

Humic Acid Stimulation of Growth and Optimization of Biochemical Profiles on Two Microalgal Species Proposed as Live Feeds in Aquaculture

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ABSTRACT

A series of batch culture experiments on two marine microalgae species, *Dunaliella salina* Teodoresco and *Nannochloropsis salina* Hibberd, was conducted at various humic acid (HA) concentrations (0.0, 0.1, 0.2, 0.3, 0.4, 0.5 mgL⁻¹), in order to evaluate the stimulatory potential of HA on microalgae growth (expressed as a biomass concentration), pigment production (chlorophyll *a* and carotenoids) and carbon to nitrogen (C:N) ratio. The impact of HA on the proximate composition (moisture, ash, dietary fiber, crude lipid, available carbohydrates, crude protein, and energy content) was also considered. Results demonstrated a highly significant positive effect of HA on growth, pigment production, and proximate analysis ($P \leq 0.01$). The excellent response of the two investigated microalgae to HA recommends it as a low-cost, high-yield investment, despite the finding that the C:N ratio in *D. salina* showed a gradual decrease upon addition of HA. A slight increase in the C:N ratio was observed upon addition of HA in *N. salina*.

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INTRODUCTION

Microalgae have an important role in aquaculture as a means of enriching zooplankton before feeding to fish and other larvae, providing energy that is transferred through the food chain to higher trophic levels via the zooplankton intermediates (Brown 2002). Microalgae are characterized as having high nutrient value, containing proteins, lipids, carbohydrates (Renaud *et al.* 1999); sterols (Mohammady 2004); polyunsaturated fatty acids (Sargent *et al.* 1997); and minerals (Fabregas and Herrero 1986). The biochemical composition of microalgae can be manipulated readily by changing the growth conditions (Brown *et al.* 1997). Nutrient enrichment and optimization is a biochemical attribute of practical use to aquaculturists, who may grow microalgae 'designed' to deliver the specific levels of nutrients needed by the animal. The enrichment of a microorganism's nutrient medium with humic substances (HS) was studied by Muller-Wegener (1988). This organic matter is commonly distributed in natural habitats such as water, soil and sediments (Coates *et al.* 2002). It is readily available as compost (Canellas *et al.* 2002) that can be applied to different crops (Mackowiak *et al.* 2001, Canellas *et al.* 2002) to provide the widely recognized benefits attributed to the numerous functional groups that have been characterized within HS (Coates *et al.* 2002). HS have been extensively studied for their possible contribution to phytoplankton growth as a source of nitrogen (Carlsson *et al.* 1995) and carbon (Doblin *et al.* 1999). Other studies (Doblin *et al.* 2000) attributed the benefits observed in microalgae treated with HS to its content of metal-binding ligands that modulate the availability of trace elements. Furthermore, a combination of metabolic (Doblin *et al.* 1999) and membrane permeability alterations (Vigneault *et al.* 2000) triggered by HS may also enhance algal growth.

Humic acid (HA) is a fraction of HS that has a variety of chemical functions (Sun *et al.* 2005). It has been shown to positively affect the growth rate and biomass production of *Chlorella vulgaris* (Brown 1969), the dinoflagellate *Alexandrium tamarense* (Gagnon *et al.* 2005), and some diatom species (Granéli *et al.* 1999).

Dunaliella (Dunal) salina Teodoresco is a marine microalgae used in the aquaculture industry as a live feed for copepods, *Acartia tonsa*, (Veloza *et al.* 2006), while *Nannochloropsis (Monallantus) salina* Hibberd is used in feeding both *Brachionis plicatilis* and *Artemia* spp. (Brown *et al.* 1997,

Brown 2002). Both species have a nanoplankton size (2-20 μm) with a cellular composition of high nutritional value (Brown *et al.* 1997). They have extensive production of valuable pigments (Fried *et al.* 1982, Lubián *et al.* 2000) and polyunsaturated fatty acids (Fried *et al.* 1982, Sukenik *et al.* 1989).

For these reasons, the objective of this study was to evaluate the stimulatory potential of HA extracted from garden soil for increasing biomass and improving different biochemical profiles of *D. salina* and *N. salina*. Mohammady and Fathy (2007) showed that HA has no effect on the fatty acid composition of these two strains. Data were obtained on biomass concentration, pigments (chlorophyll *a* and carotenoids), carbon to nitrogen ratio and the proximate components (moisture, ash, dietary fiber, crude lipid, available carbohydrates, crude protein, and energy content).

