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RESEARCH ARTICLE

EFFECT OF GARLIC (*ALLIUMSATIVUM*), ADMINISTERED IN A BALANCED RATION, ON THE GROWTH AND SURVIVAL OF WHITELEG SHRIMP (*LITOPENAEUSVANNAMEI*, BOONE 1931)

González García Rubí, Capistrán BarradasAscención, Díaz Inocencio Diana Laura and Eduardo Alfredo Zarza Meza*

Facultad de Ciencias Biológicas y Agropecuarias, Región Poza Rica-Tuxpan, Universidad Veracruzana, Carr. Tuxpan-Tampico Km. 7.5, Tuxpan, Veracruz, 92850, México

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ABSTRACT

Aquaculture is an alternative method for the production of organisms for society; however, on occasions, this commercial activity is affected by the appearance of virus or bacteria in said organisms, diseases treated by the use of artificial chemicals, such as antibiotics, which may affect people's health. Research is currently underway on alternative means, taken from natural medicine, of preventing viral and bacterial propagation in aquaculture production, including the present study, which was undertaken on a white leg shrimp (*Litopenaeus vannamei*) culture for eight weeks. The experimental design comprised seven fish tanks divided between two treatments containing fresh garlic at different concentrations and a control. The first treatment comprised 2.5 g garlic per kg of feedand the second comprised 5 g of garlic per kg, while the control treatment comprised commercial feed only. It should be noted that the first and second treatments were repeated twice. Treatments 1 and 3 presented 3% mortality, while 2% mortality was observed for Treatment 2,which presented the highest values for the length-weight correlation as well as the highest length and weight values.

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INTRODUCTION

Litopenaeus vannamei is distributed from northern to central Mexico and down to South America, to its southernmost distribution in Tumbes, Peru, in regions where water temperatures are normally>20 °C throughout the year. Shrimp culture in Mexico began, in the mid-1980s, with the production of the species *Litopenaeus stylirostris* along the length of the north east Pacific coast. Due to the easy handling ofthe species, Mexico produced 150,005 tons of *Litopenaeus. Vannamei* in 2017 (CONAPESCA, 2017), with shrimp culture increasing each year. In 1996 and 1998, problems occurred in shrimp farming due to viral diseases such as Taura syndrome and the white spot syndrome virus (Santiago *et al*., 2009). Favorable results have been obtained by research conducted on the use of garlic (*Alliumsativum*) in aquaculture. Garlic contains condensed carbohydrates/glucides, such as fructoses, andglutamyl dipeptides with sulphur radicals, which are formed by glutaminic acid and *S*-Allylcysteine, with the latter, known as allicin, a source of these antibiotic properties (García-Alonso, 1980). Various studies have been conducted on the use of natural products. Arcienegas-Hartman (2014) evaluated the variables of growth, survival, and production cost in red tilapia fingerlings in the sexual reversion phase. He administereda commercial sex reversion treatment, containing 45 % protein, with garlic paste, at 5 g/kg concentrations in the first treatment and at 2.5 g/kg concentrations in the second treatment. The subsequent two treatments contained a gram-positive bacillus at 2.5 g/kg and 1.25 g/kg and a control. Having installed plastic netting sub-divisions in a pond, Arcienegas-Hartman used a randomized experimental design comprising 12 experimental units, in which four treatments and three repetitions were administered over a 30-day period. Silva-Blanco (2013) added 2 and 4 % garlic to a balanced ration administered to goldfish (*Carassius auratus*)andguppy (*Poecilia reticulata*), taking into account variables related to juvenile growth and survival. Each fish species was separated into three groups, a control group, a group fed 2% garlic in the ration, and another group fed 4% garlic in the ration, with three tanks or fish tanks each. The statistical analysis of the results did not find significant differences in the parameters evaluated among the groups (Silva-Blanco, 2013).

Corresponding author: **Alfredo Zarza Meza,**
Facultad de Ciencias Biológicas y Agropecuarias, Región Poza Rica-Tuxpan, Universidad Veracruzana, Carr. Tuxpan-Tampico Km. 7.5, Tuxpan, Veracruz, 92850, México.

