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Bivalve mollusk shell powder: a sustainable alternative for alkalinity and pH correction in aquaculture

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Abstract. The present study aimed to evaluate the effectiveness of the shell powder of *Anomalocardia flexuosa* and *Crassostrea* spp. as correctors of the alkalinity and pH of aquaculture water. At the end of the experiment, the alkalinity and pH values of the oyster powder treatments (82.00 ± 18.00 mg.L^{-1} of $CaCO_3$ and 8.31 ± 0.08, respectively), Venus clam powder (89.33 ± 13.20 mg.L^{-1} $CaCO_3$ and 8.35 ± 0.03, respectively), and sodium bicarbonate (166.00 ± 2.00 mg.L^{-1} $CaCO_3$ and 8.67 ± 0.02, respectively) differed ($p<0.05$) from those of the control (36 ± 0.00 mg.L^{-1} $CaCO_3$ and 7.73 ± 0.01, respectively). The oyster and Venus clam powders reacted similarly ($p>0.05$), increasing the alkalinity and pH values; however, both had higher values than those of sodium bicarbonate ($p<0.05$). The reaction time of all products was 24 h after their addition, showing a significant increase in alkalinity in the first 24 h, with stabilization at the end of 15 days. In this sense, there is great potential for the use of oyster and Venus clam powders for the correction of alkalinity and the maintenance of pH in aquaculture.

Keywords: Oyster; Venus clam; $CaCO_3$; Water quality.

Pó de concha de moluscos bivalves: uma alternativa sustentável para correção da alcalinidade e do ph na aquicultura. Resumo: O objetivo do presente estudo foi avaliar a eficácia do pó da concha do *Anomalocardia flexuosa* e da *Crassostrea* spp. como corretores da alcalinidade e pH da água de cultivo. Ao final do experimento, os valores de alcalinidade e pH para os tratamentos Pó de ostra ($82,00 \pm 18,00$ mg.L^{-1} de $CaCO_3$ e $8,31 \pm 0,08$), Pó de maçunim ($89,33 \pm 13,20$ mg.L^{-1} de $CaCO_3$ e $8,35 \pm 0,03$) e Bicarbonato de sódio ($166,00 \pm 2,00$ mg.L^{-1} $CaCO_3$ e $8,67 \pm 0,02$) diferiram ($p<0,05$) do Controle ($36 \pm 0,00$ mg.L^{-1} $CaCO_3$ e $7,73 \pm 0,01$). O pó de ostra e do maçunim reagiram de forma semelhantes ($p>0,05$) na elevação dos valores de alcalinidade e pH, entretanto, ambos diferiram do bicarbonato de sódio ($p<0,05$) que obteve valores mais elevados. O tempo de reação de todos os produtos utilizados foi de 24 horas após a sua adição, observando-se um aumento expressivo da alcalinidade nas primeiras 24 h com uma estabilização ao final de 15 dias. Neste sentido, há uma grande potencialidade na utilização do pó de ostra e de maçunim para a correção da alcalinidade e manutenção do pH na aquicultura.

Palavras-chave: Ostra; Maçunim; $CaCO_3$; Qualidade de água.

Introduction

Over time, the growing demand for fish has driven the development of more efficient methodologies that are aligned with socio-environmental issues in the aquaculture production sector (FAO 2020). However, to obtain good results, it is necessary to consider a set of factors that can affect the sector's growth. According to Leira *et al*. (2017), some of these factors include the physical and chemical characteristics of the water used for cultivation, which strongly affect the growth, reproduction, health, and survival of cultivated organisms. Thus, it can be said that a large part of the success of aquaculture projects is intrinsically linked to the quality of the water that the animals are exposed to during the cultivation period.

Among the most important limnological aspects of aquatic life, most notable is the acid-base balance of water, which is directly related to pH and alkalinity (Coldebella *et al*. 2020). Water acidity problems are not the direct result of the effects of low pH, but rather the result of the effects of low total alkalinity and the presence of acidic sediments at the bottom of the ponds (Quispesivana *et al*. 2016). To counteract these effects, bicarbonates and carbonates, which are compounds responsible for buffering the pH of the water, are added. This practice prevents large variations in pH values throughout the day, which are mainly due to the temporal patterns of photosynthesis, in conjunction with oxygen consumption, and the production of carbon dioxide by respiration. In this way, the balance in the acidity values of the water is maintained (Keppel *et al*. 2016, Queiroz & Boeira 2006).