MATERIALS AND METHODS

Extraction of HA

From the top 6 cm of garden soil at the Faculty of Science (Alexandria University, Egypt), a fresh sample was collected and transported directly to the algae laboratory, where it was immediately assayed for HA extraction according to Stevenson (1982). To summarize this process, a 25 g sample of sieved uncontaminated garden soil was allowed to settle, and the aqueous-phase material was decanted and discarded. Approximately 25 mL of water was added to the soil and the slurry was allowed to sit for 30 min. The pH was then adjusted to 7.0 with the addition of 1 M NaOH. The total volume of the slurry was brought to 250 mL with the addition of 0.1 M NaOH, and the mixture was stirred for 24h. The mixture was then centrifuged and the particle-free supernatant was adjusted to pH 1.0 by adding 6 M HCl with constant stirring. This resulted in the formation of a dark brown precipitate. The suspension was allowed to stand for 12h and centrifuged to recover the precipitate. This precipitate was suspended in a solution of 0.1 M HCl and 0.3 M HF overnight to remove mineral impurities and then washed with distilled water to remove chlorides. The sample was centrifuged and the precipitated HA was freeze-dried and stored in a desiccator. HA was examined using infrared analysis (unpublished data).

Microalgae and culture media

The chlorophyte *Dunaliella salina* was obtained from the algal culture collection of the Faculty of Science (Alexandria University, Egypt). The cultures were grown axenically in 1L MH medium according to Loeblich (1982). The eustigmatophyte *Nannochloropsis salina* was obtained from the Solar Energy Research Institute (SERI) Culture Collection (Golden, CO, USA). The *Nannochloropsis* cultures were grown axenically in enriched seawater according to Boussiba *et al.* (1987). A dried stock of HA was added to the culture medium to yield the following final concentrations: 0.0, 0.1, 0.2, 0.3, 0.4 and 0.5 mgL⁻¹.

Culture conditions

All cultures were grown in triplicate 1 L glass flasks equipped with inlet and outlet tubes for aeration in a temperature-controlled room at 23 ± 1°C. Cultures were continuously agitated by bubbling with sterile air, which was also enriched with 0.5% CO₂. Illumination was provided by fluorescent lamps with an irradiance of 300 μmol m⁻² s⁻¹ at the surface of the cultures under a 16-hour regime (8-h light / 8-h dark).

Biomass recovery and estimation

According to (Mirón *et al.* 2002), the cultures were recovered daily by introducing the suspensions into a continuous centrifuge (Janetzki T24, Janetzki, Berlin, Germany) running at 1000x g for 5 min. The harvested cells were washed with saline water (0.5 M NaCl) and the optical density of the suspension was determined spectrophotometrically at 625 nm, using a Perkin Elmer Lambda Spectrophotometer (Waltham, MA, USA). Biomass concentration (C_b, gL⁻¹) was calculated as follows: C_b=0.38 x OD₆₂₅. A growth curve has been established for each alga.

The exponentially-grown cultures were subjected to the following analyses:

Chlorophyll *a*

The spectrophotometric method of Hansmann (1973) was used to estimate the chlorophyll *a* content in the algal cells. The optical density of the suspension was determined at 665, 645, and 630 nm using a Perkin Elmer Lambda Spectrophotometer. The concentration of chlorophyll *a* (mgL⁻¹) was calculated as follows:

$$Ch_a = 11.6 \times OD_{665} - 1.31 \times OD_{645} - 0.14 \times OD_{630}$$

Data were standardized as a dry weight biomass.

Total carotenoids

Carotenoids were determined according to the method of Whyte (1987). The optical density of the suspension was determined at 444 nm wavelength using a Perkin Elmer Lambda Spectrophotometer. The concentration of total carotenoids (mgL^{-1}) was calculated as follows:

$$C_t = 4.32 \times \text{OD}_{444} - 0.0439$$

Data were standardized as a dry weight biomass.

Carbon and nitrogen

The harvested cells were introduced into the Elemental Analyzer PE2400 Series II CHNS/O System (Perkin Elmer, Waltham, MA, USA), and the C:N ratio was calculated (Reboloso Fuentes *et al.* 2000).