In Ecuador, when the Taura virus was found in shrimp postlarvae, a liquid mixture of garlic and lime was used to control it, a mixture which was also administered randomly into shrimp ponds as a natural antibiotic. Its antiseptic and mycotic effect enables disinfection, the biostimulation of the immune system, and the ability to combat the viruses, bacteria, and parasites that attack crustaceans, obtaining favorable results in terms of low mortality at low cost via this natural non-contaminating product (Villar-Ochoa, 2013). Another study evaluated the effect of *Allium sativum* and *Matricaria chamomilla* on *Poecilia latipinna,* which is attacked by one of the infections produced by *Ichthyophtirius multifilus*, which is more frequent in ornamental fish. The experiment was divided into two groups, with Group A receiving a 0.1 g/L concentration of garlic and Group B receiving 0.4 g/L *Matricaria chamomilla*. The best result was obtained for Group A, although both treatments were found to be viable for treating ectoparasites and, above all, for substituting the use of chemical treatments for the multiple benefits of using plant extracts (Gholipour *et al.*, 2012).

Garlic can be added in emulsion form to enable easy adhesion, a technique that has been used to evaluate tilapia productivity. Two treatments, supplemented with 2.5 and 5% garlic, as well as a control, were applied, evaluating weight, length, width, body condition score, and food conversion. The study found that much more positive results were obtained via the first treatment (Franco-Cabrera, 2012). Garlic is able to prevent and cure *Aeromonas* infections, a property studied in Thai catfish damaged by *Aeromonas hidrophila* in an *in vitro* experiment, in which garlic was administered in 1, 2, 5,10, 25, and 50 mg/ml doses, with 25 mg/mL found to be the most effective in preventing infection (Yuhana-Normalina, 2008). Shalaby *et al.* (2006) compared the effects on tilapia of both the antibiotic chloramphenicol and garlic, at respective doses of15, 30, and 45 g/kg and 10, 20, 30, and 40 g/kg. The two treatments were administered for a 90-day period in a balanced diet, while a control diet containing neither chloramphenicol nor garlic was also administered for same period. The results obtained revealed that the 30mg/kg garlic treatment achieved a higher feed conversion ratio, as well as higher growth and survival rates, than either the cloramphenicol or control treatments. Colorni *et al*. (1998) studied the histopathological and serological effects of an experimental *Mycobacterium marinum* infection on European bass (*Dicentrarchus labrax*). The study involved intraperitoneally injecting garlic, both separately and in combination with streptomycin, once every two weeks for a 12-week period. However, favorable results were not obtained.

Whiteleg shrimp production in Mexico has been impacted by *Vibrio parahaemolyticus*. Survival rate, growth, immune system gene expression, stress, and digestion were studied in whiteleg shrimp (*L. vannamei*) infected with *Vibrio parahaemolyticus* and administered 2, 6, and 10 g/kg garlic feed treatments combined with *Bacillus lincheniformis* BCR 4–3 g feed treatments, along with a control. Said study aimed to seek treatment alternatives without the use of artificial chemicals, by isolating and characterizing the strains causing early mortality syndrome in the hepatopancreas of *L. vannamei*. The most effective treatment was a combined feed treatment comprising 6 g garlic/kg (1x10 UFC) and *B. licheniformis* BCR 4-3/g (López, 2015).

The use of natural bactericides is cheaper than chemical solutions and, moreover, it does not negatively impact either consumer health or the environment. López and Rivera (2013) studied the effect of three natural bactericides (garlic, onion, and ginger) on populations of *Vibrio cholerae*, a pathogen occurring in juvenile *L. vannamei.* Simultaneous to the preparation of an agar (TCBS) culture for the bacterial culture, an external analysis of the organism was carried out, as were the extraction and maceration of the hepatopancreas and the bacterial culture and count. The following results were obtained: garlic – 78,000 to 3,333 colony-forming units, color yellow, per gram, and 99,000 to 1,000 colony-forming units, color green, per gram; onion – 183,000 to 19,000 colony-forming units, color yellow, per gram, and 78,000 to 6,666 colony-forming units, color green, per gram; and, ginger – 10,000 and 27,000 colony-forming units, color yellow, per gram, and 22,222 to 6,666 colony-forming units, color green, per gram. The level of growth in *Macrobrachium rosenbergi* postlarvae was determined by means of the dietary incorporation of *Allium sativum* (garlic), *Zingiber officinale* (ginger), *Cúrcuma longa* (turmeric), and *Trigonella foenum-graecum* (fenugreek), obtaining a higher survival rate, 69–80%, than the 66% obtained for the control. The following parameters were also considered: condition factor; feed conversion ratio; conversion efficiency; protein conversion efficiency; metabolizable energy; and, gross energy. Garlic obtained the most statistically significant results, of $p < 0.05$, for these parameters (Poongodi *et al.,* 2012). Research conducted for six weeks on *Procambarus clarkii* studied the efficacy of probiotics, prebiotics, and stimulants, via a 1%, 2%, and 3% Biogen (probiotics) concentration, *Allium sativum* (garlic), *Cynodon dactylon* (as an immunostimulant), a calcium alginate concentration (3g/l) as a prebiotic, and a control. *C. datylon* and *A. sativum* obtained significantly higher increases in survival and growth rates than the other treatments (Mona *et al.,* 2015).