Currently, different products are used to control the acidity and alkalinity of growing waters, with ground limestone rocks composed of calcite ($CaCO_3$), magnesia ($MgCO_3$), and/or dolomite ($CaCO_3$.Mg CO_3) being more commonly used (Leira *et al*. 2017). However, the extraction of these ores is responsible for significant environmental and social externalities, such as environmental changes, land-use conflicts, depreciation of surrounding properties, generation of degraded areas, and disturbances to urban traffic. Specifically, the extraction of limestone, which negatively impacts the speleological heritage, also uses explosives and generates excessive amounts of dust, which can be dangerous for the society surrounding the mining company. This is a concern because this mineral is usually produced in areas close to urban centers (Ferreira *et al* 2006, Rezende 2016). In light of the impacts of mineral extraction, it is necessary to find alternatives to produce new compounds that meet the needs of aquaculture, while being aligned with environmental sustainability.

With this in mind, we sought to highlight the application of different compounds rich in $CaCO_3$, such as lime, sodium bicarbonate, and powder from the shells of bivalve mollusks. The use of *Anomalocardia flexuosa* shells, according to studies by Lo Monaco *et al*. (2015), and *Crassostrea rhizophorae* shells, according to Freire *et al*. (2009), yielded suitable amounts of calcium carbonate, with values of 95.40% in *A. flexuosa*, and between 81.45% and 83.43% in *C. rhizophorae*. Another very relevant characteristic of the powder from these shells is its solubility of 0.1 $g.L^{-1}$, which presents values 10 times greater than the solubility of mineral limestone, which is 0.01 $g.L^{-1}$ (Cysy 2019). The lower solubility of mineral limestone can cause even more increases in production costs. The amount of limestone normally used in cultivation environments is 0.1 to 0.3 kilograms of limestone per m^2 (Faria & Morais 2019, Senar 2017) at a cost of $ 0.05 per kilo. Therefore, such intrinsic characteristics make it possible to suggest that shell powder can potentially be used as a corrective for the acidity and alkalinity of the water used for cultivation.

In addition, the increase in the amount of bivalve mollusks produced has enlarged the volume of discarded shells (IBGE 2020), which is responsible for the transformation of local landscapes (Mottola *et al*. 2020). Therefore, the reuse of these shells can also directly mitigate the effects of important environmental and health issues. From this perspective, this study aimed to evaluate the efficacy of powder from native oysters (*Crassostrea* spp.) and Venus clam (*A. flexuosa*) shells, as alkalinity and pH correctors of the water used in aquaculture.

Material and Methods

The study was conducted in the Carcinology Laboratory of the Federal University of Alagoas at the Penedo Teaching Unit. The experiment consisted of four treatments with three replicates each, totaling 12 experimental units (EUs), supplied with 20 L of water each. The experimental design was completely randomized and divided into four treatments: Control (without the addition of any product), Venus Clam Powder (addition of 0.22 $g.L^{-1}$ of Venus clam shell powder), Oyster Powder (addition of 0.22 $g.L^{-1}$ oyster shell powder), and Sodium Bicarbonate (addition of 0.22 $g.L^{-1}$ sodium bicarbonate). The product concentration of the treatments was determined according to the recommendation of the sodium bicarbonate manufacturer to increase alkalinity to approximately 160 $mg.L^{-1}$. All the EUs were maintained in a static system with constant aeration for 15 days.

The shells of bivalve mollusks (Venus clam and oysters) were obtained from shellfish collectors in the municipality of Barra de São Miguel/AL-Brazil. After collection, the samples were washed under running water and dried at room temperature for a period of 24 h. After this procedure, they were sent to the Soil Remediation Laboratory of the Agricultural Engineering Department of the Federal University of Sergipe, where they underwent a grinding process in a Technal Wiley knife mill to obtain powder from the shells. Thereafter, in the Benthic Ecology Laboratory of the Federal University of Alagoas (LAB – UFAL), the powder was sieved to extract the desired fraction of 0.063 mm. Following the protocol described by Lo Monaco *et al*. (2015), this powder was placed in an oven at 80ºC for a period of 48 h to eliminate moisture.

