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Chemical Origin Antimicrobial And Antibiotic Based Shrimp Toxicity; Biochemical And Water Quality Assessment.

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ABSTRACT

Shrimp farming is a grown aquaculture industry worldwide. The massive production of various species of shrimp including freshwater and marine in the different part of world require a large number of resources including feed, nutrient and agents to restrict infections in growing animal. Both marine and freshwater shrimps are prone to microbial and viral infections leading to massive loss of crop. To protect larvae and adult animals from infection and associated diseases a large number of chemicals based antibiotics, antimicrobial and pesticides are used. The molecules have dual effect on shrimp farming; one effective reduce microbial infections and disease and at same time also affect growth of animal. The immediate impact of chemical-based antibiotics and antimicrobials may alter biochemical parameters of growing larvae and animals as well. Further, the long term effects of chemical-based antibiotics and antimicrobial reported on water quality. As shrimp farming requires a bulk amount of water and massive use of pesticides, antibiotics and antimicrobials entirely deteriorate water quality. These toxic molecules remain in effluent and pollute water resources. The study was aimed to investigate effect of chemical-based antibiotics and antimicrobials on shrimp growth precisely biochemical analysis. Our work is focused on effects of antibiotics and antimicrobials on the growth and vital metabolic parameters of growing animals. We also emphasize risk of bioaccumulation and biomagnifications of chemical-based antibiotics and antimicrobials. The cellular toxicity was estimated in shrimps feed with antibiotics and antimicrobial with control study. Further, work also emphasizes water quality analysis and presence of active antibiotics and antimicrobials in effluent from shrimp farming.
Keywords; Shrimp farming, antibiotics and antimicrobials, toxicity, water quality and environment.

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INTRODUCTION

Aquaculture is a fully grown commercial seafood industry worldwide and in recent decade global annual production reaches a record 4.3 million tons during 2018-2019. The freshwater shrimp farming represents more than half of world shrimp production i.e. 60%. On world map Southern Asian countries including China, India, Thailand, Indonesia and Bangladesh produce more than 85% of world shrimp need. Record export of freshwater shrimp from India during 2018-2019 to the United States showed production capacity of modern fresh water shrimp farming (Fernandes et al 2019). Though there are more than 300 different species of shrimps including fresh and marine water for commercial production only a few of them are available including *Acetes japonicus, Penaeus monodon, Pandalus borealis and Trachysalambria curvirostris.* The commercial freshwater shrimp production utilizes multiple approaches including indoor and outdoor pond system methods. Both of them have own advantage and disadvantage in large scale shrimp production. Compare to marine shrimp production, freshwater shrimp farming recognized as more efficient and valuable means of aquaculture. Considering demand and nutritional values *Litopenaeus vannamei* (Pacific white shrimp) and *Penaeus monodon* (giant tiger prawn) represent 32.14% of all farmed shrimp (Ninawe and Selvin 2009). Shrimp farming can be defined as modern aquaculture business for large scale production of shrimps and other similar aquatic animal for human consumption (Moriarty 1997, 2005). Technically freshwater shrimp farming is entirely different from marine shrimp production that is precisely harvesting shrimp from marine environment.

Freshwater shrimp farming actively started in the late 1970s and crustaceans group of animals were selected for large scale production. United States is most significant market for shrimp towards consumption of seafood of different varieties of shrimps. On world map, South Asian countries including Thailand, Indonesia and India are competent freshwater shrimp producers. However, China is leading in fresh water shrimp production due to implementation of modern aquaculture and agriculture methods (*Bui et al., 2012*). Many countries including Thailand, Bangladesh, Indonesia and China have revolutionized and updated ways of shrimp farming that enhanced annual production to several folds. Considering commercial value of seafood, Crustacea is a dominant and diverse group of aquatic animals, among which the decapods (order Decapoda) comprise members of major ecological significance in marine and freshwater habitats and include many species with high economic values, such as shrimp, crabs, lobsters, and crayfish. In the world shrimp production crustacean group alone dominates more than 95% of total species (Melgar et al 2013). In particular, the penaeid shrimp (family Penaeidae) represents the most important group in fisheries and aquaculture and has therefore attracted considerable research attention. There is huge variation in shrimps species opted for commercial farming due to aquatic habitat and benthic adaptations. Despite a large number of shrimps species reported worldwide nearly 300 only a few of them are commercially available especially for freshwater shrimp farming purposes (Lui et al 2009).

