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ORIGINAL PAPER

Homeopathy outperforms antibiotics treatment in juvenile scallop *Argopecten ventricosus*: effects on growth, survival, and immune response

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Background: Mortality from vibriosis in mollusk production is attributed to pathogenic bacteria, particularly _Vibrio alginolyticus_. Use of increasingly potent antibiotics has led to bacterial resistance and increased pathogenicity. Alternatives in sanitation, safety, and environmental sustainability are currently under analysis. To-date, homeopathy has been investigated in aquaculture of freshwater fish, but not in marine mollusks. The effect of the homeopathic complexes in the growth, survival, and immune response of the Catarina scallop _Argopecten ventricosus_ were assessed.

Methods: A bioassay to assess the potential of homeopathy in improving cultivation of juvenile _A. ventricosus_ was conducted for 21 days, with a final challenge of 120 h with _V. alginolyticus_. The experimental design included two homeopathic formulas The homeopathic complex Passival, consisting of Passiflora incarnata 30 CH, Valeriana officinalis 30 CH, Ignatia amara 30 CH and Zincum valerianicum 30 CH plus Phosphoricum acid 30 CH (treatment TH1) or Silicea terra 30 CH (TH2), two antibiotics (ampicillin = AMP, oxytetracycline = OXY), and two reference treatments (without homeopathic or antibiotic treatment = CTRL, ethanol 30° GL = ETH). Additionally, a negative control CTRL− (untreated/uninfected) is included in the challenge test. Juvenile scallops (4.14 ± 0.06 mm, 13.33 mg wet weight) were cultivated in 4 L tanks provided with aerated, filtered (1 μm), and UV-sterilized seawater that was changed every third day. They were fed a blend of the microalgae _Isochrysis galbana_ and _Chaetoceros calcitrans_ (150,000 cells mL^{-1} twice a day). All treatments were directly added to the tank water and then 500 mL challenge units were inoculated with 1×10^7 CFU/mL (LD_{50}) of _V. alginolyticus_.

Results: Juveniles grew significantly larger and faster in height and weight with TH2 compared to the ETH and CTRL ($P < 0.05$, ANOVA). Higher concentrations of proteins occurred in scallops exposed to TH2 (160.57 ± 7.79 mg g^{-1}), compared to other treatments and reference treatments. Higher survival rate during the challenge bioassay occurred with TH1 (85%), compared to AMP (53%), OXY (30%), and CTRL (0%), and superoxide dismutase ($P < 0.05$) was significantly higher in scallops treated with TH1, compared to other treatments and reference treatments.

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Conclusions: **Homeopathic treatments improved growth and survival and enhanced survival against *V. alginolyticus* in juvenile *A. ventricosus*. This suggests that homeopathy is a viable treatment for this mollusk to reduce use of antibiotics in scallops and its progressive increase in pathogenicity in mollusk hatcheries.** *Homeopathy* (2017) **106**, 18–26.

Keywords: Aquacultural homeopathy; Hatchery cultivation; Bivalve health; Antibiotics; *Vibrio alginolyticus*; Immune response

Introduction

The Catarina scallop *Argopecten ventricosus* (Sowerby II, 1842) is a medium-size pectinid that ranges from the Baja California Peninsula in Mexico to Peru.[1] It is a fishing resource with aquaculture potential[2] and, similar to other pectinids, has increasing market demand, particularly in the Gulf of California.[3] During hatchery production, some authors have addressed problems, such as susceptibility of larvae to bacterial diseases,[4] phenol oxidase activity in larvae, juveniles, and adults,[5] use of antibiotics in larval cultivation,[6] and assessment of water quality during larval development to optimize cultivation protocols.[7]

Despite advances in cultivation in hatcheries to ensure continuity in juvenile (spat) production, commercial yield is still hindered by high die-offs of larvae and juveniles.[8,9] Currently, the most relevant problems faced by growing diverse species of bivalves, including *A. ventricosus*, is the recurring presence of infectious diseases and parasites.[10,11] Vibriosis is one of the most frequently reported diseases leading to economic losses in facilities raising bivalve mollusks.[12] Among some species, *Vibrio alginolyticus* and *V. parahaemolyticus* are reported as the most virulent strains that are naturally present in coastal systems.[13,14] For several decades, these diseases were controlled with antibiotics, but its indiscriminate use to intensify production and obtain higher profitability favored antimicrobial resistance to treatment.[15] Higher doses of new generation antibiotics, combined with chlorine and disinfectants, have generated environmental problems for to floating fish farms and effluent discharges from shrimp farms.[16] Homeopathic treatments are potential alternatives to control diseases in cultivated animals, aquatic and terrestrial. It is known that homeopathic treatments reduced or eliminated some antibiotics,[17] improved general health of animals by strengthening their immune system,[18] and even controlling the spread of endoparasites and ectoparasites.[19–22]

Homeopathy has been successfully applied to including chickens and pigs as growth promoters,[23,24] to control pathogenicity of *Escherichia coli* in pigs,[25] improve rabbit production,[26] and facilitate chicken vaccination.[27] Some homeopathic treatments (*Fator Vermes*[20]; *Ferrum phosphoricum*, *Arsenicum album*, and *Calcarea carbonica*[21]) are used for controlling nematodes and helminths in small ruminants, mainly reducing stress that improves development and survival of the host.[22] Likewise, homeopathic complexes, such as sulfur, staphysagria, calendula, and oscillococcinum have shown a bactericidal effect that is useful for treating *in vitro* plants of sugar cane infected with the bacteria *Xanthomonas albilineans* in intensive agricultural production, surpassing results using antifungal and antibacterial chemicals.[28] Homeopathy has not been widely explored in aquaculture, but recent results demonstrate its potential. The Nile tilapia *Oreochromis niloticus* has been treated with homeopathic complexes, such as Homeopatila™ to increase reproductive performance and success of adults,[29] and increase muscle quality in juvenile stock.[30,31] Treatments applied to tilapia *O. niloticus* are able to increase morpho-functional response and the content and quality of fatty acids in muscle,[32,33] as well as to modulate content of cortisol in muscle.[34] In another important edible fish, the pacu *Piaractus mesopotámicus*, physiological response to stress was successfully modulated with homeopathy.[35] In the ornamental fish, the molly *Poecilia* spp., homeopathic treatment induced spawning and improved the reproductive response.[36,37] Finally, in the American bullfrog *Lithobates catesbeianus*, homeopathic treatment modified the rate of metamorphosis.[38]

The aim of this study was to assess two homeopathic complexes and two antibiotics, the latter are regularly used in aquaculture.[39] We measured growth, survival, biochemical composition, and immunological response of juvenile Catarina scallops.

Materials and methods

Ethics

Care of animals met CIBNOR's institutional guidelines.

