI: 10.4025/actascianimsci.v33i1.10686

Calcium-free and low-calcium water production for aquaculture research

Paloma Damasceno Pinheiro, Davi de Holanda Cavalcante, Manuella Gazzineo de Moraes and Marcelo Vinícius do Carmo e Sá*

*Laboratório de Ciência e Tecnologia Aquícola, Departamento de Engenharia de Pesca, Centro de Ciências Agrárias, Universidade Federal do Ceará, Campus do Pici, 60356-000, Fortaleza, Ceará, Brazil. *Author for correspondence. E-mail: marcelo.sa@ufc.br*

ABSTRACT. The present work aimed to obtain a mathematical expression to calculate the amount of EDTA required to produce calcium-free and low-calcium water ($[Ca^{2+}] < 5$ mg L^{-1}). Initially, a pilot test was carried out to confirm the effectiveness of EDTA as a chelating agent of waterborne calcium and to evaluate its effect on water pH. Five application rates of Na-EDTA (25, 50, 75, 100 and 125 mg L^{-1}) were tested. The concentration of calcium in water was determined initially (before the application of EDTA) and after one hour. Linear regression analysis was performed to find out the relationship between the desired calcium concentration in water and the required EDTA: initial calcium hardness ratio. The expression $\hat{y} = -0.0911x + 3.3628$ ($R^2 = 0.933$) was derived from the linear regression analysis carried out, in which *x* represents the desired calcium concentration in water and *y* the required EDTA: initial calcium hardness ratio.

Key words: EDTA, calcium hardness, fish culture.

RESUMO. Produção de água mole e isenta de cálcio para experimentação em aquicultura. O presente trabalho teve como objetivo determinar a quantidade de EDTA necessária para se produzir água sem cálcio e água mole ($[Ca^{2+}] < 5$ mg L^{-1}) para experimentação em aquicultura. Inicialmente, realizou-se teste-piloto para confirmar a efetividade do EDTA como agente complexante do cálcio presente na água e avaliar seu efeito no pH da água. No teste principal, cinco taxas de aplicação de Na-EDTA (25, 50, 75, 100 e 125 mg L^{-1}) foram avaliadas. A concentração de cálcio da água foi determinada inicialmente (antes da aplicação de EDTA) e após 1h. Análise de regressão linear foi feita para se descobrir a relação entre a concentração desejada de cálcio na água e a relação EDTA/dureza cálcica inicial da água necessária. A expressão $\hat{y} = -0,0911x + 3,3628$ ($R^2 = 0,933$) foi obtida a partir da regressão realizada na qual x representa a concentração desejada de cálcio na água e y a relação EDTA/dureza cálcica inicial da água.

Palavras-chave: EDTA, dureza cálcica, piscicultura.

Introduction

Calcium-free water is a prerequisite to determine the dietary calcium requirement of finfish because they are able to absorb calcium directly from water (NIYOGI; WOOD, 2004). With that purpose, experiments were carried out in finfish (ROBINSON et al., 1984, 1986, 1987). Currently, the most used chemical to experimentally produce calcium-free water is EDTA (ethylenediaminetetraacetic acid). EDTA is a synthetic compound that complexes itself with free calcium in water and withdraws it from solution (APHA, 1999).

In addition to calcium, EDTA is also capable of chelating with several others elements, including some that may be toxic to animals (OVIEDO; RODRÍGUEZ, 2003). Currently, EDTA is used routinely in the intensive culture of penaeid shrimp larvae to increase both the percentage of eggs that hatch and the survival of larvae (ROBINSON et al., 2005). Its use, however, must be with caution because EDTA can also be toxic to aquatic animals at higher concentrations (NOWACK, 2002).

Another area of research in which EDTA and calcium-free water would be important is water hardness, which is an index that indicates the concentrations of calcium and magnesium in water (SILVA et al., 2003). Likewise nutritional studies, experimental works on water hardness would also require calcium-free water and low-calcium water in order to correctly evaluate the effects of the different calcium materials on fish growth performance. Therefore, the basic knowledge about the preparation of calcium-free and low-calcium water would be valuable for aquaculture science and deserves research efforts (HOLANDA-CAVALCANTE et al., 2009).

The present work aimed to answer the following methodological question on calcium-free and low-calcium water technology: how much EDTA is required to produce calcium-free water and low-calcium water ($[Ca^{2+}] < 5$ mg L^{-1})? The effect of EDTA application on water pH was also observed.

Material and methods

The trials were performed at the Laboratório de Ciência e Tecnologia Aquícola – LCTA (Departamento de Engenharia de Pesca, Universidade Federal do Ceará, Fortaleza, Ceará State, Brazil). Initially, a preliminary trial was carried out to evaluate the effect of EDTA on water pH. In this trial, water samples of 1000 mL were treated with Na-EDTA analytical grade at 100, 250 and 500 mg L^{-1} and seven periods of observation after the EDTA application were carried out (15 min., 1, 6, 24, 48, 72h and one week after).

