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Supplementing commercial media with shrimp fecal waste enhances productivity in Salicornia grown in a hydroponic system

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Abstract

The aim of this work was to evaluate the use of shrimp waste as a bioavailable source of nutrients for the growth of *Salicornia europaea*, before and after a cavitation process and either in combination with or without commercial fertilizer. Fecal wastes were collected from the drum filter in a recirculating system for shrimp *Litopenaeus vannamei* culture. The two-factorial experimental design was pretreatment of fecal waste with cavitation (and control without cavitation) and nutrient source (commercial nutrient media (NM) or fecal waste (FW)) at different concentration ratios (100%/0%; 65%/35%; 35%/65% and 0%/100% of nutrient media or fecal waste). The growth experiment lasted 62 days. Media composition had a significant effect on final biomass, and yields ranged from 0.29 to 0.62 kg m^{-2} at 0%/100% and 65%/35%, respectively. The commercial media evaluated does not appear to be well suited for cultivation of Salicornia, and performance can be significantly increased if the commercial media is partially replaced with fecal waste (65%/35% ratio). However, whether pretreatment of fecal sludge is necessary to make nutrients available to Salicornia cannot be answered because it had no significant effect on final biomass.

1. Introduction

Over the past fifty years, the decrease in freshwater and groundwater has led to a continuing degradation of agricultural land by salinization (Panta et al. 2014; Cárdenas-Pérez et al. 2021). Therefore, there is some interest in conducting further studies with plants typically referred to as halophytes because they are more salt resistant than conventional crops (Ventura et al. 2011). Halophytes are plants that grow in environments affected by periodic flooding by salt water, such as mangroves and salt marshes, and not only can they tolerate high salinity but also require salt for better growth (Flowers and Colmer 2008; Ventura and Sagi 2013).

The halophyte genus *Salicornia*, popularly known as sea asparagus, samphire, or glasswort, is of great interest in the world market of haute cuisine for its slightly salty taste and high nutritional value (Barreira et al. 2017). These plants have a nutritional profile suitable for human consumption, containing protein and *n-3* polyunsaturated fatty acids, and are also a valuable source of natural antioxidants and phenolic compounds for the food and pharmaceutical industries (Essaidi et al. 2013; Bertin et al. 2014; Barreira et al. 2017; Costa et al. 2018). Regarding cultivation, different degrees of success have been observed in different parts of the world. Salicornia species are being farmed at a commercial scale for biodiesel, animal feed, and salt and oil extraction (Cybulska et al. 2014; Taghipour et al. 2021; Laveille et al. 2022). Therefore, integrating food production systems can reduce input and waste and increase yield and sustainability (FAO 2018).

As global warming threatens to submerge more and more land masses and fresh water becomes increasingly scarce, switching to saline crops could be a viable option (Roy et al. 2014). Some authors have obtained positive results producing vegetables irrigated with low salinity effluents from shrimp farms (Dufault and Korkmaz 2000; Dufault et al. 2001; Miranda et al. 2008; Mariscal-Lagarda et al.

2012). The successful development of Salicornia in marine aquaponics with shrimp *Litopenaeus vannamei* in biofloc system (Pinheiro et al. 2017; Poli et al. 2019; Doncato and Costa 2021), and also the treatment of marine aquaculture effluent using halophyte plants has already been demonstrated (Webb et al. 2012, 2013; Shpigel et al. 2013; Buhmann et al. 2015).

The development of recirculating aquaculture systems (RAS) in Europe allows the recovery and utilization of fecal wastes for fertilizer purposes since the solid fraction can commonly carry up to 32% and 84% of the total nitrogen and total phosphorus in the wastewater, respectively (Cripps and Bergheim 2000). However, fecal composition also differs among species, feed quality, and feed management. Despite all the differences between the culture methods, most of the nitrogen waste (60–90%) is in dissolved form (mainly ammonia), whereas the phosphorus is excreted within the fecal waste (25–85%) (Van Rijn 2013). Furthermore, more than 50% of the feed nutrients given to aquatic animals are not retained in their bodies and can be potentially available plant nutrients, and there are substantial proportions of essential nutrients bound in solids relative to the respective amounts that appear dissolved (Strauch et al. 2018; Lennard and Goddek 2019).