In aquaculture, bacterial diseases have emerged as a serious problem and represent the most important challenge facing this industry (Morales, 2004; Holmstrom *et al.,* 2003). Bacterial microorganisms can also cause destructive infections, such as the diseases caused by bacteria of the *Vibrio* genus and the bacteria that cause necrotizing hepatopancreatic (NHP). These are the main diseases responsible for infections in shrimp farms (Roque *et al.,* 2001). The causative agent of necrotizing hepatopancreatic (NHP) is a gram-negative, pleomorphic, obligate intracellular pathogen. The predominant is a non-flagellated, rod-shaped, Rickettsia-like form that occasionally, exhibits a transverse constricted zone indicative of replication by binary fission (Vincent & Lotz, 2007). Infections due to necrotizing hepatopancreatic have been virtually eliminated in the Americas, but the International Office of Epizootics (IOE) has stated some concerns due to the potential that this disease possesses that could be extended to the entire world. Further, the mortalities caused in shrimp due to necrotizing hepatopancreatic may include up to 95% of the organisms infected in the pond, causing large losses during the crop cycle (Vincent & Lotz, 2007). Antibiotics are commonly used in aquaculture during the production cycle, both in the larval and growth phases.

The use of antibiotics in aquaculture is associated with environmental and human health problems, including bacterial resistance, the persistence of the disease in the aquatic environment, and effects on the biogeochemical composition of the sediment. The accumulation of antibiotic residues in the edible tissues of shrimp may also alter the human intestinal flora and cause food poisoning or allergy problems (Ma *et al.,* 2006). The antibiotics, most frequently used in aquaculture to combat bacterial diseases include oxytetracycline, florfenicol, sarafloxacin, and enrofloxacin (Roque *et al.,* 2001; Soto- Rodríguez *et al.,* 2006).

Globally, other antibiotics such as chlortetracycline, quinolones, ciprofloxacin, norfloxacin, oxolinic acid, pefloxacin, sulfamethazine, gentamicin, and tiamulin are used (Holmstrom *et al.,* 2003).The use of chemical-based antibiotics and antimicrobial also has negative impact on shrimp as well. These drugs affect growth profile and biochemical parameters of growing shrimp larvae and long term use may result in accumulation in toxic levels. The consumption of shrimps produced under larger dose of antibiotics and antimicrobial may have serious effect on human health as well. Here in present study we aimed to investigate effect of antibiotics on shrimp growth and biochemical parameters.

MATERIALS AND METHODS

The Shrimps strains were collected from local shrimp farming firms at Vijayawada, Andhra Pradesh, India. *Litopenaeus vannamei* (Pacific white shrimp) and *Penaeus monodon* (giant tiger prawn) collected for research purposes only. All the consumables and chemicals used in present work were purchased from Sigma Aldrich India and Hi-Media India of research-grade. The shrimps were maintained at grown conditions prescribed by the Indian council of agriculture research. No probiotics were fed to growing shrimps for control study. During the study standard microbiological and molecular protocols were followed. The bacterial species were stored at -40^{0}C and discarded after experiment by autoclave. The conventional media and growth parameters opted during entire study.

Experimental Design

The study was carried out in an isolated indoor pond system with two shrimp species including *Litopenaeus vannamei* (Pacific white shrimp) and *Penaeus monodon* (giant tiger prawn). The fully grown shrimps (15-15gm) were allowed for one month for growth and reproduction in ideal nutrient conditions. Here in study three indoor pools were designed with capacity of 100 L freshwater with added minerals equivalent to seawater (Pool 1- animal fed with antibiotics, pool 2 animals fed with GUTFOLD 99 and pool three animals fed with only commercial-grade feed). Shrimps were fed with commercial-grade feed and herein present study we have used AIM Shrimp-E 130g Natural Fish Food thrice in day with a regular interval of 8 hours. Shrimps were also fed with high content of soybean and GUTFOLD 99 a probiotic formulation. Here in present study 30 animals were divided into five groups where four were test and one as control. The control batch was fed as mentioned above without antibiotics and antimicrobial agents. On the contrary, four groups were with commercial feed and probiotics (three animals each from group) and antibiotics (three animals from each group). Here in the present study we have used two tanks and shrimps fed with antibiotics and probiotics were grown separately in similar growth conditions for 90 days with control group. Here in present study we have used four different classes of antibiotics including beta-lactam (ampicillin 100 mg/ml), tetracycline (oxytetracycline 50mg/ml), fluoroquinolones (ciprofloxacin 50mg/ml) and aminoglycosides (streptomycin 100mg/ml). The animals were grown for 90 days, growth profile, biochemical parameters were recorded in test and control group. The hemolymph was isolated and collected from animal of both the group and evaluated for concentration of various antibiotics (Oie 2006).