In the main trial, five application rates of EDTA (25, 50, 75, 100 and 125 mg L^{-1}) were tested. The concentration of calcium in water was determined initially and after one hour. There were three replications (1,000 mL-beakers) per treatment.

Tap water was used as the initial water. The initial concentrations of calcium (13.2 ± 1.1 mg L^{-1}), calcium hardness (32.9 ± 2.6 mg L^{-1} $CaCO_3$), total hardness (70.7 ± 0.64 mg L^{-1} $CaCO_3$), total alkalinity (48.0 ± 2.8 mg L^{-1} $CaCO_3$) and pH (7.2 ± 0.04) of the tap water were determined through standard methods (APHA, 1999). The same analytical methodologies were applied in the experimental water.

The pH data were analyzed by one-way analysis of variance (ANOVA) to detect whether there were significant differences between the EDTA's application rates. When the differences were significant, the means were compared two-by-two using Tukey's test (ZAR, 2009). Linear regression analysis was performed to discover the relationship between the desired concentration of calcium in water and the required EDTA: initial calcium hardness ratio. The statistical analyses were carried out with the aid of the SigmaStat 2.0 statistical software (JANDEL STATISTICS, 1995) and Microsoft Office Excel 2007 (Microsoft Corporation). The level of significance of 5% was adopted in all statistical analyses.

Results and discussion

After 15 minutes of EDTA application, the water pH decreased from 7.2 ± 0.04 to 6.14 ± 0.06 (100 mg EDTA L^{-1}), 5.19 ± 0.01 (250 mg EDTA L^{-1}) and 4.92 ± 0.03 (500 mg EDTA L^{-1}; Figure 1). The initial drop in water pH after EDTA application was already expected since EDTA is an acidic compound (HARRIS, 2007). The results of water pH for these three EDTA application rates (100, 250 and 500 mg L^{-1}) were statistically different from one another in each observation ($p < 0.05$). In addition, the statistical differences between the water pH for the three EDTA application rates remained unchanged throughout the observation period. The water pH remained stable over the time for the EDTA treatments 250 mg L^{-1} and 500 mg L^{-1} but increased over time in the EDTA application rate of 100 mg L^{-1} (Figure 1).

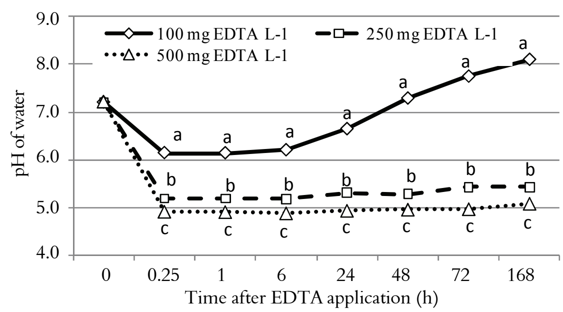

Figure 1. Water pH after Na-EDTA application in three different rates (100, 250 and 500 mg L^{-1}). Initial pH of water = 7.2 ± 0.04. In each time, means with different letters are statistically different between themselves by Tukey's test ($p < 0.05$). Each value represents the mean of three replications.

The expressive increase in water pH after 24h and beyond just for the EDTA application rate of 100 mg L^{-1} can be explained by the reaction between CO_3^{-2} and H_2O. In this case, as the EDTA withdrew Ca^{2+} from solution, CO_3^{-2} became free to react with H_2O and release OH-, according to the following reaction: $CO_3^{-2} + H_2O \rightarrow HCO_3^- + OH^-$ (BOYD, 1979). The same, however, did not happen in the EDTA application rates of 250 and 500 mg L^{-1} because the significantly higher concentrations of H^+ in the water were capable to neutralize the effect of OH^- in lowering the pH of water.

Schmidt and Brauch (2004) have also observed that sodium salts of aminopolycarboxylates, such as the one used in the present work, may have an alkaline reaction with water. As EDTA produces significant effects on water pH, it is important to use it with caution in waters intended to experimentally raise fish. Another alternative is to correct the pH of water after the EDTA application with some acidic or alkaline solution, depending on the case.

The effect of increasing EDTA application rates on water calcium hardness was performed to establish a valid mathematical expression that represented the relationship between the desired calcium concentration in water and the required EDTA: initial calcium hardness ratio. Based on the obtained data, the expression $y = -0.0911x + 3.3628$

(R^2 = 0.933) was derived from the linear regression analysis carried out (Figure 2).

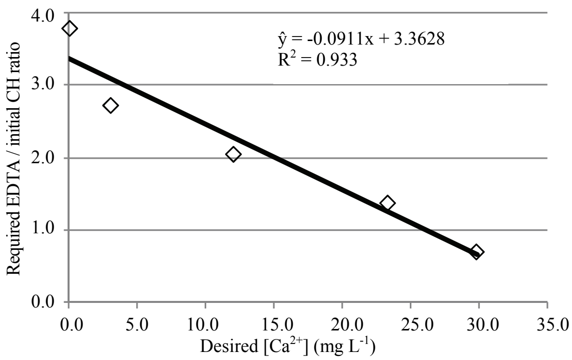

Figure 2. Relationship between the desired calcium concentration in water (mg L^{-1}) and the required EDTA: initial calcium hardness (CH) ratio. Each point represents the mean of three replications.