In experimental studies, supplemented aquaponic nutrient solution (i.e., after adding missing nutrients) has been shown to promote plant growth compared to hydroponics (Delaide et al. 2016). Therefore, sludge mineralization is also a promising way to improve aquaponics system performance, as the recovered nutrients are used to supplement the aquaponics solution (Delaide et al. 2019). Hence, the combined use of cavitation and biological processes for wastewater treatment is economically and ecologically very promising (Bhat and Gogate 2021).

Cavitation is the process of formation, growth, and collapse of vapor cavities within microseconds. Intense shock waves are generated, causing the nanobubbles to implode and decompose the organic material by releasing high pressure and temperature (Gogate and Pandit 2005). In this way, when a cell of the fecal waste is lysed due to cavitation, its intracellular material, which contains soluble organic matter and nutrients, is released to the liquid phase and becomes available as a growth substrate (Lambert et al. 2022). Fecal waste could therefore be an effective mineral substitute if used in crop production.

Shrimp farming has recently acquired great attraction due to its high price and market demand. Reproduction and rearing technologies are available, and more and more shrimp farms are starting to produce throughout Europe (Emerenciano et al. 2022). Since typical European shrimp farming is based on RAS, an increasing amount of shrimp feces will be produced in the near future. Thus, this work aimed to evaluate the use of shrimp waste as a bioavailable source of nutrients for the growth of Salicornia, before and after the cavitation process, and either in combination or not with commercial fertilizer.

2. Materials And Methods

2.1. Origin of plants, fecal waste, and commercial nutrient media

Fecal waste originated from the culture of shrimp *Litopenaeus vannamei* fed a commercial shrimp feed (Le Gouessant Aquaculture). Fecal waste was collected from drum filter residue in two-day intervals and several times prior to the experiment and stored at -4°C before use. The nutrient media used in this study was the "Blaumarke" manufactured by YARA GmbH & Co. KG (Dülmen, Germany). The nutrient composition of both fecal waste (after cavitation) and nutrient media is presented in Table 1.

Table 1
Composition of the nutrient sources tested: fecal waste and commercial nutrient media (Blaumarke, Yara GmbH).

Nutrient composition (mg L^{-1})	Nutrient source	
	Shrimp fecal waste	Commercial nutrient media
NO_3-N	40.0	119.0
NH_4-N	250.0	71.0
Phosphorus (P), total	52.5	60.0
Potassium (K)	216.5	166.03
Sodium (Na)	3271.0	-
Iron (Fe)	0.049	0.7
Copper (Cu)	0.0135	0.1
Molybdenum (Mo)	< 0.0005	0.04
Zinc (Zn)	0.4535	0.25
Cobalt (Co)	-	-
Manganese (Mn)	0.083	0.4
Magnesium (Mg)	455.0	18.0
Boron (B)	0.4535	0.25
Calcium (Ca)	187.0	-
Sulfur (S)	164.0	24.0

Values for NO_3-N, NH_4-N and Phosphorus (P) obtained by a single measurement according to DIN ISO 6878 2004, DIN EN 6878 / D11. All other values were analyzed in duplicate samples, according to DIN by Labor Iben GmbH.

The seeds of *Salicornia europaea* were obtained from the company Rühlemann's (Horstedt, Germany). They were sowed in December 2019 and transferred to the experimental units in May 2020. In total, 24 growth plastic trays measuring 30 x 20 x 7 cm (OBI Eurobox-System Tauro Box Vollwand, Wermelskirchen, Germany) were setup on a table in a greenhouse by random order. Trays were covered

with shading nets (70% light removal) about 20 cm above the trays. Seeds were planted on growth taps made from coconut fiber (n = 5 per tap) (Appendix A - Figure A1).