MATERIALS AND METHODS

Two thousand *L. vannamei* postlarvae, received via donation, were transferred to the bioassay laboratory at the Faculty of Biological, Agricultural, and Livestock Sciences, at the Poza Rica-Tuxpan Region campus of the *Universidad Veracruzana.* The subjects were acclimated for 15 days in 800L tanks, under recirculation and oxygenation conditions, with 294 juveniles then selected and placed in seven 102 L fish tanks containing salt water extracted from the sea and subject to water changes every 15 days. The subjects' feed, received in four portions per day, comprised balanced commercial feed containing 40 % crude protein, to which two different quantities of garlic, 2.5 g and 5 g per kilo of feed, were added, while the control comprised commercial feed without garlic. Biometries and mortality rates were taken every week, while the temperature and physico-chemical parameters of oxygen and salinity level were taken daily. Bromatological analysis was undertaken on both the garlic-supplemented and commercial feed, while moisture levels were also determined and the protein levels were measured via the Kjeldahl technique (1883). Said technique was also used, via three phases (digestion, distillation, and titration), to measure the total nitrogen content in the sample, while the percentage of fat was also calculated. Finally, the shrimp were dissected in order to observe the anatomical structures (hepatopancreas, intestine, gills, and

telson, etc) and ascertain whether the garlic had achieved positive results and had also not damaged the organs.

The formula for obtaining the feed conversion rate was:

$$\text{Feed conversion rate} = \frac{\text{Feed consumed (g)}}{\text{Weight gained (g)}}$$

$$\text{Growth rate} = \frac{\text{Final average weight} - \text{Initial average weight}}{\text{Weight gained (g)}}$$

RESULTS

The final average length after eight weeks was 6.02 ± 10 cm for the 336 individuals measured, with minimum and maximum lengths of 3.0 cm and 9.5 cm, respectively. The values for weight (g) were 3.33 ± 0.7 g, with minimum and maximum weights of 1.4 g and 6 g, respectively. The length to weight correlation for the 2.5 g garlic treatment was r=0.99, p 0.01 (Figure 1), while this was r=0.98, p 0.01 for the 5 g garlic treatment (Figure 2), and r=0.98, p0.01 for the control. The organisms gained weight as they grew (Figure 3). While the survival rate was found to be high, by the last week of the experiment, 3 % mortality was observed for the 5 % garlic treatment and 2 % mortality was observed for the 2.5% garlic treatment. The salinity observed in the present study ranged from 30 to 31 psu, while temperature ranged from 28 to 29 ° C and the dissolved oxygen from 5.6 to 6 ml/L. The complete bromatological analysis for each treatment shows minimal differences in terms of both the 2.5 % (Table 1) and 5 % (Table 2) garlic treatments, in contrast with the control (Table 3), which may be due to the low garlic concentration in the two garlic treatments.