Source of juvenile stock

Juvenile *A. ventricosus* was raised in the hatchery, using routine methods described by Mazón-Suástegui.[2] Conditioned and sexually mature broodstock were induced to spawn by thermal shock (18–28°C) and the gametes were fertilized to obtain embryos and larvae. Veliger larvae were cultivated (15 days) in 1500 L fiberglass cylinder-conical tanks, filled with filtered (1 μm), UV sterilized, and gently aerated seawater, kept at 23 $\pm$ 1°C and salinity of 37 $\pm$ 0.5. Scallop larvae were fed a 1:1 mix of cultivated microalgae whose cell concentration progressively increased from 20 to 40 $\times$ 10^3 cells mL^{-1}). Microalgal strains of *Isochrysis galbana* v. aff galbana (code UTEX LB 2307) and *Chaetoceros calcitrans* (origin from IFREMER, France) were obtained from CIBNOR's collection. These microorganisms were cultivated in glass and transparent polycarbonate tanks (2000 L), in filtered

(0.5 μm) and UV sterilized seawater at 22°C and 37 ± 0.5 salinity. Culture medium (f/2) was used for the flagellate *I. galbana* and silicates for the diatom *Ch. calcitrans*.[40] Harvesting of microalgae was made during its exponential growth phase.

Settlement of pediveliger larvae took place in plastic mesh collector substrates deployed within the same culturing tanks. Recently recruited juveniles (spat) were cultivated on the collector substrate and fed the same microalgal blend at 80−100 × 10^3 cells mL^{-1}. After two weeks, all collectors with early juveniles were manually shaken in seawater to detach them. Then, the spat were transferred to a fiberglass nursery unit (2000 L), each containing 12 vertical cylinders (20 L). These units were designed to recirculate seawater with microalgae, by means of air-lift devices to ensure continuous up-welling flow.[2] The nursery phase lasted four weeks at the same salinity and temperature, and included a daily total water exchange (only in this phase) to eliminate wastes and uneaten microalgae and a continuous feeding regime at a concentration of 150 × 10^3 cells mL^{-1}.

Treatments and experimental design

The homeopathic treatments *Passival®/Phosphoricum acidum* (TH1 = Pass/Pha) and *Passival®/Silicea terra* (TH2 = Pass/Sit) were assessed as growth promoters in two different trials. Passival™ is a commercial homeopathic medicinal complex consisting of Passiflora incarnata 30 CH, Valeriana officinalis 30 CH, Ignatia amara 30 CH and Zincum valerianicum 30 CH. These components are Similia™ homeopathic dynamizations prepared in ethanol 87° GL mixed in equal volumetric proportion.

TH1 consists of Passival™ 30 CH and Phosphoricum acid 30 CH (1:1 in volume; ethanol 87° GL). TH2 consists of Passival™ 30 CH and Silicea terra 30 CH (1:1 in volume; ethanol 87° GL). All products were manufactured by Farmacia Homeopática Nacional (México).[41] Additionally, the antibiotics oxytetracycline (OXY; #05750, Sigma−Aldrich, St. Louis, MO) and ampicillin (AMP; #A-9518, Sigma−Aldrich) were tested. We used ethanol from the Farmacia Homeopática Nacional® (www.similia.com.mx) to prepare homeopathic medicines (87° GL). A general diagram of the experimental design is shown in Figure 1.

In the first trial, we used 4 L plastic containers with 2 L filtered (1 μm) and UV-sterilized seawater at 23 ± 1°C and 37 ± 0.5 salinity. Juveniles (4.14 ± 0.06 mm mean shell height; 13.33 ± 0.03 mg live wet weight) were divided into six groups of 120 scallops each (in triplicate) and treated for 21 days with: TH1 or TH2 at 100 μL L^{-1} and OXY or AMP at 10 ppm.[42,43] One reference treatment was homeopathic ethanol 30° GL (ETH), diluted with distilled water and the other reference treatment was without homeopathy or antibiotic (CTRL). Treatments were administered after each water exchange (every 48 h) and before feeding. The measured variables were: survival, growth (shell height and wet weight), and biochemical composition of the stock. Assays were done with 30 scallops collected at random from each triplicate (see below details).

In the second trial, the effect of TH1 and TH2 were tested on immune response of juvenile scallops. After the end of the first trial (24 h after the last dose of treatment), the effect of a challenge with the pathogen bacteria *V. alginolyticus* CAIM57 (www.ciad.mx/caim) was measured for each previous treatment. Apart from the groups treated by homeopathy (TH1, TH2), antibiotics (OXY, AMP), and reference treatments (ETH, CTRL), an additional untreated and uninfected group (CTRL−) was included to eliminate false positives or other factors not related to the effect of the pathogen in survival during the challenge. The trial was performed in duplicate and 30 juvenile scallops from each treatment in Trial 1 (see Figure 1) were collected at random (5−6 mm shell height) and placed in 500 mL experimental units filled with 300 mL filtered seawater. This bioassay took place in a laboratory designed for working with bacteria under high biosecurity. Pure stock cultures (1 × 10^9 CFU mL^{-1}) of *V. alginolythicus* were cultured in trypticase soy broth medium (TSB; #257107, BD Biosciences, San Jose, CA) for 24 h at 35°C. A single initial infection dose was added to the water, adjusted to a final concentration of 10 × 10^6 CFU mL^{-1}. This dose was defined by the mean lethal dose (LD_{50}), previously determined by Probit analysis (logistic regression analysis), based on the dose−response model described by Finney,[44] using software.[45]

Survivors were counted at 0, 24, 48, 72, and 120 h after the challenge. Superoxide dismutase (SOD) enzyme activity was measured before the challenge and 48 and 72 h afterward (see below). During the challenge, no water was exchanged. In both trials, aeration was continuous and all juveniles were fed twice daily with the same microalgae mix at 80 × 10^3 cells mL^{-1}.

Growth and survival (Trial 1)

At the beginning of the first trial, 30 juveniles were photographed and images were processed (Image-Pro Plus 6.0, Media Cybernetics, Bethesda, MD)[46] to determine initial shell height (0.1 mm). Juveniles were also weighed on an analytical scale (±0.01 g) to determine initial wet weight with shell. Subsequent measurements of size and wet weight were taken on days 7, 14, and 21 to determine absolute growth (mm, mg) and growth rates (mm d^{-1}, mg d^{-1}) for each treatment. During samplings, survivors were counted to determine survival rates (%).