2.2. Experimental design

The growth trial was performed for 62 days, between 11.05.2020 and 22.07.2020. All nutrient media were filled into 1-liter bottles and stored at -4°C prior to use. Individual bottles were removed from the freezer and thawed one day prior to deployment into the experimental units. The experiment was completely randomized in a two-factor (2x2) experimental design, with three replicates totaling 24 experimental units. The factors were a pretreatment of fecal waste with cavitation (and control without cavitation) and the nutrient source (commercial nutrient media (NM) or fecal waste (FW)) at different concentration ratios (100%/0%; 65%/35%; 35%/65% and 0%/100% of nutrient media or fecal waste).

Prior to the experimental period, trays were randomly assigned to treatments and filled with nutrient media or fecal waste. On the first day of the experiment, each Salicornia seedling was weighed, randomly assigned, and transferred to an experimental tray (9 plants per tray). The mean initial weight of the plants (including tabs) was 38.25 ± 4.38 g (SD).

Trays were filled with thawed nutrient at a rate of 0.4 L per tray (so that the bottom of each tray was covered with nutrient by about 1 cm). The liquid that evaporated during the week was manually replaced with fresh water and the volumes were recorded. Nutrient sources were replaced with fresh media once per week. For weekly biometrics, plants were removed from trays prior to application of the new media and excess water was drained from the tabs for one minute. Then each plant (including the tabs) was weighed and returned to the original tray, and the fresh media was applied.

2.3. Plant production indexes

At the end of the experimental time, all plants were weighed individually. The average final weight (g), final biomass (kg), specific growth rate (SGR), and production (kg m^{-2}) were then calculated. SGR was calculated according to the formula $SGR = 100*(\ln(FW_{end}) - \ln(FW_0))/d$, where FW_{end} is the final mean fresh weight of plant per pot, FW_0 is the initial mean fresh weight of plant per pot, and d is the number of experimental days.

2.4. Statistics

There was a total of 207 observations, distributed similarly among the different subgroups. However, for the 100%/0% NM/FW treatment, it is worth noting that all the observations collected (58) were accumulated in the control factor, as there was no shrimp fecal waste to treat in this ratio combination. The descriptive analysis showed bimodality of the data, indicating that the variances of the collected observations in each group were not homogeneous. The difference between the pretreatment and control groups was caused by this pronounced bimodality. Therefore, a two-sample Kolmogorov-Smirnov (KS) test (Massey 1951) was performed in R (R Core Team 2018). Multiple testing was adjusted using the Holm-Bonferroni correction (Holm 1979). All tests were performed at a significance level of 0.05.

3. Results

Media composition significantly affected final biomass. Except for 100/0 versus 0/100 ($P = 0.18$), all direct treatment comparisons were significantly different ($P < 0.03$) (Fig. 2). Pretreatment had no significant effect on final Salicornia biomass ($P = 0.18$) (Fig. 3).