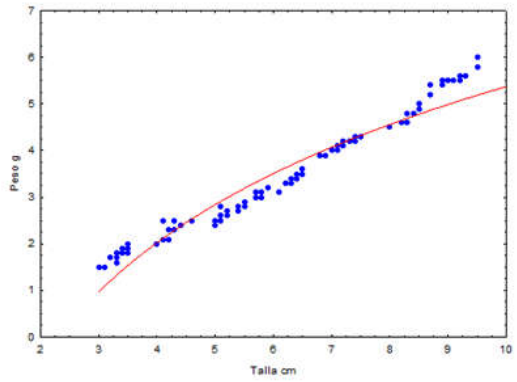

Figure 1. Length to weight correlation for the 2.5 g garlic treatment

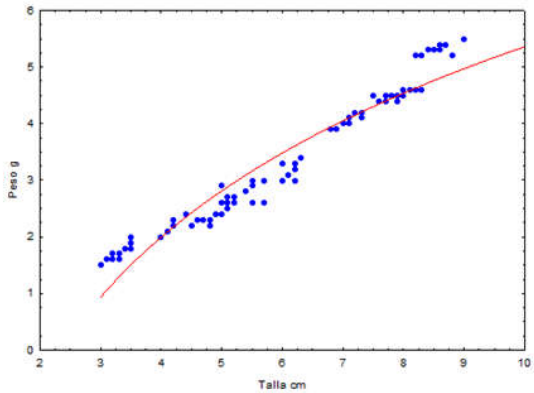

Figure 2. Length to weight correlation for the 5g/kg treatment

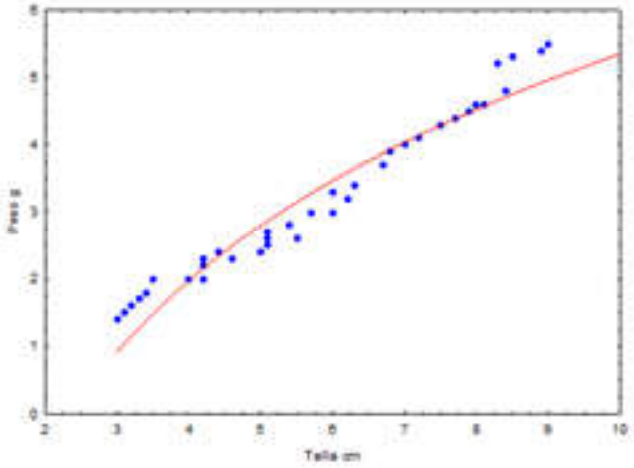

Figure 2. Length to weight correlation for the control treatment

DISCUSSION

The results obtained for *L. vannamei* in the present study, by means of the 2.5 % garlic treatment, are relatively similar to those obtained by Silva and Blanco (2013), who conducted a study on ornamental fish and reported greater lengths in goldfish (*Carassius auratus*) administered feed containing 2 and 4 g garlic. These results contrasted with the shorter lengths found for *Poecilia reticulata*. Andino and Romero (2014) obtained better results via the use of a 6g/kg garlic feed treatment in *L. vannamei.* Franco-Cabrera (2011) administered a 2.5 % garlic treatment, obtaining the best length results for Nile tilapia (*Oreochromis niloticus*), a finding coinciding with the result obtained in the present study for the 2.5% garlic treatment, in that the best length results were obtained at this concentration. Arcienegas (2014) administered three different garlic treatments per kilo to three-day-old tilapia (*Oreochromis sp.*) fingerlings, with a 5 g/kg treatment obtaining the best average length results, 25.07 mm, compared to the other two treatments (24.65 mm and 24.59 mm).

Silva and Blanco (2013) obtained, in *Poecilia reticulata* and *Carassius auratus,* the best results for weight with a 4 g garlic treatment, a finding which does not coincide with the results of the present study, in which the best weight result for *L. vannamei* was obtained with Treatment 1 (2.5 g garlic). It can be concluded from these results that a lower garlic concentration may lead to a higher level of palatability. Similarly, Arcienegas (2014), who supplemented commercial red tilapia (*Oreochromis sp*) feed with garlic, found that treatment with 5 g garlic/kg obtained the best final average weight gains, a finding which does not concur with the results obtained by the present study for *L. vannamei*. The present study found that the length to weight correlation remained constant for each week of the experiment, which concurs with the results found by Fraga *et al.* (2002) for weekly growth in *Litopenaeus schmitti*. Moreover, the seeding density was similar to that used in the present study, with the same or similar growth results obtained. This is due to the good practice applied in both studies as well as other factors that were not considered in the present study/by Fraga *et al.*, such as temperature, given that temperatures over 34 C may cause dwarfism (Carvajal and Bolaños, 2013). Aside from its aseptic effects, the use of garlic may have collateral beneficial effects, with, for example, Poongodi *et al.* (2012) and Andino and Romero (2014) recording a survival rate of up to 85 % in *L.*