Biochemical composition (Trial 1)

The biochemical composition of their visceral masses under each treatment was determined with triplicate samples at the beginning and end of the first trial. Samples were stored at −80°C and then lyophilized, re-hydrated in 3 mL cold saline solution (35%), and homogenized to obtain crude extracts. Crude extracts were used to determine: (1) total carbohydrates according to Van Handel,[47] which uses a reagent blank and dextrose solution as the standard (#G8270, Sigma−Aldrich); absorbance was read

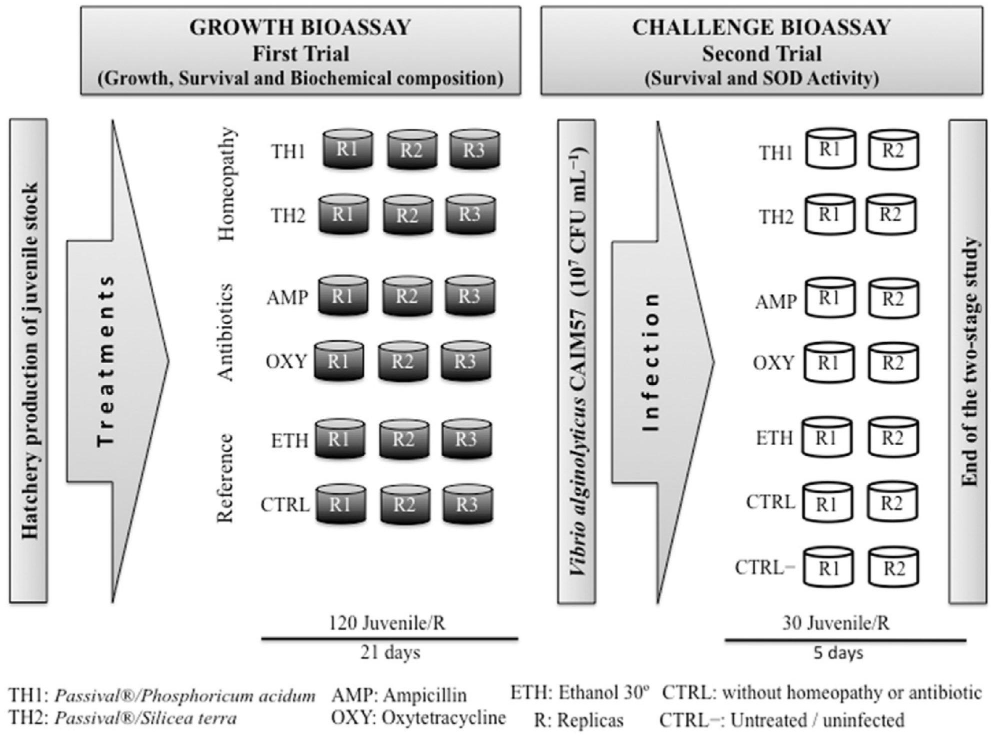

Figure 1 Details of the experimental design in using homeopathic complexes TH1 (*Pass/Pha*) and TH2 (*Pass/Sit*); antibiotics OXY (oxytetracycline) and AMP (ampicillin), and reference treatments ETH (ethanol), CTRL (without homeopathy or antibiotic) and CTRL− (untreated/uninfected) in the juvenile Catarina scallop *Argopecten ventricosus*.

at 620 nm; (2) total proteins according to Bradford,[48] which uses a reagent (#B6916, Sigma−Aldrich) and bovine serum albumin (#9048-46-8, Sigma−Aldrich) as the standard; absorbance was read at 595 nm; and (3) total lipids, following a modified version of Bligh,[49] using a microplate with 20 μL supernatant extract and 200 μL enzyme reagent (Randox Laboratories, Antrim, UK); absorbance was set at 560 nm.

Superoxide dismutase activity (Trial 2)

In the second trial (challenge against *V. alginolyticus*), activity of SOD was determined. For each treatment, soft tissues (100 mg) from six juveniles were weighted and 500 μL phosphate buffer (pH 7.5) were added. The tissues were homogenized and centrifuged at 9327×*g* for 10 min at 4°C, recovering the supernatant and storing it at −20°C until further analysis. SOD activity was determined with a commercial kit (SOD Assay Kit-WST #19160, Sigma−Aldrich). Results were expressed as an indirect measure of SOD activity as a percent of the water soluble tetrazolium salt (WST-1) formazan complex inhibition.

Statistical analysis

For each treatment group, normality was initially analyzed with the Kolmogorov−Smirnov test and then confirmed with the Levene test for homogeneity of variances. Thereafter, one-way ANOVA assessed significant differences in growth, biochemical composition for Trial 1, and survival and SOD activity in challenged scallops in Trial 2, as a function of the treatment. As needed, *post hoc* multiple range mean comparisons with Tukey's test (HSD) were included. Level of significance was set at $P < 0.05$ for all analyses.

Results

Growth, survival, and biochemical composition

From Trial 1, juveniles were significantly larger (Figure 2A) and grew faster (Figure 2B) in shell height when treated with TH2 (6.99 ± 0.09 mm; 0.08 mm d^{-1}) and TH1 (6.22 ± 0.11 mm; 0.05 mm d^{-1}), compared to the ETH (6 ± 0.14 mm; 0.05 mm d^{-1}) and CTRL (5.05 ± 0.10 mm; 0.02 mm d^{-1}). Higher wet weight biomass (Figure 3A) and daily gain (Figure 3B) occurred with TH2 (41.16 ± 0.35 mg; 1.3 mg d^{-1}), compared to the ETH (33.66 ± 0.10 mg; 0.9 mg d^{-1}) and CTRL (24.33 ± 0.10 mg; 0.5 mg d^{-1}). Survival was 100% in all treatments.

The biochemical composition of juvenile scallops in Trial 1 was significantly affected by the treatment (Table 1). Concentrations of protein were higher in scallops exposed to TH2 (160.57 ± 7.79 mg g^{-1}), compared to the CTRL (109.91 ± 1.06 mg g^{-1}). Lipid reserves were highest in the TH1 treatment (53.38 ± 3.27 mg g^{-1}); carbohydrate content was high in all treatments except the CTRL group (11.98 ± 0.83 mg g^{-1}).

Challenge with *V. alginolyticus*

Untreated juvenile scallops (CTRL) had 0% survival at 72 h after challenge with *V. alginolyticus*, while untreated