4. Discussion

The current yields of 2.92 to 6.17 tons ha^{-1} are similar to yields previously reported for *Salicornia brachiata* in the growing season in tidal marshes (2.51 to 6.07 tons ha^{-1} (Rathore et al. 2016). However, much higher values are also given in the literature. For example, yields of 10 to 20 tons ha^{-1} have been reported for *S. europaea* grown in aquaculture effluent (Brown et al. 1999), and yields as high as 23.7 tons ha^{-1} have been reported for *Salicornia sp.* (Lyra 2017). These differences in yields can be manifold, including species, cropping system, season, nutrient supply, flooding interval, salinity, or pH (Cárdenas-Pérez et al. 2021). When growing seasons are included, the results become clearer. Current yields in 62 days ranged from 0.29 to 0.62 kg m^{-2} (in 0%/100% and 65%/35%, respectively). For *S. ambigua* grown in an aquaponic system with the marine shrimp *L. vannamei*, the average production yield was 8 kg m^{-2} of fresh biomass after 73 experimental days (Pinheiro et al. 2017). The genus *Salicornia* grown in hydroponic culture with fertilizer-enriched seawater yielded a production of 6 kg m^{-2} (Ventura et al. 2011). In an experimental culture of *S. ambigua* irrigated with wastewater from shrimp farms in Brazil, up to 2 kg m^{-2} of fresh biomass was obtained after 150 days of culture (Izeppi 2011). In our study, only nine plants were grown in each container, and the yield per unit area could be increased by increasing the planting density. Another factor that may have contributed to the low overall performance compared to the literature is the high variation in weights of individual plants per pot (data not shown). Some individuals show severely inhibited growth or even no growth, which reduces the overall yield. This could be due to poor seed quality. In fact, the seedlings showed that only about 10% of the seeds germinated.

Growth rate is of great importance when evaluating the commercial potential of a species of interest. Species of the genus *Sarcocornia*, for example, are characterized by slow growth and low productivity when irrigated with seawater. It was not the scope of this study to maximize yields. Such studies require a complete nutrient screening of all media. Rather, the goal was to find simple solutions to reduce reliance on commercial media and to utilize aquaculture waste streams that are becoming increasingly available. It is therefore quite interesting to note that a) commercial media does not seem to be well suited for culturing Salicornia and b) performance can be significantly increased if commercial media is partially replaced by fecal waste (65%/35% ratio). The average final biomass in this treatment was almost 3 times higher than in all other treatments (Fig. 2). The poor performance of Salicornia grown in fully commercial media cannot be fully explained by a lack of salinity. Salinity present in form of sodium ions in fecal waste (Table 1) is known to be a key factor affecting the growth of Salicornia. Optimal growth of *Salicornia europaea* is between 200 and 400 mM NaCl, while growth is limited at 0, 800, and 1000 mM NaCl (Cárdenas-Pérez et al. 2022). Adequate nutrient composition may have counteracted this positive

effect on salinity in these treatments. Thus, we conclude that the observed differences in growth performance are indeed due to the positive effects of fecal sludge.

Fecal sludge from aquaculture consists of nutrients and other promoting substances such as bacteria, which are known to enhance the transfer of nutrients to plant roots. The present system was set up in a greenhouse during the months from May to July and water temperature in the hydroponic system ranged between 26.6 and 28.1°C. These conditions and organic substances from the fecal waste may have favored bacterial growth in the nutrient film (Khiari et al. 2019) and therefore may have hence contributed to nutrient liberalization.

The lack of a pretreatment effect known to increase the bioavailability of organic sources to plants may be related to bacterial mineralization (Rakocy 2012). Cavitation can release organically bound nutrients in fish feces (Bhat and Gogate 2021), but in this case did not result in improved growth of Salicornia. However, the effect of higher nutrient levels may have been counteracted by inadequate experimental culture conditions. At the same time, the lack of a positive effect of cavitation could be related to the fact that this method does not release enough ions or releases only selective ions. The lack of an effect can be explained by several effects. Not all nutrients are released during the application of the cavitation method and are increasingly available to the plants. For example, although the concentration of orthophosphate and iron doubles, the concentration of other elements such as K, Mg or Mn increases only slightly (unpublished data). Potassium, in particular, is needed as a macronutrient that limits growth when it is present in small amounts in the growth media.

Whether fecal sludge needs pre-treatment to make nutrients available to Salicornia cannot be answered. Organically bound nutrients in fecal sludge could have resulted in slow release, as bacterial mineralization was sufficient to support nutrient supply. However, numerically there was a tendency for Salicornia to grow better in pretreated fecal sludge than Salicornia growing in commercial media. This could be related to the weekly supply of new media, which does not allow substantial release of elements by bacteria.