In that expression, *x* represents the desired calcium concentration in water and *y* the required EDTA: initial calcium hardness ratio. Therefore, once the desired calcium concentration in water is defined, the above expression gives the required EDTA: initial calcium hardness ratio, which approximately leads to it. For example, if the initial calcium hardness of water is 50.0 mg L^{-1} $CaCO_3$ and it is desired to produce calcium-free water (x = 0), the probable application rate of reagent grade Na-EDTA needed is equal to 3.3628 * 50.0 or 168.1 mg L^{-1}. If the aim is producing low-calcium water at 5 mg L^{-1} (x = 5), it would require 2.9073 * 50.0 or 145.4 mg L^{-1} of EDTA.

Conclusion

It was concluded that the required amount of EDTA to produce calcium-free and low-calcium water for aquaculture research can be derived from the following expression: y = -0.0911x + 3.3628, where x represents the desired calcium concentration in water and y the required EDTA: initial calcium hardness ratio.

References

APHA-American Public Health Association. **Standard methods for the examination of water and waste water**. 20th ed. New York: American Public Health Association, 1999.

BOYD, C. E. **Water quality in warmwater fish ponds**. Auburn: Auburn University, 1979.

HOLANDA-CAVALCANTE, D.; SOUSA-POLIATO, A.; RIBEIRO, D. C.; MAGALHÃES, F. B.; CARMO-E-SÁ, M. V. Effects of $CaCO_3$ liming on water quality and growth performance of fingerlings of Nile tilapia, *Oreochromis niloticus*. **Acta Scientiarum. Animal Sciences**, v. 31, n. 3, p. 327-333, 2009.

HARRIS, D. C. **Quantitative chemical analysis**. 7th ed. New York: W. H. Freeman and Company, 2007.

JANDEL STATISTICS. **SigmaStat statistical user's manual**. Version 2.0. San Rafael: Jandel, 1995.

NIYOGI, S.; WOOD, C. M. Kinetic analyses of waterborne calcium and cadmium transport and their interactions in the gills of rainbow trout (*Oncorhynchus mykiss*) and yellow perch (*Perca flavescens*), two fish species differing greatly in acute cadmium sensitivity. **Journal of Comparative Physiology B**, v. 174, n. 19, p. 243-253, 2004.

NOWACK, B. Environmental chemistry of aminopolycarboxylate chelating agents. **Environmental Science and Technology**, v. 36, n. 19, p. 4009-4016, 2002.

OVIEDO, C.; RODRÍGUEZ, J. EDTA: the chelating agent under environmental scrutinity. **Química Nova**, v. 26, n. 6, p. 901-905, 2003.

ROBINSON, C. B.; SAMOCHA, T. M.; FOX, J. M.; GANDY, R. L.; MCKEE, D. A. The use of inert artificial commercial food sources as replacements of traditional live food items in the culture of larval shrimp, *Farfantepenaeus aztecus*. **Aquaculture**, v. 245, n. 1-4, p. 135-147, 2005.

ROBINSON, E. H.; LABOMASCUS, D.; BROWN, P. B.; LINTON, T. L. Dietary calcium and phosphorus requirements of *Oreochromis aureus* reared in calcium-free water. **Aquaculture**, v. 64, n. 4, p. 267-276, 1987.

ROBINSON, E. H.; RAWLES, S. D.; BROWN, P. B.; YETTE, H. E.; GREENE, L. W. Dietary calcium requirement of channel catfish *Ictalurus punctatus*, reared in calcium-free water. **Aquaculture**, v. 53, n. 3-4, p. 263-270, 1986.

ROBINSON, E. H.; RAWLES, S. D.; YETTE, H. E.; GREENE, L. W. An estimate of the dietary calcium requirement of fingerling Tilapia aurea reared in calcium-free water. **Aquaculture**, v. 41, n. 4, p. 389-393, 1984.

SCHMIDT, C. K.; BRAUCH, H.-J. Impact of aminopolycarboxylates on aquatic organisms and eutrophication: overview of available data. **Environmental toxicology**, v. 19, n. 6, p. 620-637, 2004.

SILVA, L. V. F.; GOLOMBIESKI, J. I.; BALDISSEROTTO, B. Incubation of silver catfish, *Rhamdia quelen* (Pimelodidae), eggs at different calcium and magnesium concentrations. **Aquaculture**, v. 228, n. 1-4, p. 279-287, 2003.

ZAR, J. H. **Biostatistical analysis**. 5th ed. New York: Prentice Hall, 2009.

Received on July 19, 2010.

Accepted on October 20, 2010.