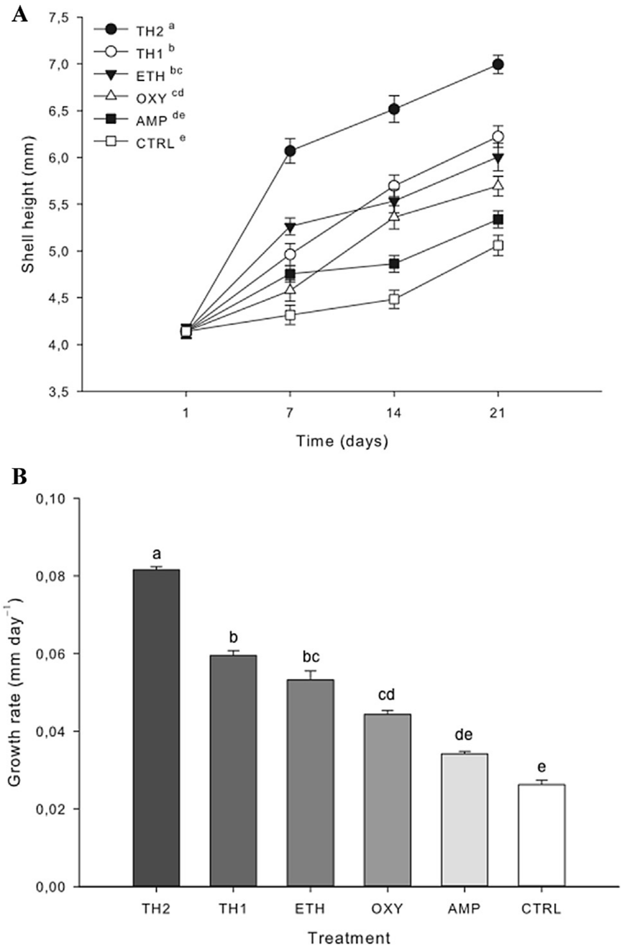

Figure 2 Absolute growth in shell height (**A**) and growth rate (**B**) in juvenile Catarina scallop *Argopecten ventricosus* treated with homeopathic complexes TH1 (*Pass/Pha*) and TH2 (*Pass/Sit*), antibiotics OXY (oxytetracycline) and AMP (ampicillin), and reference treatments ETH (ethanol) and CTRL (without homeopathy or antibiotics). Data show the mean ± SE. Treatments in legends are presented in the same order as in the figure. Different superscript letters denote significant differences ($P < 0.05$).

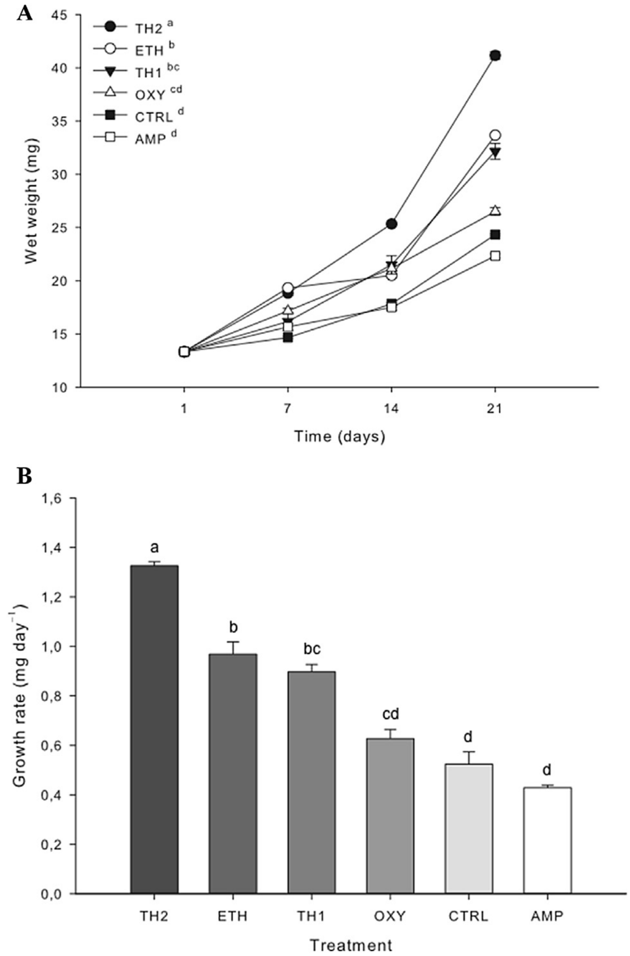

Figure 3 Absolute growth in total wet weight (**A**) and growth rate (**B**) in juvenile Catarina scallop *Argopecten ventricosus* treated with homeopathic complexes TH1 (*Pass/Pha*) and TH2 (*Pass/Sit*), antibiotics OXY (oxytetracycline) and AMP (ampicillin), and reference treatments ETH (ethanol) and CTRL (without homeopathy or antibiotics). Data show the mean ± SE. Treatments in legends are presented in the same order as in the figure. Different superscript letters denote significant differences ($P < 0.05$).

and uninfected juvenile (CTRL−) had 95%. Juveniles treated with TH1 and TH2 had >80% survival. At the end of the challenge (120 h), exposure to TH1 significantly reduced mortality, with a high of 85 ± 0.7% survival, compared to treatments with AMP at 53 ± 1.4% and OXY at 30 ± 1.4% (Figure 4).

SOD activity

Before the challenge, juveniles treated with OXY produced significantly more SOD (86 ± 1.06%) than the CTRL (63 ± 0.71%). The lowest SOD activity occurred in scallops treated with ETH (44 ± 1.84%). However, after the challenge, SOD activity significantly increased at 48 h with ETH (58.24 ± 0.71%) and decreased in OXY (9.34 ± 0.07%) and CTRL (15.38 ± 0.28%). At 72 h in the group treated with TH1 (81 ± 1.20%), SOD activity significantly increased over the other treatments and the CTRL. For the CTRL, no scallop survived to allow measuring SOD activity at 72 h (Figure 5).

Table 1 Changes in biochemical composition in juvenile Catarina scallop *Argopecten ventricosus* treated with homeopathic complexes TH1 (*Pass/Pha*) and TH2 (*Pass/Sit*); antibiotics OXY (oxytetracycline) and AMP (ampicillin); and reference treatments ETH (ethanol) and CTRL (without homeopathy or antibiotics)

Treatment	*Concentration (mg g^{-1})*		
	Total carbohydrates	*Total lipids*	*Total proteins*
Before treatment	8.21 ± 0.43^{c}	28.42 ± 0.32^{c}	65.25 ± 1.29^{d}
TH1	17.48 ± 0.18^{ab}	53.38 ± 3.27^{a}	76.49 ± 1.19^{d}
TH2	20.49 ± 1.15^{a}	42.54 ± 1.17^{ab}	160.57 ± 7.79^{a}
ETH	21.07 ± 0.29^{a}	31.45 ± 1.04^{bc}	132.35 ± 4.81^{b}
OXY	16.82 ± 4.09^{ab}	46.73 ± 8.37^{a}	141.16 ± 2.22^{b}
AMP	23.23 ± 1.34^{a}	42.61 ± 1.36^{ab}	122.68 ± 1.69^{bc}
CTRL	11.98 ± 0.83^{bc}	51.94 ± 0.05^{a}	109.91 ± 1.06^{c}

Data show the mean ± SE. Different superscript letters denote significant differences ($P < 0.05$).