Proximate composition

The following analyses were individually processed as components of the proximate composition:

Moisture: The moisture was determined by drying a representative 2 g sample of the pellet in an oven at 100-105°C for 40 h (Reboloso Fuentes *et al.* 2000).

Ash: Total ash was determined by incineration of a representative 0.5 g sample of the pellet in an oven at 450°C for 48 h (Reboloso Fuentes *et al.* 2000).

Dietary fiber: Fiber content was determined by the neutral detergent extraction procedure (Goering and Van Soest 1970).

Crude lipid: Lipid content was determined on the weighted extract obtained with chloroform/methanol (2:1, v/v), according to Kochert (1978).

Available carbohydrates: The anthrone-sulfuric acid method was applied according to Osborne (1985). A calibration curve was prepared for each experiment using D-glucose dissolved in distilled water. The glucose concentration (C_g , mgL^{-1}) was calculated from the optical density at 630 nm according to the following equation: $C_g = 0.536 \times \text{OD}_{630} + 0.0028$.

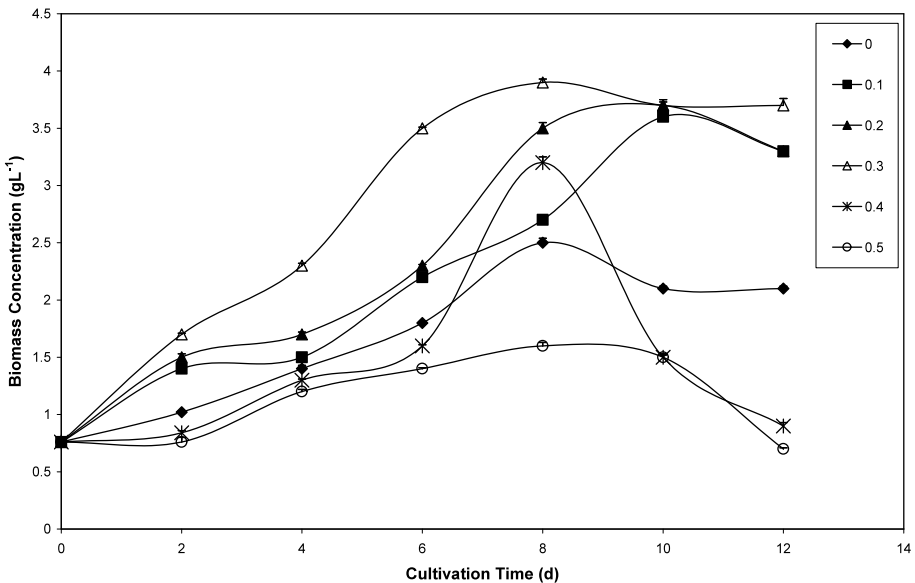
Crude protein: Total protein was calculated by multiplying the N_2 value (obtained using the Elemental Analyzer PE2400 Series II CHNS/O System) by 6.25 according to Becker (1994).

Energy: The energy content of the biomass was calculated as the sum of the values obtained after multiplying the values obtained for crude protein, available carbohydrates, and crude lipid by 4.00, 3.75 and 9.00, respectively (Reboloso Fuentes *et al.* 2000).

Statistical analysis

The concentration values were standardized to dry weight and data were analyzed using two-way analysis of variance (ANOVA), using COSTAT 2.0 statistical analysis software (CoHort Software, Monterey, CA, USA). Means were tested with least square difference (LSD), where the difference of $P \leq 0.01$ was highly significant. The mean value of triplicate data and the standard deviations (SD) were also calculated.

Figure 1. Growth response of *D. salina* to various humic acid concentrations, values are means \pm SD ($n = 3$).



RESULTS

HA dose-response curves for a maximum of parameters:

Biomass concentration

The growth of *D. salina* (Figure 1) expressed as biomass concentration (gL^{-1}) showed a significant positive effect ($P \leq 0.01$) from the addition of the various HA concentrations, up until day 8. The highest value ($3.9 \pm 0.3 \text{ gL}^{-1}$) was observed in cells grown at 0.3 mgL^{-1} HA. The biomass declined by the 12th day in all cultures treated with 0.1 and 0.2 mgL^{-1} HA, although the value was still higher than the control. Biomass concentration was reduced to 42% and 33% of control value in 0.4 and 0.5 mgL^{-1} HA grown cells, respectively.