Table 1. Bromatological analysis of commercial feed with 2.5% garlic

Content	WET BASIS (%)	DRY BASIS (90) %	DRY BASIS (100 %)
Dry material 92.5%			
Moisture 7.05%			
Crude protein	42.26	40.92	45.47
Ether extract	10.6	10.27	11.41
Total ashes	9.69	9.38	10.42
Crude fiber	3.74	3.62	4.02
Nitrogen-free elements	26.62	25.81	28.68
% of the total digestible nutrients	75.02	72.64	80.71
Digestible energy K cal/Kg	3.55	3.44	3.82
Metabolizable energy K cal/Kg	3.13	3.05	3.39

Table 2. Bromatological analysis of commercial feed with 5% garlic

Content	WET BASIS (%)	DRY BASIS (90) %	DRY BASIS (100 %)
Dry material 91.89%			
Moisture 8.11%			
Crude protein	41.24	40.39	45.47
Ether extract	10.51	10.29	10.44
Total ashes	9.02	8.84	9.82
Crude fiber	5.33	5.22	5.8
Nitrogen-free elements	25.79	25.26	28.06
% of the total digestible nutrients	72.22	72.69	80.77
Digestible energy K cal/Kg	3.56	3.48	3.87
Metabolizable energy K cal/Kg	3.17	3.11	3.45

Table. 3 Bromatological analysis of commercial feed (control)

Content	WET BASIS (%)	DRY BASIS (90) %	DRY BASIS (100 %)
Dry material 91.35%			
Moisture 8.65%			
Crude protein	40.57	39.96	44.4
Ether extract	13.84	13.64	15.15
Total ashes	8.07	7.95	8.84
Crude fiber	3.38	3.33	3.7
Nitrogen-free elements	25.49	25.12	27.91
% of the total digestible nutrients	77.23	76.09	84.54
Digestible energy K cal/Kg	3.72	3.67	4.08
Metabolizable energy K cal/Kg	3.34	3.29	3.66

vannamei via the administration of diets supplemented with garlic. These results depend on many factors, but mainly good aquacultural practice (Carvajal and Bolaños,2013). Similarly, López and Rivera (2013) found garlic to be a natural bactericide which obtained better results than a chemical bactericide. Arcienegas (2014) found optimal results in red tilapia fingerlings treated with 2.5 g garlic, while Shalaby *et al.* (2006) used garlic and chloramphenicol and found that feed supplemented with a 30 g/kg garlic concentration obtained the highest feed conversion rate in *Oreochromis sp* juveniles. Mona *et al.* 2015 conducted a study in which *Macrobrachium rosenbergii* larvae were administered feed containing garlic and biogenic and probiotic material, finding high survival rates with the garlic treatment, which concurs with the findings of the present study for *L. vannamei*.

Conclusion

A survival rate of 97 % was obtained for Treatment 2 (5 g/kg of garlic) and the control, while a 98 % survival rate was obtained for Treatment 1 (2.5 g/kg of garlic). Under the conditions used in the present study, low concentrations of fresh garlic in the feed, above all at concentrations of 2.5 g per kg of feed, were found to obtain the best results in terms of weight and length gain for whiteleg shrimp. Moreover, as it was found that the use of garlic does not negatively affect organism growth and survival, it can be concluded that garlic is a viable feed alternative in shrimp culture, given that it functions as a natural antiseptic, providing organisms protection against virus and bacteria. Larva survival rates were consistently high, with only relative decreases observed towards the final stages of the study.

REFERENCES

Andino L. A., Romero R. F. 2014. Efecto de dos tratamientos contra enfermedades bacterianas: Virkon vs. Ajo, sobre el crecimiento del camarón *Litopenaeus vannamei*. Tesis de Ingeniería. Universidad Nacional Autónoma de Nicaragua Unan- León Facultad De Ciencias y Tecnología. Carrera de Ingeniería Acuícola, Nicaragua.

Arcienegas-Hartman O. 2014. Determinación del efecto de la pasta de ajo (*Allium sativum*) y un bacilo gram positivo (*Bacillus amyloliquefaciens*), en el alimento comercial, sobre el crecimiento y supervivencia de postlarvas de tilapia roja (*Oreochromis* sp; Trewavas 1983). Tes00is de ingeniería en producción acuícola. Pasto, Colombia.