Effect of growth profile

The effect of antibiotics on selected species of shrimps under given growth condition was evaluated in the term of body weight of Shrimps during 90 days of the experiment. The growth of growing shrimps was measured daily during the experiment. The growth profile of shrimps was evaluated as Mean weight, Weight gain, Daily weight gain (DWG) and relative gain rate (RGR), Specific growth rate (SGR). The growth profile of shrimps supplemented with probiotics was compared with shrimps without probiotics. The daily increase in growth of growing shrimps was calculated as ratio of total growth weight gain and duration of study in 90 days. The formula for specific and relative growth weight gain is given in table below as per finding carried out Sandeepa et al 2016.

Isolation of haemolymph

For the evaluation of antibiotic effect and toxic profile, haemolymph found the most suitable body fluid in shrimp. It also acts as reservoir of several bacterial species as gut microbiota. Haemolymph is key body fluid in shrimps rich in several biochemical parameters including immune cells and molecules. The haemolymph was collected from four week grown shrimps fed with commercial food AIM Shrimp-E 130g

Natural Fish Food. The haemolymph (1ml) was isolated via sterile syringe from first abdominal segment (ventral sinus). Haemolymph was collected from each group of growing shrimp along with control study as well. The anticoagulant was added in haemolymph to avoid any chance of clotting. The plasma was removed from haemolymph by brief spin at 4000rpm for 15 min at 4^0C. The haemolymph separated from plasma from each group was stored in sterile tubes at -80^0C for further studies.

Antibiotics load

For antibiotic load evaluation haemolymph collected from four tests and control groups was used. A comparative microbial growth inhibition assay was carried out using haemolymph as test and standard antibiotic as positive control against E coli DH5 α strain. The haemolymph was diluted 1; 10, 1; 100 and 1; 1000 for microbial growth inhibition assay. A standard solution of used antibiotic including beta-lactam (ampicillin 100 mg/ml), tetracycline (oxytetracycline 50mg/ml), fluoroquinolones (ciprofloxacin 50mg/ml) and aminoglycosides (streptomycin 100mg/ml) was used as positive control. The 0.25µl of haemolymph was added in each well plated with bacterial strain and incubated for overnight. The zone of inhibition was measured and results were compared with standard antibiotics.

Effects of biochemical parameters

The haemolymph was used for the analysis of various biochemical parameters in growing shrimps and control studies as well. The animal fed with commercial feed along with antibiotics may change the glucose utilization, lipid profile and total protein content in experimental and control studies. To analyze the effect of antibiotic mixed commercial feed on biochemical parameters readymade kits for glucose estimation, lipid profile and protein estimation was used. The analysis was made as per the protocol recommended by supplier. The haemolymph of different study groups and control studies was subjected to biochemical analysis. For glucose estimation we used here readymade kit CBA086 from Sigma Aldrich. For serum triglycerides level TR0100 from Merck and TP0100-1KT from sigma Aldrich was used for total protein estimation.

Water quality analysis

To analyze water quality during the experiment where shrimps were fed with antibiotics along with AIM Shrimp-E 130g Natural Fish Food and GUTFOLD 99 three parameters were selected. The dissolved oxygen (DO), chemical oxygen demand (COD) and trace of antibiotic in pool were estimated in experiment and control group before and after experiment. Here we used dissolved oxygen test kit model OX-2P with given protocol. The kit allows a rapid test method for dissolved oxygen measurement. For biochemical oxygen demand Aqua XL kit was used for estimation. A set of rapid detection readymade kits for selected four antibiotics (AntiBioR) were used for estimation of traces of antibiotics in the pond before and after experiment. The specific gravity and turbidity of water were also measured using spectroscopic analysis with fresh water as control (pre-added minerals equivalent to marine water). Here, water samples from both the pool were evaluated for dissolved oxygen, chemical oxygen demand and presence of antibiotic (pool 1), specific gravity and suspended matter.

RESULTS AND DISCUSSION

Effect of growth profile

growth profile of shrimp in both the pools fed with antibiotics and commercial probiotics mixed in commercial-grade feed AIM Shrimp-E 130g Natural Fish Food shown positive signs in both test and control groups as well. The finding demonstrates use of antibiotics and antimicrobial possibly reduce risk of infection and associated disease and promotes animal growth provided required supplements (Sakai 1998). A comparative analysis was made between animals fed with antibiotics and probiotics to understand benefits and risks. The pool 2 fed with probiotic (GUTFOLD 99) specially designed for shrimp had shown a significant improvement in body weight during last week of experiment (Samuel 1996) in both shrimp species *Litopenaeus vannamei* and *Penaeus monodon*. As the result shown in figure 1 and table 1 the growth profile was not much significant as body weight of both the groups reported a minimal difference (Santosh et al 2017).