In *N. salina*, biomass concentration (Figure 2) gradually increased in the $0.0, 0.1, 0.2,$ and 0.3 mgL^{-1} HA grown cells up to the 10th day. The highest value (3.8 gL^{-1}) was observed in cells grown at 0.2 mgL^{-1} HA on days 8 and 10. In all cultures, the biomass declined on the 12th day, but the biomass concentrations of the $0.1, 0.2, 0.3 \text{ mgL}^{-1}$ HA grown cells were still higher than the control. Nevertheless, the biomass concentration was reduced to 50% and 33% of control value at 0.4 and 0.5 mgL^{-1} HA, respectively.

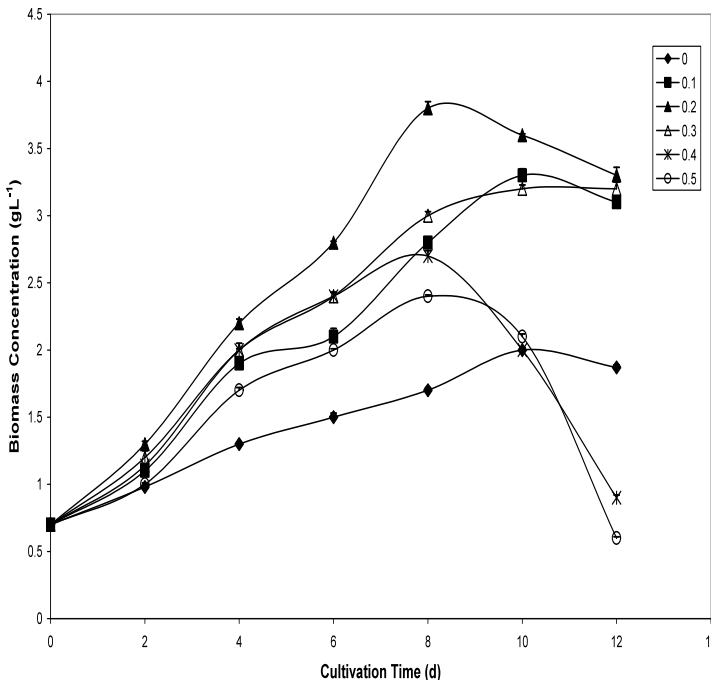


Figure 2. Growth response of *N. salina* to various humic acid concentrations, values are means \pm SD ($n = 3$).

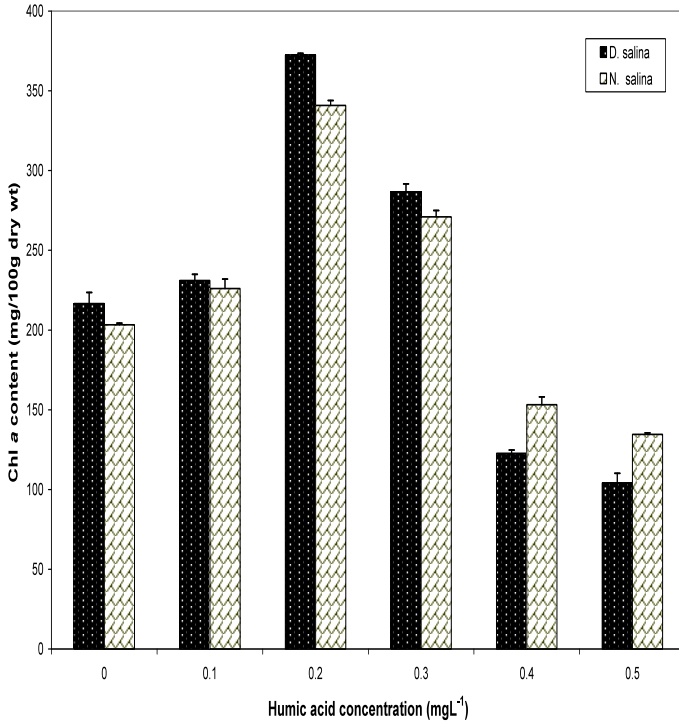


Figure 3. Standardized chlorophyll a content of *D. salina* and *N. salina* in response to various HA concentrations, values are means \pm SD ($n = 3$).