For the chemical characterization of the powder, the samples were sent to the Technological and Research Institute of the State of Sergipe (ITPS), where the CaO, MgO, and $CaCO_3$ contents were quantified using the titrimetric method. Its neutralization power (NP) was also quantified according to the method suggested by MAPA (2017). The total relative neutralizing power (TRNP) of these products was calculated using Equation 1. The reactivity (RE) was 100% as the powder used had a particle size of less than 0.30 mm, ABNT n° 50 (MAPA 2006).

$$\text{TRNP (\%)} = \text{NP} \times \text{RE}/100 \qquad \text{(Eq. 1)}$$

The addition of compounds to the EUs occurred at the beginning of the experiment. Analyses of the alkalinity and pH parameters were carried out daily and were always performed at the same time to ensure that the reaction time of the products and the levels of $CaCO_3$ incorporated into the water were precisely quantified. For this, 60 mL of water was removed from each EU with the aid of beakers; from this, 50 mL was used for pH analysis and 10 mL was for alkalinity testing. The total alkalinity was estimated by potentiometric titration using a 0.01 N sulfuric acid solution (Mackereth *et al*. 1978), and the pH was estimated with a bench-top pH meter (Alfakit, AT-355, pH meter microprocessor).

The pH and alkalinity values were subjected to normality (Shapiro-Wilks) and homoscedasticity of variance (Levene's) tests. When a normal distribution was observed, the obtained means were investigated using an analysis of variance (ANOVA – *one way*) and, in case of a significant difference, the means were compared using Tukey's test at 5% significance. The BioStat software was used.

Results

The powder from oyster and Venus clam shells was found to be rich in calcium carbonate, with concentrations above 94%, and showed high neutralizing power with values above 94% (Table I).

During the experiment, it was possible to verify the reaction time of the products. Both products showed an exponential reaction in the first 24 h after application, as shown in Figures 1A and 1B. The alkalinity of the water supply in the EUs was 28.00 ± 0.00 $mg.L^{-1}$ of $CaCO_3$ and, after 24 h of the application of oyster shell powder, Venus clam powder, and

Table I. Chemical characterization of the powder from oyster shells (Crassostrea spp.) and Venus clam shells (*Anomalocardia flexuosa*).

Sample	CaO	MgO	$CaCO_3$	NP	RE	TRNP
Oyster shell powder	53.05%	0.82%	94.73%	94.57%	100%	94.57%
Venus clamshell powder	53.44%	0.02%	95.43%	96.52%	100%	96.52%

* NP: neutralization power; RE: reactivity; TRNP: total relative neutralizing power

sodium bicarbonate, the alkalinity became 62 ± 2.00; 56.67 ± 0.94; and 153 ± 3.00 mg.L^{-1} of $CaCO_3$, respectively. This indicates that there was an increase of 34.00; 28.67; and 125.00 mg.L^{-1} of CaCO3 in the treatments Powder of Oyster, Venus Clam Shell Powder, and Sodium Bicarbonate, which translates to an increase of 121.43%, 102.39%, and 446.43%, respectively, in the first 24 h.

In all the treatments, a significant increase in alkalinity was observed during the first 24 h. After this first moment, a gradual increase occurred. When the alternative products were used, the alkalinity values were statistically lower ($p<0.05$) than those treated with sodium bicarbonate. The total reaction capacity of these products was higher than that of sodium bicarbonate, being representative until the 15th day, as shown in the results presented in Figure 1A.

When compared, oyster shell powder and Venus clam shell powder presented statistically similar results for alkalinity ($p>0.05$). However, these results were statistically different from those of sodium bicarbonate ($p<0.05$), which showed the highest efficiency with an increase of 492.85% of mg.L^{-1} $CaCO_3$ in the water, with respect to the initial alkalinity of the experiment water. Nevertheless, the alternative products proved to be quite effective in increasing the total alkalinity of the water, as the oyster shell powder presented an increase of 192.86% and the Venus clam powder showed an increase of 219.03% mg.L^{-1} $CaCO_3$ at the end of the 15 day-period of testing (Table II).