Faecal waste has the potential to reduce the environmental impact of Salicornia production. Conventional phosphate fertilizer used in mineral media contributes 27MJ/kg P_2O_5 to the non-renewable cumulative energy input from fossil and nuclear resources (KEA), 1.2kg CO_2 eq/kg P_2O_5 to 100a global warming potential (GWP), 19g SO_2 eq/kg P_2O_5 to terrestrial acidification potential (TAP), 24g P eq/kg P_2O_5 to freshwater eutrophication potential (FEP), 0.4g N eq/kg P_2O_5 to marine eutrophication potential (MEP), and 4.5kg 1,4-DCBEq/kg P_2O_5 to human toxicity potential (HTP) (Kraus et al. 2019). In the present study, 19.2 liters of the nutrient media were used for the highest yield during the experimental period of 60 days. The 65%/35% media contained 0.21 mg P_2O_5 less than the full conventional media (100%/0%). This corresponds to a reduction of 18.7 mg P kg^{-1} fresh weight of Salicornia biomass produced, which is equivalent to 0.02 kg P per ton of fresh biomass produced.

5. Conclusion

Present results showed that Salicornia growth performance can be significantly increased if the commercial media is partially re-placed with fecal waste (65%/35% ratio). Fecal waste can reduce the environmental impact of Salicornia production by 18.7 mg P kg^{-1} fresh weight equivalent to 0.02 kg P per ton of fresh Salicornia biomass produced.

Declarations

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Author Contributions: Conceptualization, SSWE and JH, methodology, SSWE, validation, MJM and RM, formal analysis, MJM and RM, investigation, THE and GJ, resources, JH, data curation, SSWE, writing—original draft preparation, SSWE and IP, writing—review and editing, SSWE, IP and JH, visualization, MJM and RM, supervision, SSWE, project administration, JH, funding acquisition, JH. All authors have read and agreed to the published version of the manuscript.

Data Availability Statement: The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request