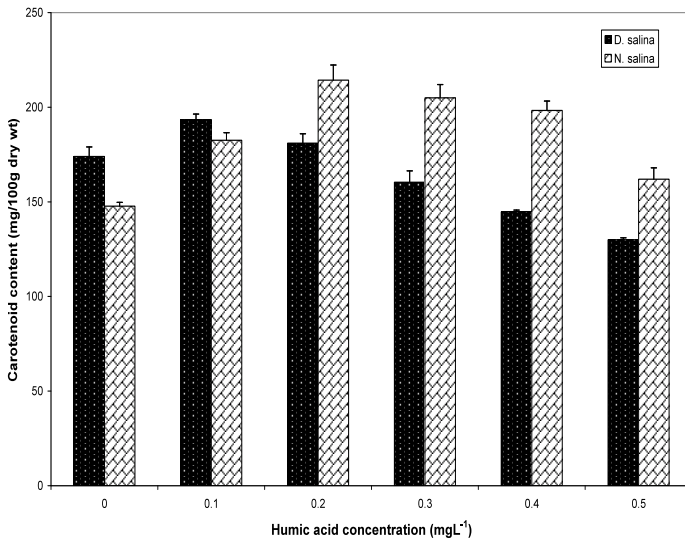


Figure 4. Standardized carotenoid content of *D. salina* and *N. salina* in response to various HA concentrations. Values are means \pm SD ($n = 3$).

Chlorophyll *a* content

In *D. salina*, chlorophyll *a* (Figure 3) was gradually increased from 216.6 ± 7 to 372.46 ± 1 mg /100g dry wt at 0.2 mgL^{-1} HA treated cells, which represents a 1.72 fold increase. At 0.5 mgL^{-1} HA, chlorophyll *a* content decreased to approximately 48% of the control value. On the other hand, in *N. salina*, the content increased from 203.3 ± 1 to 340.9 ± 3 mg/100g dry wt, which represents a 1.67 fold increase over the control. At 0.5 mgL^{-1} HA, chlorophyll *a* declined to about 66% of the amount produced by the control cells.

Carotenoid content

A maximum carotenoid content (Figure 4) of 193.42 ± 3 mg/100g dry wt was detected in *D. salina* cells grown at 0.1 mgL^{-1} HA, while in *N. salina*, the maximum content (214.24 ± 8 mg/100g dry wt) was observed in cells grown at 0.2 mgL^{-1} HA. This represents approximately a 1.5 fold increase in the content of the control value.

C:N ratio

In *D. salina*, C:N value in control condition is recommended, but it gradually decreased with the impact of addition of HA to reach its minimum value (2.9 ± 1) at 0.5 mgL^{-1} HA, which represents 0.32% of

Figure 5. Impact of HA addition on C/N ratio for *D. salina* and *N. salina*, values are means \pm SD ($n = 3$).

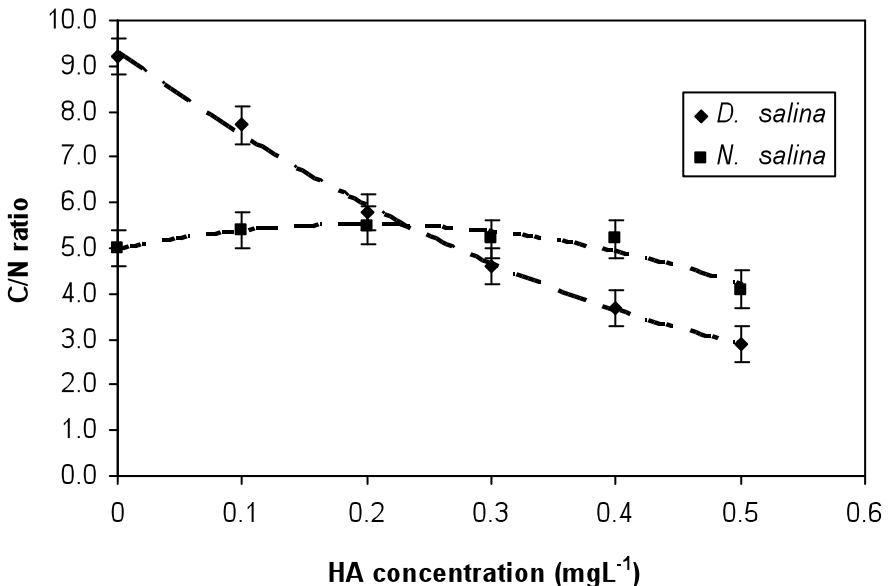


Table 1. Impact of HA addition on proximate composition for *D. salina* (data are expressed as g/100g dry wt). Values are means \pm SD (n = 3).