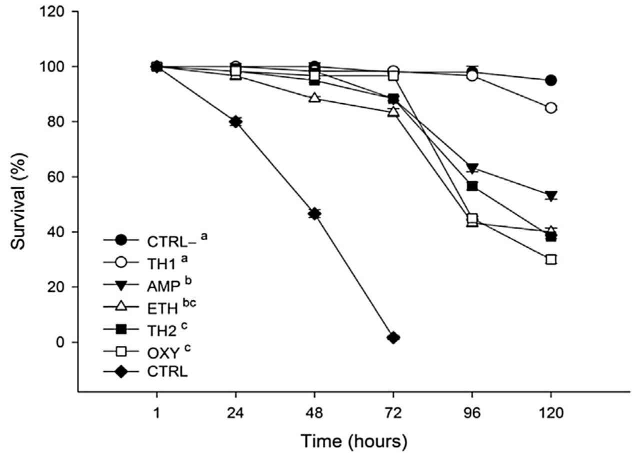

Figure 4 Survival rate in juvenile Catarina scallop *Argopecten ventricosus* treated with homeopathic complexes TH1 (*Pass/Pha*) and TH2 (*Pass/Sit*); antibiotics OXY (oxytetracycline) and AMP (ampicillin), and reference treatments ETH (ethanol) and CTRL (challenged without homeopathy or antibiotics) after challenge with pathogenic bacteria *Vibrio alginolythicus* strain CAIM57. A negative control CTRL− (untreated/uninfected) is included. Data show the mean ± SE. Treatments in legends are presented in the same order as in the figure. Different superscript letters denote significant differences ($P < 0.05$).

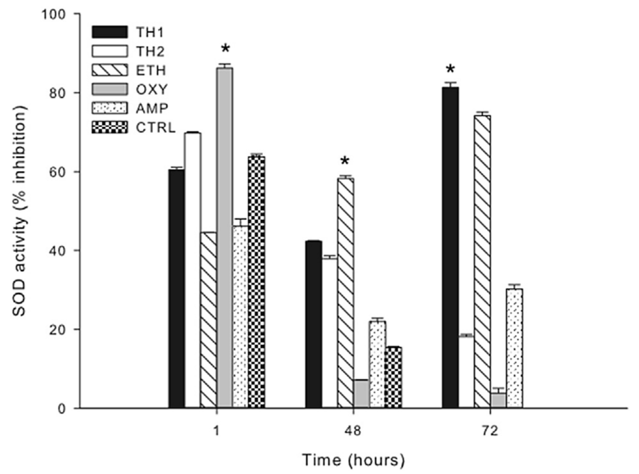

Figure 5 Changes in SOD activity determined by percentage inhibition during formation of the water-soluble tetrazolium complex (WST-1 formazan) in juvenile Catarina scallop *Argopecten ventricosus* treated with homeopathic complexes TH1 (*Pass/Pha*) and TH2 (*Pass/Sit*); antibiotics OXY (oxytetracycline) and AMP (ampicillin), and reference treatments ETH (ethanol) and CTRL (challenged without homeopathy or antibiotics) after challenge with pathogenic bacteria *Vibrio alginolythicus* strain CAIM57. Data show the mean ± SE. * denotes significant differences ($P < 0.05$).

Discussion

Juvenile *A. ventricosus* treated with homeopathic complexes TH1 and TH2 promoted growth and maximized survival. Both treatments included the *Passival*® formula used in human treatments as a tranquilizer to reduce stress and improve sleep[50]; thus it appears it may have an anti-stress action. Our results indicate that TH1 and TH2 treatments have different action spheres from the second component in the formula; TH1 includes *P. acidum*, a homeopathic medicine made from phosphoric acid obtained after calcination of phosphorus, which has the ability to treat gastrointestinal conditions that suggest malnutrition and poor food assimilation, and it is used for promoting exhaustion strength and general vigor. TH2 is one of the most valuable homeopathic medicines, as it includes *Silicea terra*, a mineral derived from quartz that has the potential to cure many different diseases, including poor nutrient absorption. In our study, TH2 yielded the fastest growth in shell length and weight gain and the highest protein content as well.[51] Regarding biochemical composition, scallops require high levels of lipids and proteins in the diet, but the protein content is a more relevant factor. For instance, in the Chilean scallop *A. purpuratus*, an increase in the protein content of microalgae by manipulation of the culture medium, significantly increases larval growth.[52] Increased protein content in microalgae also promotes better growth in juvenile scallops, likely due to an increase in the efficiency of absorption of such microalgal protein.[53] The enhanced response with TH2 is tied to the high protein content in juveniles, likely from better assimilation of nutrients and gain in soft tissue mass. This action is similar to the action of *S. terra*, used in humans for treating rickets, cachexia, malnutrition, and gastrointestinal conditions because it improves assimilation of nutrients.[54]

S. terra has been used for promoting growth in poultry[23] and swine.[24] Homeopathic treatments based on *Tuberculinum*, *Luesinium*, *Medorrhinum*, and *Ignatia* have led to increased weight of rabbits.[26] *China officinalis* has been used with a similar objective in swine infected with *E. coli*,[25] and *Calendula officinalis* to increase body weight in broiler chickens.[27]

Feeder fish, such as the white molly *Poecilia sphenops* and goldfish *Carassius auratus*, treated with *Natrum muriaticum* led to increased growth. *N. muriaticum* is a homeopathic medicine used mainly for dehydration and malnutrition in humans, and in the case of these ornamental freshwater fish, it significantly increased weight gain during the first spawning cycle.[36,37,55] The homeopathic complex *Homeopatila 100*® was used in juvenile and adult Nile tilapia *O. niloticus*, but significantly improved survival in juveniles.[30] In adult Nile tilapia that received *HomeoAqua Mega 3*®, no significant differences in growth and survival occurred, but lipid content was lower in muscle, which provided low fat fillets in human diets.[33]

In vivo challenge with pathogenic bacteria is a useful tool to understand the relative importance of the diverse defense factors of a species.[56] Experimental challenges are also used to assess and select possible treatments that confer protection to from pathogenic bacteria, as is performed in selecting probiotics[57] and vaccines.[58] Infection when a host's immune system is weak is common.[17] Antibiotics have been widely used to fight against pathogenic bacteria, applying the principle of contraries, which characterizes allopathic therapy and generally acts with non-specific bactericide effects and often harms the host's beneficial microbiota.[17,59] It includes commensal microorganisms, normally found in the digestive tract, that are essential for life, since they occupy specific niches that avoid and displace pathogenic bacteria.[60,61] In our study, juvenile *A. ventricosus* treated with either of the antibiotics had higher mortality after the bacterial challenge, particularly with oxytetracycline, as well as a significant decrease in the SOD activity after the challenge. This probably led to increased oxidative stress during the challenge. A similar effect is reported in the juvenile pleasure oyster *Crasssostrea corteziensis* treated with oxytetracycline,[62] likely from disturbance of the host's endogenous intestinal microbiota. This microbiota and some key environmental factors are crucial in modulating the immune system of animals.[63] Stimulation or suppression of SOD activity depends on the concentration of the antibiotic,[64] but the sensitivity and response of each species may be different for a specific antibiotic. In our study, juvenile *A. ventricosus* grew slower when treated with antibiotics, similar to increased mortality and reduced growth in larvae of the Chilean scallop *A. purpuratus* treated with oxytetracycline.[65] We also believe that some negative effects of antibiotics may be related to microalgae−bacteria interactions.[66]