Effect on biochemical parameters

To understand effects of antibiotics on the biochemical parameters glucose utilization, total protein content and serum triglycerides were estimated in different groups of both the pool. The shrimps fed with antibiotics shown a rapid glucose utilization (serum level of glucose in haemolymph) compare to second group fed with GUTFOLD 99 specially designed for shrimps (Schock et al 2013). The control animal has similar pattern but glucose utilization was comparatively week from both test groups. A maximum glucose utilization as function of level of glucose in haemolymph was reported 27.45±0.60 and 28.10±0.43mg/dl) in both shrimp species *Litopenaeus vannamei* and *Penaeus monodon in pool 2 compare to pool 1 and control study pool 3* (table 2 a and figure 2 a. The healthy animals are capable of promoting metabolic activity and hence various anabolic mechanisms get activated. As a result high protein content was reported in both the group in growing shrimp over control study. As the result shown in figure 2 b and data presented in table 2 b we have reported highest protein content (78.54±0.70 and 79.55±0.74 mg/ml) in haemolymph in both shrimp species *Litopenaeus vannamei* and *Penaeus monodon in pool 2 compare to pool 1 and control study pool 3*. The serum level of total triglycerides demonstrates impact of antibiotics on shrimp growth profile. Compare to second pool where animals were fed with GUTFOLD 99 not much difference was reported. In both the pool animal fed with antibiotics and (GUTFOLD 99 serum level were raised as duration of study goes over control study group. As the result shown in table 2c and figure 2c in both shrimp species *Litopenaeus vannamei* and *Penaeus monodon in pool two compare to pool 1 and control study pool 3*. (47.32±0.65 and 49.70±0.16 mg/dl respectively). These findings demonstrate probiotic supplementation had a beneficial role in shrimps growth and metabolic activities allowing animal to opt anabolic events and synthesize essential biomolecules.

Antibiotics load

The effect of a massive dose of antibiotics on shrimp was studied as bioaccumulation in body fluid. The haemolymph was used for estimation of traces of antibiotics and microbial growth inhibition plate assay was used for active residues of antibiotics (Jager et al 2018). As result shown in table 3 and figure 3 we found here an active antibiotic residues present in the haemolymph of grown shrimp after 90 days or harvesting time. The higher traces of antibiotics reported in haemolymph of grown shrimp were found in ampicillin and ciprofloxacin as comparative larger zone of inhibition. The study also report traces of oxytetracycline and streptomycin as well however zone of inhibition was comparatively smaller. These findings demonstrate that use of antibiotic at higher dose for longer duration of time trigger deposition of drugs in animal tissue. Every animal top an effective excretion system, however, the aquatic life for shrimp could be a restriction as osmotic system might fail to remove all antibiotics from tissue to pool due to persistence higher concentration in the reservoir (Uyaguari et al 2009. Tu et al 2010). There is another aspect of drug deposition of antibiotics in animal tissue i.e. entry into food chain. The fully grown animal having a deposit of antibiotics consumed by human and other animal have antibiotic driven side's effects and complications. Further, shrimp haemolymph is reservoir for many good bacteria as symbiotic. The persistent concentration of antibiotics and many other antimicrobial agents may increase risk of drug resistance to gut microbiota (Landsman et al 2019). Use of antibiotics and antimicrobials from biological origin might have beneficial impact on shrimp farming and environment as well (Verma et al, 12, 13, 2016, 2017). There are several studies shown that enzyme could work in multiple ways by improving growth and quality of shrimps (Sobha et al 2014, 2017). Further, use of enzyme from biological origin also improves antioxidant and related property beneficial for shrimp farming (Verma 2013).