Humic acid concentration (mgL ⁻¹)	Moisture	Ash	Dietary fiber	Crude lipid	Available carbohydrates	Crude protein	Energy (KJ)
0 (control)	1.7 \pm 0.1	15 \pm 2	0.38 \pm 1	6.4 \pm 0.3	20 \pm 3	53.2 \pm 2	1377 \pm 32
0.1	1.5 \pm 0.1	3 \pm 0.2	0.59 \pm 0.2	8 \pm 0.5	22 \pm 0.5	59.9 \pm 2	1649 \pm 39
0.2	1 \pm 0.1	1 \pm 0.1	0.49 \pm 1	5.5 \pm 0.6	23 \pm 0.4	63 \pm 8	1778 \pm 82
0.3	1.1 \pm 0.2	2 \pm 0.1	0.40 \pm 2	5.1 \pm 0.7	19.7 \pm 0.2	66.4 \pm 8	1734 \pm 41
0.4	1.8 \pm 0.3	10.5 \pm 0.2	0.30 \pm 0.2	4.8 \pm 0.2	18.4 \pm 3	60.1 \pm 5	1513 \pm 36
0.5	6 \pm 0.1	27 \pm 0.2	0.28 \pm 0.1	4.2 \pm 0.2	18.4 \pm 3	44.3 \pm 6	1226 \pm 29

Table 2. Impact of HA addition on proximate composition for *N. salina* (data are expressed as g/100g dry wt). Values are means \pm SD (n = 3).

Humic acid concentration (mgL ⁻¹)	Moisture	Ash	Dietary fiber	Crude lipid	Available carbohydrates	Crude protein	Energy (KJ)
0 (control)	1.6 \pm 0.1	12 \pm 0.4	0.59 \pm 0.3	9.2 \pm 0.1	31 \pm 3	39.89 \pm 0.5	1500 \pm 35
0.1	1.4 \pm 0.1	6 \pm 0.6	0.59 \pm 0.3	12 \pm 0.3	33 \pm 3	42.95 \pm 1	1689 \pm 40
0.2	1.1 \pm 0.1	4 \pm 0.6	0.62 \pm 0.1	9 \pm 0.4	36 \pm 3	44.36 \pm 1	1731 \pm 61
0.3	2 \pm 0.1	18.5 \pm 1	0.69 \pm 0.1	9 \pm 1.6	26 \pm 0.2	36.64 \pm 2	1378 \pm 32
0.4	3.7 \pm 0.1	20 \pm 2	0.69 \pm 0.1	8.5 \pm 1.5	26 \pm 0.1	33.63 \pm 4	1291 \pm 30
0.5	7.2 \pm 0.3	32 \pm 6	0.70 \pm 0.3	6 \pm 1.7	14 \pm 0.1	31.3 \pm 0.2	969 \pm 23

the control value. However, in *N. salina*, although a slight increase of the ratio was observed, the ratio was reduced to 4.14 ± 1 at 0.5 mgL^{-1} HA (Figure 5).

HA impact on proximate composition

Data on proximate composition are shown in Tables 1 and 2. In both microalgal species, moisture content decreased upon the addition of HA concentrations up to 0.2 mgL^{-1} . This was followed by an increase (6.0 ± 0.1 and $7.2 \pm 0.3 \text{ g/100 dry wt}$) in the 0.5 mgL^{-1} HA grown *D. salina* and *N. salina*, respectively.

Ash content in both microalgae showed a dramatic decrease at 0.1 HA mgL^{-1} compared to the control value. However, at the 0.4 and 0.5 HA mgL^{-1} concentrations, the ash content of both species sustained significant increases that reached a 1.8 fold increase for *D. salina* and a 2.7 fold increase for *N. salina* in 0.5 mgL^{-1} HA grown cells.