At the end of the experiment, there was no significant difference ($p>0.05$) in the water pH values between the treatments with oyster and Venus clam powders, which both showed an increase in the initial values of 1.31 and 1.35, respectively. In comparison with the control, both treatments using shell powder showed a significant difference ($p<0.05$), showing higher pH values than in the control treatment (Table III).

During the experiment, the treatments using the shell powder from oysters and Venus clams reached the highest pH value (8.42 ± 0.02) after 24 h of applying the products. This value decreased by an average of 0.07 ± 0.01 until the 3rd day. From the 8th day onwards, the mean daily variation was similar to that of the treatment with bicarbonate (0.02 ± 0.01) when it stabilized. The treatment with sodium bicarbonate reached its highest value (8.92 ± 0.01) 48 h after the application of the product, which then decreased by an average of 0.38 ± 0.40 until the 4th day (Figure 1B). The variation in water pH throughout the experiment was greater with the use of bicarbonate than with the alternative products.

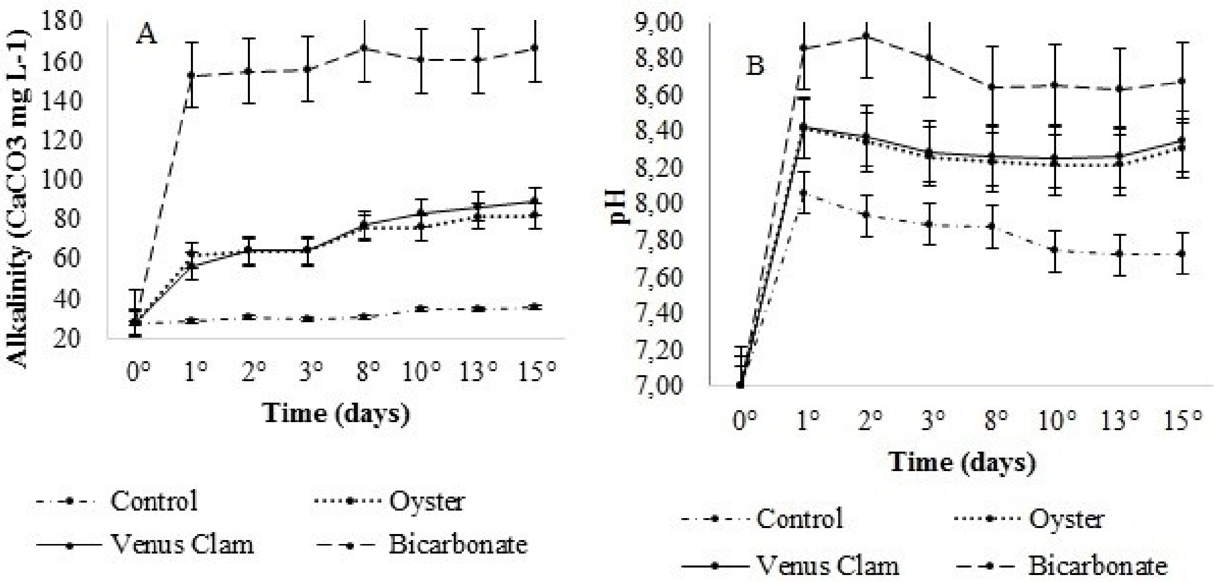

Figure 1. (A) Water alkalinity over 15 days of product reaction: oyster shell powder, Venus clam shell powder, and sodium bicarbonate, each at a concentration of 0.22 g.L^{-1}, and the control; (B) pH of water over 15 days of product reaction: oyster shell powder, Venus clam shell powder, and sodium bicarbonate, each at a concentration of 0.22 g.L^{-1}, and the control.

Table II. Water alkalinity values (± standard deviation) at the end of 15 days of product reaction: oyster shell powder, Venus clam shell powder, and sodium bicarbonate, each at a concentration of 0.22 g.L^{-1}, and the control.

Treatment	Alkalinity (mg.L^{-1} de $CaCO_3$) 0° day** / 15° day
Control	28.00 ± 0.00 / 36.00 ± 0.00 c
Sodium Bicarbonate	28.00 ± 0.00 / 166.00 ± 2.00 a
Oyster Shell Powder	28.00 ± 0.00/ 82.00 ± 18.00 b
Venus Clam Powder	28.00 ± 0.00 / 89.33 ± 13.20 b

* Different letters in the same column indicate statistically significant differences according to Tukey's test at 5% probability. **Alkalinity of water before product application.