Water quality analysis

A comparative analysis of water quality in all three pools was carried out. We have reported here pool 1 water quality reaches to extreme level compare to pool 2 and pool 3. The water quality of all three pools after the experiment was affected as compare to zero time. The water quality parameters selected in the present study shown in table 4 suggest pool 1 must subject to water treatment before release to open environment (Kulkarni et al 2017). The high concentration of antibiotic residues was reported in pool 1 after completion of experiment. The level of dissolved oxygen was reported in pool one than pool two and pool 3. A similar pattern was seed in chemical oxygen demand as well where pool 1 reported in the higher order of COD compare to pool 2 and pool 3. The specific gravity of all three pools was estimated and compared (Biao et al 2004). The dissolved and suspended matter in all three pools was determined and reported pool 1 at higher level. The study suggests freshwater shrimp farming needs a paradigm shift in method of shrimp cultivation. As water act as major resource of shrimp farming and hence treatment system must be applied before release to external environment (Barraza-Guardado et al 2015). The use of probiotics has reduced water pollution and risk of contaminants as shown in present work. Hence, in new approaches use of antibiotics must be cut and or replaced with probiotics (Berry et al 2019). The use of probiotics also reduces risk of bioaccumulation and biomagnifications of medicines and similar toxic chemicals in animal which are growing threat to human health.

Growth and development

Table 1; Effect of various antibiotic and commercial probiotics on the growth and development of shrimp supplemented with commercial feed after 90 days of cultivation. The shrimps were grown in three different pools with identical growth conditions however, feed and supplement were varied as pool 1 animals were fed with antibiotics, and pool 2 animals were fed with probiotics and pool 3 only with feed for 90 days.

Shrimp species	Body weight (gm)		Control
	Pool 1	Pool 2	Pool 3
Litopenaeus vannamei	16.54±0.52	17.12±0.24	15.25±0.40
Penaeus monodon	15.85±0.20	16.14±0.02	14.85±0.74

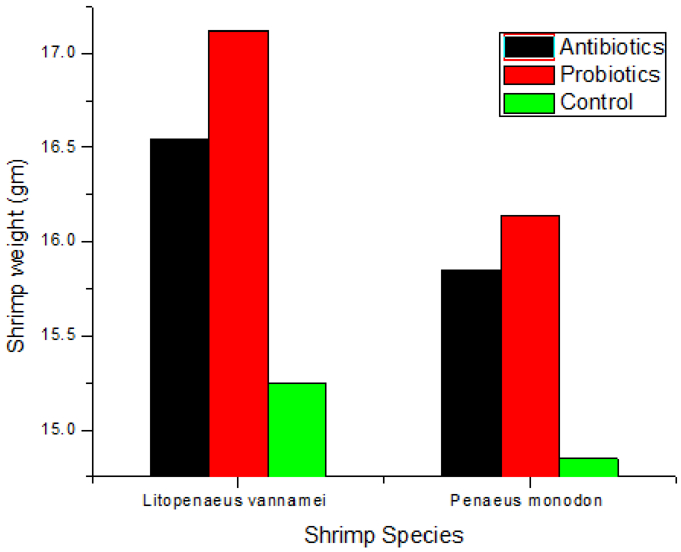

Figure 1; The figure demonstrates a comparative analysis for the effect of antibiotics and probiotics on the growth and development of shrimp. Here in the study we have used commercial grade feed with antibiotics in pool 1, with probiotics in pool 2 and only feed in pool 3 as control for 90 days.

Biochemical parameters

Table 2 a; Effect of various antibiotic and commercial probiotics on the glucose utilization of shrimp supplemented with commercial feed after 90 days of cultivation. The shrimps were grown in three different pools with identical growth conditions however, feed and supplement were varied as pool 1 animals were fed with antibiotics, and pool 2 animals were fed with probiotics and pool 3 only with feed for 90 days.

Shrimp species	Glucose level (mg/dl)		Control
	Pool 1	Pool 2	Pool 3
Litopenaeus vannamei	25.12±0.66	27.45±0.60	24.12±0.30
Penaeus monodon	26.25±0.05	28.10±0.43	24.45±0.36

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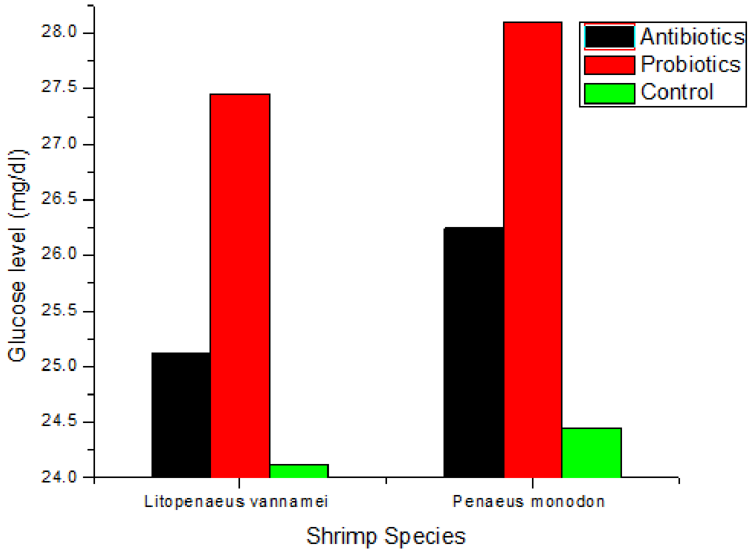

Figure 2 a; The figure demonstrates a comparative analysis for the effect of antibiotics and probiotics on the glucose utilization (level of glucose in haemolymph) of shrimp. Here in the study we have used commercial grade feed with antibiotics in pool 1, with probiotics in pool 2 and only feed in pool 3 as control for 90 days.