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Figures

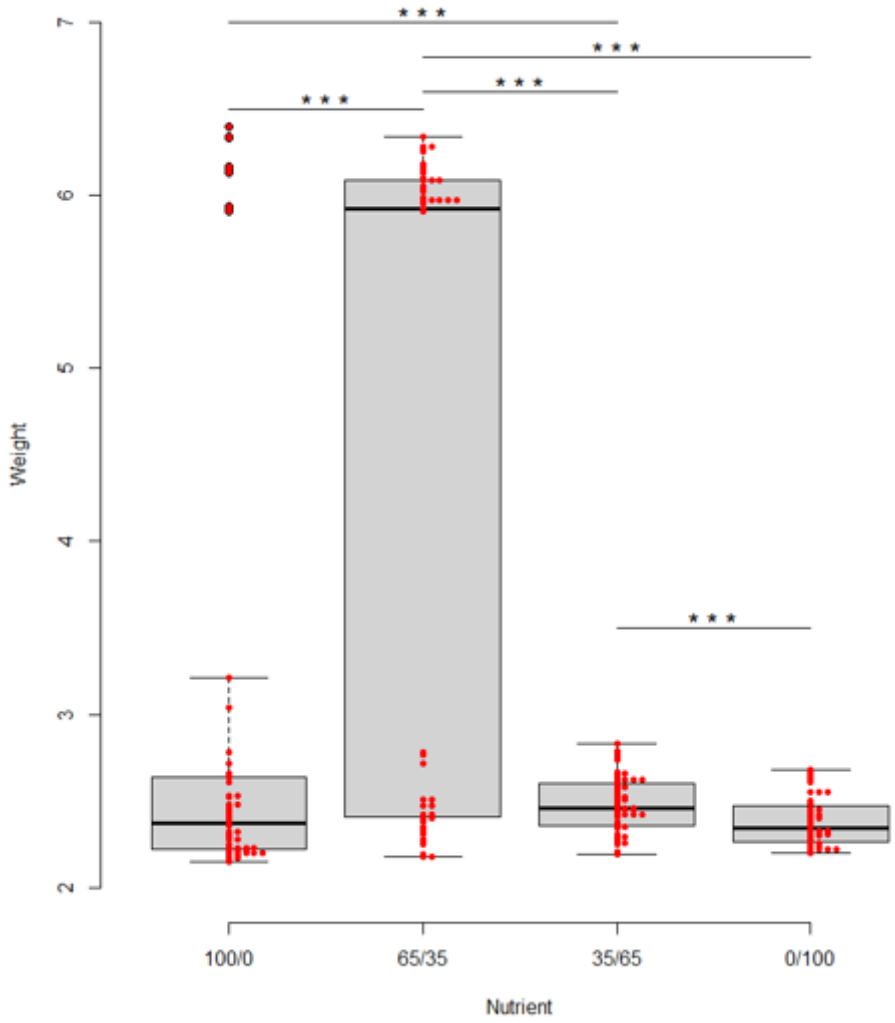


Figure 1

Results of the Kolmogorov-Smirnov test after adjustment for multiple testing using the Holm correction for the nutrient ratio group. The three asterisks represent the groups for which the p-values are less than 0.05.

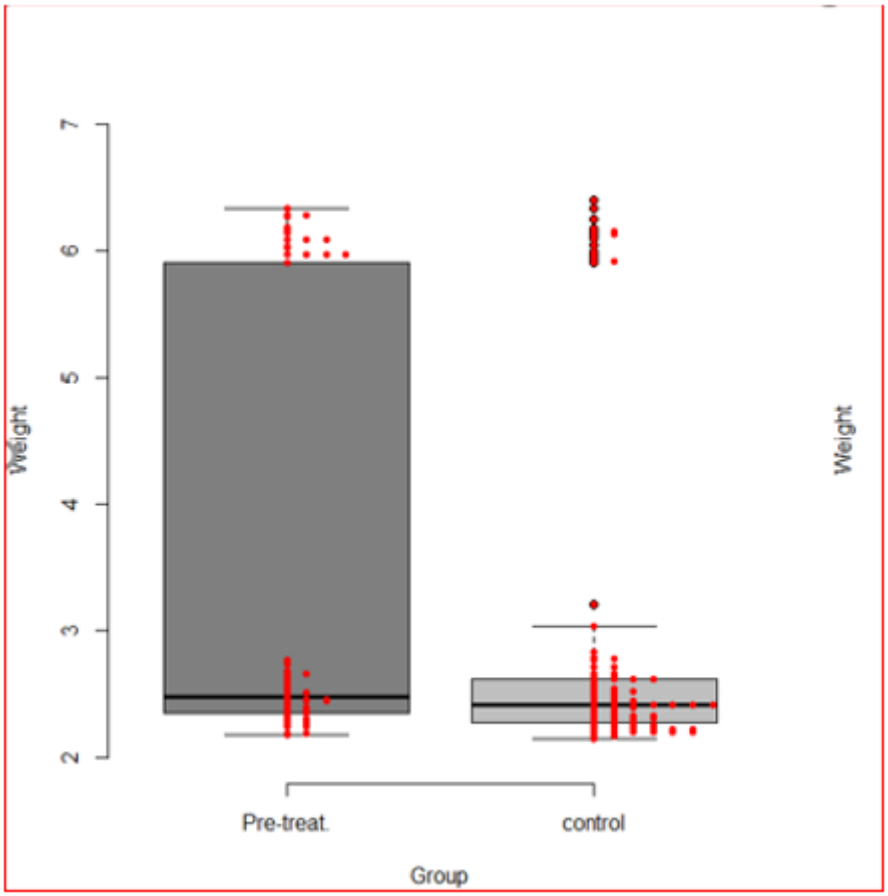


Figure 2

Box plots along with dot plots comparing the control and the pretreatment groups final weight.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- AppendixA.docx