In *D. salina*, dietary fiber content increased from 0.38 ± 1 to $0.59 \pm 0.2 \text{ g/100g dry wt}$ at 0.1 mgL^{-1} HA. The content gradually decreased to its minimum value (0.28 ± 0.1) at 0.5 HA mgL^{-1} concentration. However, in *N. salina*, HA addition produced a slight progressive increase in the fiber content, from $0.59 \pm 0.3 \text{ g/100g dry wt}$ to $0.70 \pm 0.3 \text{ g/100g dry wt}$ in 0.5 mgL^{-1} HA grown cells.

Lipid content in *D. salina* increased by 1.25-fold upon the addition of 0.1 mgL^{-1} HA compared to the control. However, at the higher HA concentrations, lipids were gradually reduced. A similar response was shown in *N. salina*, although the lipid content was relatively high compared to *D. salina*.

Compared to other microalgae, available carbohydrate was relatively low in both species grown under control conditions. Carbohydrates gradually increased with increasing HA dose to reach the maximum values (23.0 ± 0.4 and $36.0 \pm 3.0 \text{ g/100g dry wt}$) at 0.2 mgL^{-1} HA concentration for *D. salina* and *N. salina*, respectively. At the higher concentrations the values declined, especially in *N. salina*, to about half the amount of the control.

Protein content was relatively high in most cultures of *D. salina* compared to other microalgae. The maximum value ($66.4 \pm 8 \text{ g/100g dry wt}$) was observed in cells grown in 0.3 mgL^{-1} HA. On the other hand, in *N. salina*, the maximum value ($44.36 \pm 1 \text{ g/100g dry wt}$) was observed in cells grown at 0.2 mgL^{-1} HA.

Energy content increased upon the addition of HA to reach its maximum value in 0.2 mgL⁻¹ HA grown cultures. For *D. salina*, the maximum value was 1778 ± 82 (a 23% increase over control), while for *N. salina*, the maximum value was 1731 ± 61 KJ /100g dry wt (a 15% increase over control). However, *D. salina* showed a stronger response towards HA impact of up to 0.4 mgL⁻¹.

DISCUSSION

Microalgae, either as a full or partial enrichment, should be considered for improving the nutritional quality of zooplankton. In this paper, the possible benefits of using HA to improve the nutritional quality of microalgae are reported. The fact that HA had its optimum effect at the lower concentration is of economic interest.

The stimulatory capacity of HA on algal growth has been studied by many authors (Graneli *et al.* 1999, Gagnon *et al.* 2005, Sun *et al.* 2005). In the present study, the positive dose-response effect tends to decline at higher HA concentrations.

The decline in biomass concentration in both of the microalgae studied may be attributed to the ability of HS to complex trace elements in the growth media (Sunda and Huntsman 1998) until they begin to limit cell growth (Gagnon *et al.* 2005).

Our results demonstrated a positive effect from HA ($P \leq 0.01$) on pigment production in both microalgae studied. A 0.2 mgL⁻¹ concentration of HA showed maxima for chlorophyll *a* in both species. Further, the two investigated species are characterized by high carotenoid content at control condition (Fried *et al.* 1982, Lubián *et al.* 2000). Ronnestad *et al.* (1998) demonstrated that microalgae pigments transferred through to zooplankton may contribute to their nutritional value. They found the dominant pigments in the copepod *Temora* sp. were lutein and astaxanthin, whereas in *Artemia* it was canthaxanthin. Our results demonstrate a positive role for HA in pigment production, particularly in *N. salina*. Humic substances stimulated photosynthetic pigments in the green alga *Pseudokirchneriella subcapitata* (Koukal *et al.* 2003), a stimulation that was mainly dependent on Fe bioavailability (Sun *et al.* 2005). As demonstrated by Brown (1969), Fe uptake in *Chlorella vulgaris*

was roughly proportional to the concentration of water-soluble humic acid in the nutrient medium.