Table 2 b; Effect of various antibiotic and commercial probiotics on the total protein content of shrimp supplemented with commercial feed after 90 days of cultivation. The shrimps were grown in three different pools with identical growth conditions however, feed and supplement were varied as pool 1 animals were fed with antibiotics, and pool 2 animals were fed with probiotics and pool 3 only with feed for 90 days.

Shrimp species	Total protein content (mg/ml)		Control
	Pool 1	Pool 2	Pool 3
Litopenaeus vannamei	76.21±0.62	78.54±0.70	74.00±0.63
Penaeus monodon	78.45±0.52	79.55±0.74	75.50±0.10

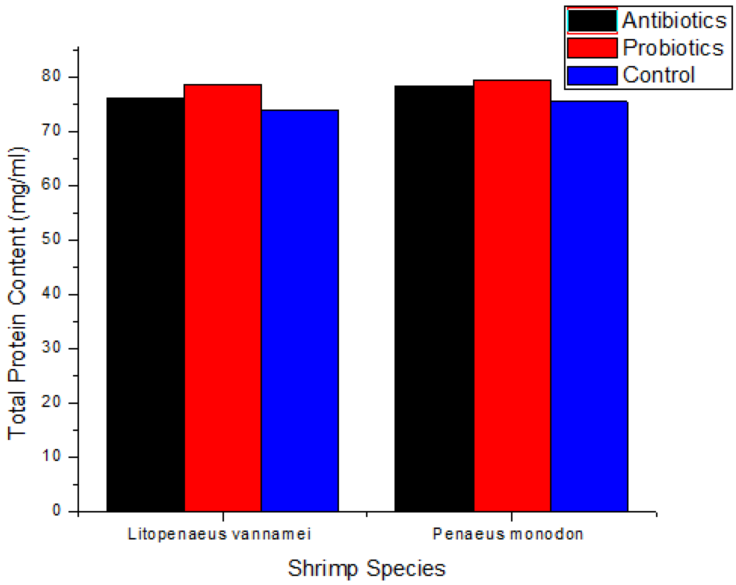

Figure 2 b; The figure demonstrates a comparative analysis for the effect of antibiotics and probiotics on the total protein content of shrimp haemolymph. Here in the study we have used commercial grade feed with antibiotics in pool 1, with probiotics in pool 2 and only feed in pool 3 as control for 90 days.

Table 2c; Effect of various antibiotic and commercial probiotics on the growth and development of shrimp supplemented with commercial feed after 90 days of cultivation. The shrimps were grown in three different pools with identical growth conditions however, feed and supplement were varied as pool 1 animals were fed with antibiotics, and pool 2 animals were fed with probiotics and pool 3 only with feed for 90 days.

Shrimp species	Serum Triglycerides level (mg/dl)		Control
	Pool 1	Pool 2	Pool 3
Litopenaeus vannamei	46.12±0.34	47.32±0.65	44.70±0.65
Penaeus monodon	48.25±0.50	49.70±0.16	45.05±0.52