In our study, juveniles treated with TH1 significantly improved growth and SOD activity (>80%) after challenge with *V. alginolyticus*. The beneficial results were higher than the treatment with antibiotics. This indicates that *P. acidum*, as the second component in the TH1 formula, effectively contributed to strengthening the innate immune system in juvenile scallops. Phosphoric acid is indicated to treat human patients with symptoms of generalized weakness, apathy, rickets, sexual weakness, malnutrition, and diarrhea. There are indications that it would increase overall performance in cultivated juvenile scallops. For that reason, we established as main response variables growth in size and weight, plus immune response when infected with a strain of *V. alginolyticus*, which is highly pathogenic to mollusks, primarily in the larval stages.

SOD is used as an indicator of immune response in marine invertebrates,[67,68] such as in challenge experiments on the whiteleg shrimp *Litopenaeus vannamei*[69,70] and Japanese oyster *Crassostrea gigas*.[71,72] Our experimental evidence indicates that homeopathic complexes can be substituted for antibiotics,[59] with favorable results in *A. ventricosus*. Similar benefits and advantages of using homeopathy in replacement of antibiotics, are reported using the *C. officinalis* and *Coli* 30 K to treat infection from *E. coli*[25,73] and *Silicea*, *Hepar sulfur*, *Belladona*, *Arnica montana*, *Mercurius solubilis*, and a homeopathic medicinal of *Staphylococcus aureus* to inhibit *S. aureus* and the *S. aureus* strain MRSA strain that is resistant to meticillin.[74]

Our results with homeopathic treatments highlight advantages of these treatments, and derivative benefits of improving sanitation, safety, and environmental sustainability in the aquaculture industry. For the first time, we report the use of these treatments in marine bivalve aquaculture, with potential to combine them with effective probiotic bacteria for eliminating indiscriminate use of antibiotics. Finally, basic and applied research must continue to establish the potential of homeopathic medicinals at a molecular/genetic level, particularly to reduce the use of antibiotics and their cumulative negative effects on cultivated edible mollusks, consumers, and coastal marine environments.

Conclusions

Juvenile Catarina scallop *A. ventricosus* treated with homeopathic complexes TH1 and TH2 improved growth (height and weight), as well as survival and enhanced immune response against a challenge with *V. alginolyticus* (only TH1).

Conflict of interest

The authors declare no competing interests.

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References

1 Keen AM (ed). *Sea Shells of Tropical West America*. Stanford, CA: Stanford Univ. Press, 1971.

2 Mazón-Suástegui JM. *Biología y cultivo de la almeja catarina Argopecten ventricosus (Sowerby II, 1842)* [Doctoral thesis]. Barcelona, Spain: Universitat de Barcelona, 2005.

3 FAO. The State of World Fisheries and Aquaculture (SOFIA). Fisheries and Aquaculture Department. http://www.fao.org/3/a-i3720e/index.html [Retrieved 2 February 2015].

4 Sainz JC, Maeda-Martinez AN, Ascencio F. Experimental vibriosis induction with *Vibrio alginolyticus* of larvae of the catarina scallop (*Argopecten ventricosus = circularis*) (Sowerby II, 1842). *Microb Ecol* 1998; **35**: 188−192.

5 Luna-González A, Maeda-Martínez AN, Vargas-Albores F, Ascencio-Valle F, Robles-Mungaray M. Phenoloxidase activity in larval and juvenile homogenates and adult plasma and haemocytes of bivalve molluscs. *Fish Shellfish Immun* 2003; **15**: 275−282.

6 Campa-Córdova AI, Luna-González A, Zarain-Herzberg M, Cáceres-Martínez CJ. Prophylactic use of antibiotics in larval culture of *Argopecten ventricosus* (Sowerby, 1835). *J Shellfish Res* 2005; **24**: 923−930.

7 Abasolo-Pacheco F, Mazón-Suástegui JM, Saucedo PE. Response and condition of larvae of the scallops *Nodipecten subnodosus* and *Argopecten ventricosus* reared at the hatchery with different seawater sources. *Aquaculture* 2009; **296**: 255−262.

8 Mazón-Suástegui JM, Maeda-Martínez AN, Robles-Mungaray M, *et al*. Avances en la producción de juveniles de *Nodipecten* spp. In: Maeda-Martínez AN, Lodeiros-Seijo C (eds). *Biología y Cultivo de los Moluscos Pectínidos del Género Nodipecten*. Mexico City: Limusa, 2011, pp 275−311.

9 Uriarte IG, Rupp G, Abarca A. Producción de juveniles de pectínidos iberoamericanos bajo condiciones controladas. In: *Los Moluscos Pectínidos de Iberoamérica Ciencia y Acuicultura*. Mexico City: Limusa, 2002, pp 147−172.