The C:N ratio of *D. salina* grown in the absence of HA was close to the Redfield ratio and within the range expected for a natural population. However, in *N. salina*, the ratio was relatively lower, a finding that is consistent with previous studies (van Bleijswijk *et al.* 1994, Mohammady *et al.* 2005). The C:N ratio depends mainly on the available carbohydrate content (Reboloso Fuentes *et al.* 2000). In this study, the gradual decrease of the C:N ratio in *D. salina* and the slight increase in *N. salina* upon HA addition is probably related either to the low amount of available carbohydrates, to a high nitrogen content in the cells, or possibly as a result of both reasons. The C:N ratio varies by a factor of almost four in *D. salina*, while the available carbohydrates undergo a small 1.1-fold reduction. Since the rigid cell wall of *N. salina* contains non-soluble polysaccharides, HS could have had more of an impact on the C:N ratio.

A variation in moisture content upon addition of HA was observed in both microalgae species, a trend that conflicted with the trend for biomass concentration. However, the observed values (less than 10%) are generally those recommended for nutritional uses (Becker 1994).

The high ash content found in cultures grown under control conditions was consistent with other studies on marine microalgae (Markovits *et al.* 1991, Canizares *et al.* 1994) and differs from that of the fresh water algae, *Scenedesmus* and *Spirulina* (Becker and Venkataraman 1982). The addition of a low concentration of HA caused a decrease in ash content in both microalgae species. However, with the impact addition, the ash content increased. The excess ash content upon increasing HA might be attributed to increased cell permeability (Vigneault *et al.* 2000) with respect to the electrogenic proton pump (Visser 1985). This leads to an increase in the nutrient uptake (Legrand and Carlsson 1998). The incineration of these nutrients may result in yields of approximately two times the ash usually found in *D. salina* and more than 2.5 times that found in *N. salina* at control conditions.

The low fiber content of *D. salina* compared to *N. salina* might be attributed to the absence of the rigid cell wall in the organism. Generally, a low fiber content is recommended for microalgae because it makes them

easier to digest, which improves their nutritional value (Reboloso Fuentes *et al.* 2000).

At control conditions, the low lipid content of both microalgae investigated may be attributed to the harvesting of cultures during the logarithmic growth phase (Piorreck and Pohl 1984). However, upon 0.1 mgL⁻¹ HA addition, the value was raised by 1.25 and 1.30 in *D. salina* and *N. salina* respectively. It is well known that HS have a negative effect on cellular membranes. This effect explains the reduction of lipid content with increasing HA dose in both species investigated.

The content of available carbohydrates was relatively low in cultures grown under control conditions. The values showed maxima upon the addition of 0.02 mgL⁻¹ HA, but were still low compared to other studies. As explained by Reboloso Fuentes *et al.* (2000), the low available carbohydrate content is a result of washing biomass.

Proteins are the fundamental building blocks for tissue biosynthesis and enzyme production in all animals. Thus, proteins must be available to meet the demands of tissue production and metabolic processes (Gatenby *et al.* 2003). Protein is generally considered most important during the rapid growth of the juvenile life stage, followed by adults undergoing gametogenesis (Kreeger and Langdon 1993, Fernández Sevilla 1995). In this study, both species were characterized by high protein content under control conditions, particularly *D. salina*. In the presence of HA, an obvious increase in protein production (25% increase in *D. salina* grown at 0.3 mgL⁻¹ HA and 13% increase in *N. salina* grown at 0.2 mgL⁻¹ HA) was detected. As postulated by Chen and Aviad (1990), HA increases nitrate uptake from the nutrient medium, which in turn increases the concentration of structural proteins inside the cell. The declines shown in the data may be due to the impact of HA in forming complexes with amino acids, peptides, and steroids (Frimmel and Christman 1988).

Energy is a property of nutrients that is released by metabolic oxidation of proteins, lipids, and carbohydrates. In this study, the energy potential of the biomass increased in response to HA addition. The maximum value for both species was recorded in cells grown at 0.2 mgL⁻¹ HA, indicating an unlikely response towards their oxidation process. However, all energy values remained within recommendations (Whyte 1987) for aquaculture purposes.

In summary, our main objective was to evaluate the stimulatory potential of HA on the biomass, pigment production, proximate analysis, and C:N ratio. The results of the present experiments demonstrate this potential. The response of the two investigated microalgal species to various HA concentrations showed the optimal effects in the lower concentrations studied, which makes it a low-cost, high-yield investment for aquaculture facility managers to consider. The addition of HA as a supplement in the nutrient media of the studied microalgae is recommended – both *D. salina* and *N. salina* showed clear benefits when low concentrations of HA were used to enhance their nutritional composition.

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