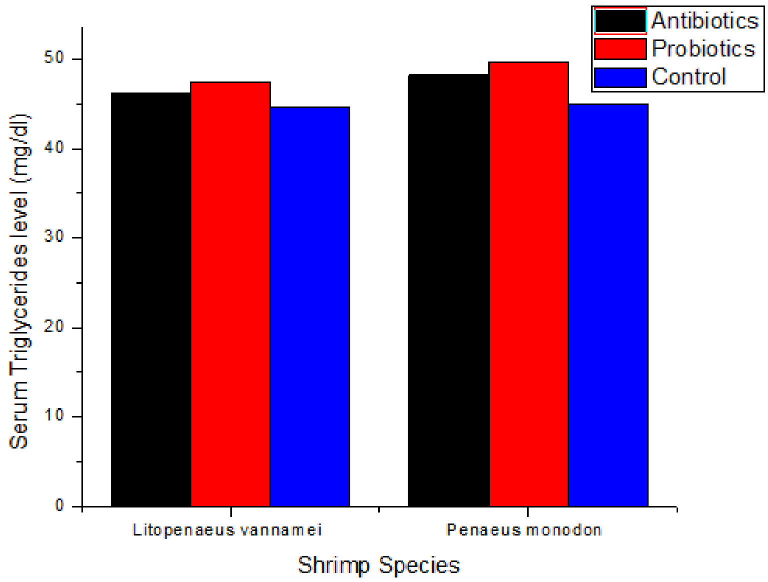

Figure 2 c; The figure demonstrates a comparative analysis for the effect of antibiotics and probiotics on the serum triglycerides level in shrimp haemolymph. Here in the study we have used commercial grade feed with antibiotics in pool 1, with probiotics in pool 2 and only feed in pool 3 as control for 90 days.

Table 3; Microbial zone inhibition assay for antibiotics traces present in haemolymph of shrimp supplemented with commercial feed after 90 days of cultivation. The shrimps were grown in three different pools with identical growth conditions however, feed and supplement were varied as pool 1 animals were fed with antibiotics, and pool 2 animals were fed with probiotics and pool 3 only with feed for 90 days.

Shrimp species	Zone of inhibition
	Pool 1 (*E coli*)
Litopenaeus vannamei	
1;10	9.5 mm
1;100	7.1 mm
1:1000	5.12 mm
Standard antibiotics	12.0 mm
Penaeus monodon	
1;10	11 mm
1;100	7.4 mm
1:1000	5.8 mm
Standard antibiotics	12.0 mm

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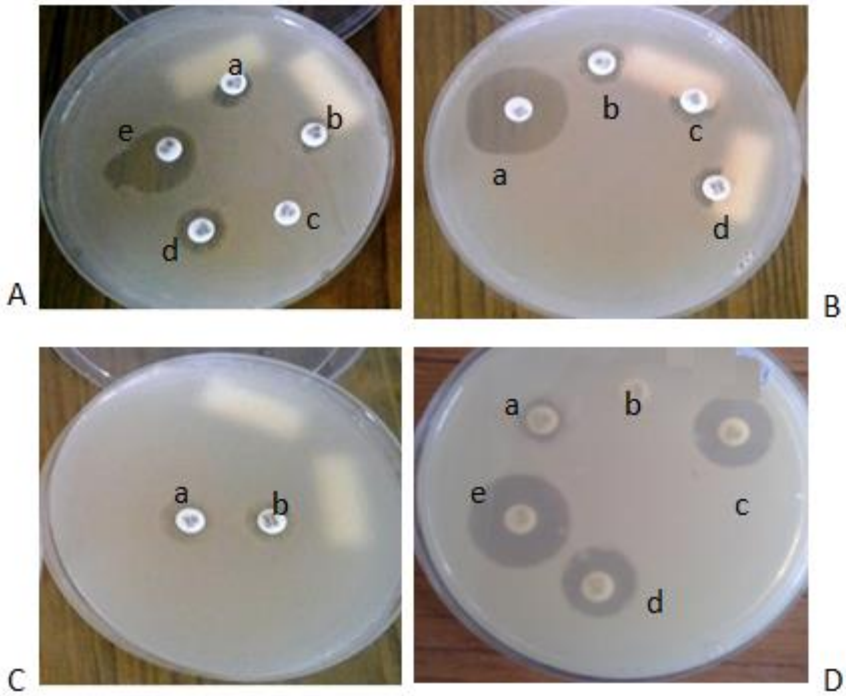

Figure 3; Bacterial growth inhibition assay for traces of antibiotics in haemolymph of shrimp after 90 days of cultivation.

Table 4; Effect of various antibiotic and commercial probiotics on the water quality of shrimp supplemented with commercial feed after 90 days of cultivation. The shrimps were grown in three different pools with identical growth conditions however, feed and supplement were varied as pool 1 animals were fed with antibiotics, and pool 2 animals were fed with probiotics and pool 3 only with feed for 90 days.