10 Barbosa V, Luna-González A, Aguirre-Guzmán G, Riquelme C, Ascencio-Valle F. Enfermedades microbianas de pectínidos cultivados en Iberoamérica. In: *Los Moluscos Pectínidos de Iberoamérica Ciencia y Acuicultura*. Mexico City: Limusa, 2002, pp 325–342.
11 Figueras A, Novoa B (eds). *Enfermedades de moluscos bivalvos de interés en Acuicultura*. Madrid: Editorial Paraninfo, 2011, pp 93–134.
12 Sainz-Hernández JC, Maeda-Martínez AN. Sources of *Vibrio* bacteria in mollusc hatcheries and control methods: a case study. *Aquac Res* 2005; **36**: 1611–1618.
13 Leyton Y, Riquelme C. Vibrios en los sistemas marinos costeros. *Rev Biol Mar Oceanogr* 2008; **43**: 441–456.
14 Luna-González A, Barbosa V, Mendes-De-Bem M. Inmunología y patología microbiana de moluscos bivalvos con énfasis en especies del género *Nodipecten* spp. In: Maeda-Martínez AN, Lodeiros-Seijo C (eds). *Biología y cultivo de los moluscos pectínidos del género Nodipecten*. Mexico City: Limusa, 2011, pp 203–225.
15 Lawrence R, Jeyakumar E. Antimicrobial resistance: a cause for global concern. *BMC Proc* 2013; **7**: 1–14.
16 Romero J, Feijoó CG, Navarrete P. Antibiotics in aquaculture—use, abuse and alternatives. In: Carvalho ED, Silva David G, da Silva RJ (eds). *Health and Environment in Aquaculture*. Croatia: InTech Europe, 2012, pp 159–198.
17 Viksveen P. Antibiotics and the development of resistant microorganisms. Can homeopathy be an alternative? *Homeopathy* 2003; **92**: 99–107.
18 Vockeroth WG. Veterinary homeopathy: an overview. *Can Vet J* 1999; **40**: 592.
19 Catto JB, Bianchin I, Feijo GLD, Araujo FR, Ramos CADN, Castelao ABC. Weight gain and control of endo-and ectoparasites of beef heifers treated with allopathic, herbal and homeopathic drugs. *Rev Bras Parasitol Vet* 2013; **22**: 502–510.
20 da Rocha RA, Pacheco RDL, Amarante AF. Efficacy of homeopathic treatment against natural infection of sheep by gastrointestinal nematodes. *Rev Bras Parasitol* 2006; **15**: 23–27.
21 Zacharias F, Guimarães JE, Araújo RR, *et al*. Effect of homeopathic medicines on helminth parasitism and resistance of *Haemonchus contortus* infected sheep. *Homeopathy* 2008; **97**: 145–151.
22 Molento MB, Veríssimo CJ, Amarante AT, *et al*. Alternative techniques for the control of gastrointestinal nematodes in small ruminants. *Arq Inst Biol* 2013; **80**: 253–263.
23 del Carmen MRR, Manuel CPM. *Efecto comparativo de la calcarea carbónica, la* Silicea terra *y la avoparcina como promotores del crecimiento en pollos de engorda* [Lic. thesis]. Guadalajara, Mexico: Universidad de Guadalajara, 1994.
24 Madan E. *El uso de Baryta Carbónica y Silicea Terra como promotor del crecimiento en la especie porcina*. La Habana: V Congreso Nacional de Ciencias Veterinarias. Palacio de las Convenciones, 2000, pp 211–212.
25 Coelho CDP, Vuaden ER, Soto FRM, *et al*. Evaluation of homeopathic treatment of *Escherichia coli* infected swine with identification of virulence factors involved: pilot study. *Int J High Dilution Res* 2014; **13**: 197–206.
26 Dodero JA, Espíndola GB, Ruipérez FH. Investigación en conejos con productos homeopáticos. *Lagomorpha* 1998; **97**: 29–34.
27 Barbour EK, Sagherian V, Talhouk S, *et al*. Evaluation of homeopathy in broiler chickens exposed to live viral vaccines and administered *Calendula officinalis* extract. *Med Sci Monit* 2004; **10**: 281–285.
28 García LRG, Villegas AB. Implicaciones médicas de las investigaciones sobre medicamentos homeopáticos realizadas en la Estación Territorial de Investigaciones de la Caña de Azúcar Villa Clara-Cienfuegos. *Rev Med Homeopat* 2014; **7**: 55–57.
29 Valentim-Zabott M, Vargas L, Ribeiro RPR, *et al*. Effects of a homeopathic complex in Nile tilapia (*Oreochromis niloticus*) on performance, sexual proportion and histology. *Homeopathy* 2008; **97**: 190–195.
30 Siena CE, Natali MRM, Braccini GL, de Oliveira AC, Ribeiro RP, Vargas L. Efeito do núcleo homeopático *Homeopatila 100*® na eficiência produtiva em alevinos revertidos de tilápia do Nilo (*Oreochromis niloticus*). *Semin Ciênc Agrár* 2010; **31**: 985–994.
31 Junior RP, Vargas L, Valentim-Zabott M, Ribeiro RP, Da Silva AV, Otutumi LK. Morphometry of white muscle fibers and performance of Nile tilapia (*Oreochromis niloticus*) fingerlings treated with methyltestosterone or a homeopathic complex. *Homeopathy* 2012; **101**: 154–158.
32 Braccini GL, Marçal MR, Ribeiro RP, *et al*. Morpho-functional response of Nile tilapia (*Oreochromis niloticus*) to a homeopathic complex. *Homeopathy* 2013; **102**: 233–241.
33 Andretto AP, Manfroi-Fuzinatto M, Braccini GL, *et al*. Effect of a homeopathic complex on fatty acids in muscle and performance of the Nile tilapia (*Oreochromis niloticus*). *Homeopathy* 2014; **103**: 178–185.
34 Merlini LS, Vargas L, Piau R, Pereira R, Merlini NB. Effects of a homeopathic complex on the performance and cortisol levels in Nile tilapia (*Oreochromis niloticus*). *Homeopathy* 2014; **103**: 139–142.
35 de Oliveira FKC, Aparecido JP, Sampaio DAJ. Physiological responses of pacu (*Piaractus mesopotamicus*) treated with homeopathic product and submitted to transport stress. *Homeopathy* 2013; **102**: 268–273.
36 Sudha C. Study on induced breeding in ornamental fish, *Poecilia sphenops*. *Eur J Exp Biol* 2012; **2**: 1250–1255.
37 Sudha C, Gokula V. Reproductive response of the guppy fish *Poecilia reticulata* for homeopathic medicine, *Natrum muriaticum*. *Biolife* 2014; **2**: 932–935.
38 Guedes JRP, Ferreira CM, Guimaraes HMB, Saldiva PHN, Capelozzi VL. Homeopathically prepared dilution of *Rana catesbiana* thyroid glands modifies its rate of metamorphosis. *Homeopathy* 2004; **93**: 132–137.
39 Gastalho S, da Silva G, Ramos F. Uso de antibióticos em aquacultura e resistência bacteriana: impacto em saúde pública. *Acta Farmacêutica Portuguesa* 2014; **3**: 29–45.
40 Guillard RRL. Culture of phytoplankton for feeding marine invertebrates. In: Smith WL, Chanley MH (eds). *Culture of Marine Invertebrate Animals*. New York: Plenum Press, 1975, pp 26–60.
41 Similia. Distribuidora Homeopática Nacional (Farmacia Homeopática Nacional) y Propulsora de Homeopatía. http://www.similia.com.mx [Retrieved 19 February 2015].
42 Aftabuddin S, Kader A, Kamal AM, Zafar M. Present status on the use of antibiotics and chemicals in shrimp hatcheries and grow-out ponds and their environmental implications in Bangladesh. *AACL Bioflux* 2009; **2**: 369–379.
43 Abasolo-Pacheco F, Saucedo PE, Mazón-Suástegui JM, *et al*. Isolation and use of beneficial microbiota from the digestive tract of lions-paw scallop *Nodipecten subnodosus* and winged pearl oyster *Pteria sterna* in oyster aquaculture. *Aquac Res* 2015; http://dx.doi.org/10.1111/are.12754.
44 Finney DJ (ed). *Probit Analysis*. London: Cambridge University Press, 1971.
45 International Business Machines Corp. (IBM). *SPSS statistics*. Ver. 20 para Mac, http://www14.software.ibm.com; 2011.
46 Media Cybernetics. *Image-pro plus 6.0*. Silver Spring, MD: Media Cybernetics, http://www.mediacy.com/; 2006.
47 Van Handel E. Estimation of glycogen in small amounts of tissue. *Anal Biochem* 1965; **11**: 256–265.
48 Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 1976; **72**: 248–254.
49 Bligh EC, Dyer WJ. A rapid method of total lipid extraction and purification. *Can J Biochem Phys* 1959; **37**: 911–917.
50 Laboratorios médicor. http://www.medicor.com.mx/insomnio.html [Retrieved 19 February 2015].