Table III. Water pH values (± standard deviation) at the end of 15 days of product reaction: oyster shell powder, Venus clam shell powder, and sodium bicarbonate, each at a concentration of 0.22 g.L^{-1}, and the control.

Treatment	pH 0° day** / 15° day
Control	7.00 ± 0.00 a / 7.73 ± 0.01 c
Sodium Bicarbonate	7.00 ± 0.00 a/ 8.67 ± 0.02 a
Oyster Shell Powder	7.00 ± 0.00 a / 8.31 ± 0.08 b
Venus Clam Powder	7.00 ± 0.00 a / 8.35 ± 0.03 b

* Different letters in the same column indicate statistically significant differences according to Tukey's test at 5% probability. **pH of the water before product application.

Discussion

The high neutralizing power of the alternative products in the present study gives them a high TRNP value, which characterizes them as being suitable for use as water acidity correctors. The TRNP of oyster shells and Venus clam shell powders observed in the present study were similar to those found by Lo Monaco *et al*. (2012) for the shells of *A. flexuosa* (95.4%) and were superior to that of the shells of *C. rhizophorae* (86.5%) (Lo Monaco *et al*. 2015).

According to the proportion of MgO found, it is possible to classify oyster shell powder and Venus clam shell powder as calcitic limestone, because they had a percentage of MgO that is equal to or less than 5% (Primavesi & Primavesi 2004; Souza *et al*. 2014). Oyster shell powder and Venus clam shell powder, when compared to products commonly used in aquaculture to correct the alkalinity and pH of water, have more satisfactory CaO, NP, and TRNP values. This makes the shell powders superior to commercial calcitic limestones. According to a study by Parizotto *et al*. (2018), the shell powders have approximately 32.9% CaO, 64.0% NP, and 37.8% TRNP, which are higher and/or similar to the values found in dolomitic limestones, which have 35–38% CaO, 98–102% NP, and 85–90% TRNP (Pivotto 2020). The chemical composition of the alternative products presents specific and usable characteristics for use in aquaculture, and can be considered great correctives for alkalinity and pH, with the ability to replace traditionally-used products.

In the present study, the use of alternative products at concentrations of 0.22 mg.L^{-1} ensured water alkalinities above 80 mg.L^{-1} $CaCO_3$ after 15 days of reaction. This value is sufficient to guarantee a safe buffer power that can be used in the production of aquatic organisms (Arana 1997). Other authors observed good zootechnical performances in environments with alkalinity values similar to this one, such as Cavalcante *et al*. (2009) who evaluated the performance of fingerlings of *Oreochromis niloticus* and found better indices for animals cultivated in environments with an alkalinity > 50 mg.L^{-1} $CaCO_3$. Similarly, in an experiment using the marine shrimp *Litopenaeus vannamei* in a biofloc system, Piérri *et al*. (2015) found that alkalinity values ranging between 40 mg.L^{-1} and 160 mg.L^{-1} of $CaCO_3$ did not compromise the zootechnical indices of the shrimp. Although there is no specific alkalinity value that is considered ideal for the cultivation of aquatic organisms, it is important that the alkalinity values remain within acceptable ranges for the cultivation of fish and marine shrimp, which is between > 40 mg.L^{-1} and ≥ 75 mg.L^{-1} of $CaCO_3$, respectively (Boyd *et al*. 2016; Furtado *et al*. 2015).

Considering the pH variation in water during the experiment, it is possible to suggest that oyster shell powder and Venus clam shell powder are more efficient agents for buffering the pH of water than sodium bicarbonate. According to Millard *et al*. (2021) and Han *et al*. (2018), the smaller the variation in this parameter, the better the water conditions become for cultivation. This is because pH variation can negatively affect the development, growth, weight gain, and survival rate of fish and shrimp.

Conclusion

The powder from oyster shells and Venus clam shells has great potential as neutralizing acids, and for raising the alkalinity and pH, which allows them to be classified as effective products for use in aquaculture. Thus, these alternative products can be considered promising in the aquaculture sector, showing the ability to replace some traditionally-used products. These findings demonstrate the potential of using bivalve mollusk shells in the water used for seafood cultivation.

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