Carvajal. J.Y. Bolaños N.B.2013. Efecto de dos tipos de dietas: Comercial y Experimental sobre el crecimiento de camarones Litopenaeus vannamei en etapa de postlarvas. Tesis de ingeniero acuícola. Universidad Nacional Autónoma de Nicaragua. León, Nicaragua.

Colorni, A.; Avtalion, R.; Knibb, W.; Berger, E.; Colorni, B.; Timan, B. 1998. Histopathology of sea bass (*Dicentrarchus*

labrax) experimentally infected with *Mycobacterium marinum* and treated with streptomycin and garlic (*Allium sativum*) extract. Aquaculture 160: 1-17.

CONAPESCA. 2017. Anuario estadístico de acuacultura y pesca. SAGARPA. Mazatlán. Sinaloa 300 p. México. 27pp.

Franco-Cabrera L. F. 2012. Evaluación de la productividad de la tilapia (*Oreochromis niloticus*) alimentada con extracto de ajo (*Allium sativum L*). Tesis de Licenciatura. Universidad de San Carlos de Guatemala. Centro de Estudios del Mar y Acuicultura, Guatemala.

Garcia-Alonso C. R 1980. El ajo. Cultivo y aprovechamientos. Mundi prensa. 205 pags.

Gholipour.H-Kanania, J. Sahandia y Taheri A. 2012. Influence of Garlic (*Allium sativum*) and mother worth (*Matricaria chamomilla*) extract on *Ichthyophtirius multifilus* parasite treatment in sail fin molly (*Poecilia latipinna*) ornamental fish. APCBEE Procedia 4:6-11.

López, L.P. 2015. Supervivencia, crecimiento y expresión de genes del sistema inmune, estrés y digestión del camarón blanco (*Litopenaus vannamei*) infectado con *Vibrio parahaemolyticus* y tratado con ajo y *Bacillus lincheniformis* BCR 4-3. Tesis de Maestría. Instituto Politécnico Nacional. Centro Interdisciplinario de Investigación para el Desarrollo Integral Regional, Guasave, Sinaloa, México. 56 pags.

López G. J.; Rivera G. A. 2013. Efecto de tres bactericidas naturales (ajo, cebolla y jengibre) sobre las poblaciones de Vibrio, patógenos de camarones juveniles *Litopenaeus vannamei* en estanques experimentales. Título de Ingeniería. Universidad Nacional Autónoma de Nicaragua UNAN-LEÓN. Facultad de Ciencias y Tecnología, Nicaragua.73 pags.

Mona.H, El-Sayed T. Rizk, Wesam M. Salama. Mal L. Younts. 2015. Efficacy of probiotics, prebiotics, and an immunostimulant on growth performance and immunological parameters of *Procambarus clarkii* juveniles. The Journal of Basic and Applied Zoology 69:17-25.

Poongodi R., Saravana Bhavan P., T. Muralisankar, S. Radhakrishnan. 2012. Growth Promoting Potential of Garlic, Ginger, Turmeric and Fenugreek on the Freshwater Prawn *Macrobrachium rosenbergii*. International Journal of Pharma and Bio Sciences 3(4): 917-926.

Shalaby, A. M., Khattab, Y. A., Rahman, A. M. 2006. Effects of garlic (*Allium sativum*) and chloramphenicol on growth performance, physiological parameters and survival of Nile tilapia (*Oreochromis niloticus*). J. Venom. Anim. Tox. 12:172-201.

Silva-Blanco, S.A. 2013. Efecto de la inclusión de extracto de ajo (*Allium sativum*) en una ración balanceada, sobre el crecimiento y la sobrevivencia de goldfish (*Carassius auratus*) y lebistes (*Poecilia reticulata*) en cultivo. Tesis de grado. Universidad de la República (Uruguay). Facultad de Veterinaria. Montevideo, Uruguay.

Villar-Ochoa C. A. 2010. Acuicultura orgánica-ecológica: aplicación de productos naturales en sustitución de químicos en los procesos de cría de camarones en cautiverio. Revista AquaTIC 10:1-9.

Yuhana, M., Normalina dan Sukenda, I. 2008. Potency of Garlic (*Allium sativum*) Extract against motile *Aeromonas septicaemia* disease caused by *Aeromonas hydrophila* in Pangasionodon hypophthalmus. Journal Akuakultur Indonesia 7(1): 95-107.

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