51 Farías A. Nutrición en moluscos pectínidos. In: Maeda-Martínez AN (ed). *Los Moluscos Pectínidos de Iberoamérica: Ciencia y Acuicultura*. Mexico City: Limusa, 2001, pp 89–104.
52 Farías A. Uriarte I. Nutrición en larvicultura de pectínidos: Relevancia de proteínas y lípidos. In: Cruz-Suárez LE, Ricque-Marie D, Tapia-Salazar M, Gaxiola-Cortés MG, Simoes N (eds). *Avances en Nutrición Acuícola VI. Memorias del VI Simposium Internacional de Nutrición Acuícola*; 2002;, pp 309–313. Cancún, Quintana Roo, Mexico.
53 Uriarte I, Farías A. Effect of broodstock origin and postlarval diet on postlarval performance of the Chilean scallop *Argopecten purpuratus*. In: Lavens P, Roelants I (eds). *Larvi'95 – Fish and Shellfish Larviculture Symposium*. Ghent, Belgium: European Aquaculture Society, Special Publication 24, 1995, pp 69–72.
54 Boericke W. Homeopathic material medica. http://www.homeoint.org/books/boericmm/s/sil.htm [Retrieved 4 February 2015].
55 Vishakan R, Balamurugan S, Maruthanayagam C, *et al*. Homeopathic induction of spawning in ornamental fish. In: Pandian TJ, Strüssmann CA, Marian MP (eds). *Fish Genetics and Aquaculture Biotechnology*. Boca Raton, FL: CRC Press, 2005, pp 119–121.
56 Allam B, Paillard C, Auffret M, Ford SE. Effects of the pathogenic *Vibrio tapetis* on defense factors of susceptible and non-susceptible bivalve species: II. Cellular and biochemical changes following *in vivo* challenge. *Fish Shellfish Immunol* 2006; **20**: 384–397.
57 Verschuere L, Rombaut G, Sorgeloos P, Verstraete W. Probiotic bacteria as biological control agents in aquaculture. *Microbiol Mol Biol R* 2000; **64**: 655–671.
58 Knight-Jones TJD, Edmond K, Gubbins S, Paton DJ. Veterinary and human vaccine evaluation methods. *Proc R Soc B* 2014; **281**: 28–39.
59 Batra M. Homeopathy: the effective alternative to antibiotics. *Townsend Lett* 2013; **360**: 51.
60 Prieur D, Mvel G, Nicolas JL, Plusquellec A, Vigneulle M. Interactions between bivalve molluscs and bacteria in the marine environment. *Oceanogr Mar Biol Annu Rev* 1990; **28**: 277–352.
61 Moriarty DJW. The role of microorganisms in aquaculture ponds. *Aquaculture* 1997; **151**: 333–349.
62 Campa-Córdova AI, González-Ocampo H, Luna-González A, Mazón-Suástegui JM, Ascencio F. Growth, survival, and superoxide dismutase activity in juvenile *Crassostrea corteziensis* (Hertlein, 1951) treated with probiotics. *Hidrobiologica* 2009; **19**: 151–157.
63 Wolowczuk I, Verwaerde C, Viltart O, *et al*. Feeding our immune system: impact on metabolism. *Clin Dev Immunol* 2008. Article ID 639803.
64 Ren L, Hemar Y, Perera CO, Lewis G, Krissansen GW, Buchanan PK. Antibacterial and antioxidant activities of aqueous extracts of eight edible mushrooms. *Bioact Carbohydr Diet Fibre* 2014; **3**: 41–51.
65 Miranda CD, Rojas R, Abarca A, Hurtado L. Effect of florfenicol and oxytetracycline treatments on the intensive larval culture of the Chilean scallop *Argopecten purpuratus* (Lamarck, 1819). *Aquac Res* 2013; **45**: 16–30.
66 Campa-Córdova AI, Luna-González A, Ascencio F, Cortes-Jacinto E, Cáceres-Martínez CJ. Effects of chloramphenicol, erythromycin, and furazolidone on growth of *Isochrysis galbana* and *Chaetoceros gracilis*. *Aquaculture* 2006; **260**: 145–150.
67 Li J, Xu Y, Jin L, Li X. Effects of a probiotic mixture (*Bacillus subtilis* YB-1 and *Bacillus cereus* YB-2) on disease resistance and non-specific immunity of sea cucumber, *Apostichopus japonicus* (Selenka). *Aquac Res* 2015; **46**: 3008–3019.
68 Song L, Wang L, Qiu L, Zhang H. Bivalve immunity. In: Söderhäll K (ed). *Invertebrate Immunity*. New York: Springer, 2010, pp 44–65.
69 Wang LU, Chen JC. The immune response of white shrimp *Litopenaeus vannamei* and its susceptibility to *Vibrio alginolyticus* at different salinity levels. *Fish Shellfish Immunol* 2005; **18**: 269–278.
70 Hsieh TJ, Wang JC, Hu CY, Li CT, Kuo CM, Hsieh SL. Effects of Rutin from *Toona sinensis* on the immune and physiological responses of white shrimp (*Litopenaeus vannamei*) under *Vibrio alginolyticus* challenge. *Fish Shellfish Immunol* 2008; **25**: 581–588.
71 Labreuche Y, Lambert C, Soudant P, Boulo V, Huvet A, Nicolas JL. Cellular and molecular hemocyte responses of the Pacific oyster, *Crassostrea gigas*, following bacterial infection with *Vibrio aestuarianus* strain 01/32. *Microb Infect* 2006; **8**: 2715–2724.
72 Gagnaire B, Gay M, Huvet A, Daniel JY, Saulnier D, Renault T. Combination of a pesticide exposure and a bacterial challenge: in vivo effects on immune response of Pacific oyster, *Crassostrea gigas* (Thunberg). *Aquat Toxicol* 2007; **84**: 92–102.
73 Camerlink I, Ellinger L, Bakker EJ, Lantinga EA. Homeopathy as replacement to antibiotics in the case of *Escherichia coli* diarrhoea in neonatal piglets. *Homeopathy* 2010; **99**: 57–62.
74 Passeti TA, Souza APM, Beltrame RL, Coelho CP, Diniz S. Homeopathy: a possible weapon against multidrug-resistant bacteria to antibiotics. *Int J High Dilution Res* 2014; **13**. 114–114.