Shrimp species and parameters	Test				Control	
	Pool 1		Pool 2		Pool 3	
	Before	After	Before	After	Before	After
Litopenaeus vannamei						
1. pH	7.5	8.4	7.5	8.0	7.5	7.2
2. DO (mg/dl)	4.2	4.9	4.2	4.6	4.2	4.8
3. COD (ppm)	50	84	50	74	50	79
4. Specific Gravity	1.250	1.658	1.250	1.524	1.250	1.601
5. Turbidity TSS (mg/l)	75	115	75	95	75	102
Penaeus monodon						
1. pH	7.5	8.4	7.5	8.0	7.5	7.2
2. DO	4.2	4.8	4.2	4.7	4.2	4.7
3. COD	50	82	50	72	50	74
4. Specific Gravity	1.250	1.701	1.250	1.525	1.250	1.625
5. Turbidity	75	120	75	92	75	105

CONCLUSION

In the present scenario global seafood production gains a record production of shrimp i.e. 4.3 million tons. The freshwater shrimp farming is grown as separate industry and annual export growing exponentially. The global demand for seafood mainly shrimp triggers commercial shrimp farming for higher productivity. As a result massive use of antibiotics and antimicrobials are in trends. The use of antibiotics and antimicrobials have revalorized shrimp industry and productivity however, the dark side of use of such toxic chemical posed new threat of human health. The bioaccumulation and biomagnifications of antibiotics and antimicrobials are key threats we are facing worldwide and entry into food chain. There is an immediate need for paradigm shift in our approach for method of freshwater shrimp farming. The use of antibiotics and antimicrobial must be restricted and or replaced with probiotics. The approach will reduce risk of entry of such toxic chemicals into food chain and also reduces pollution of water resources as well. The present study provides substantial evidence how long term use of antibiotics and antimicrobial may hamper human health and environment. At same time study also provide an experimental analysis that use of probiotic reduces water pollution a major resource for human and another animal as well.

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REFERENCES

[1] Moriarty DJW. (1997) The role of microorganisms in aquaculture ponds. *Aquaculture*. 151(1–4):333–349

[2] Moriarty DJW, Decamp OP. (2005) *Probiotics in Aquaculture*. AQUA Culture Asia Pacific Magazine; 84-88

[3] Fernandes S, Kerkar S, Leitao J, Mishra A. Probiotic Role of Salt Pan Bacteria in Enhancing the Growth of Whiteleg Shrimp, Litopenaeus vannamei. Probiotics Antimicrob Proteins. 2019;11(4):1309-1323.

[4] Ninawe AS, Selvin J. Probiotics in shrimp aquaculture: avenues and challenges. Crit Rev Microbiol. 2009;35(1):43-66.

[5] Bui TD, Luong-Van J, Austin CM. Impact of shrimp farm effluent on water quality in coastal areas of the world heritage-listed Ha Long Bay. *American Journal of Environmental Sciences*. 2012; 8(2):104–116.

[6] Melgar Valdes CE, Barba Macías E, Alvarez-González CA, Tovilla Hernández C, Sánchez AJ. Microorganisms effect with probiotic potential in water quality and growth of the shrimp Litopenaeus vannamei (Decapoda: Penaeidae) in intensive culture. Rev Biol Trop. 2013; 61(3):1215-28.

[7] Liu, C.H., C.S. Chiu, P.L. Ho and S.W. Wang (2009). Improvement in the growth hatchery probionts. *Appl. Environ. Microbiol.* 65: 2592–2597.

[8] Owens L and Hall-Mendelin (1989). Recent Advances in Australian shrimps (sic) diseases and pathology. Advances in Tropical Aquaculture, Tahiti, AQUACOP, IFREMER, Actes de Colloque 9: 103-112.

[9] Morales CMS. (Ed). (2004). Enfermedades del camarón, Editorial Trillas, ISBN-968-24-7112-5,

[10] Holmstrom K, Graslund S, Wahlström A, Poungshompoo S, Bengtsson, B. E. and Kautsky N (2003). Antibiotic use in shrimp farming and implications for environmental impacts and human health. International Journal of Food Science and Technology, 38, pp.255-266

[11] Roque A, Ruiz-Fernandez AC (2003). Shrimp aquaculture development and the environment in the Gulf of California ecoregion. Marine Pollution Bulletin 46, 806–815.

[12] Vincent GA and Lotz JM (2007). Advances in research of Necrotizing Hepatopancreatitis Bacterium (NHPB) affecting penaeid shrimp aquaculture. Reviews Fisheries Science,.15, pp.63-73

[13] Rodríguez J, Espinosa Y, Echeverría F, Cárdenas G, Román R and Stern S (2007). Exposure to probiotics and β–1, 3/1, 6–glucans in larviculture modifies the immune response of Penaeus vannamei juveniles and both the survival to White Spot Syndrome Virus challenge and pond culture. *Aquaculture* 273: 405–415.

[14] OIE. 2006. Manual of diagnostic tests for aquatic animals. 3rd edition. World Organization for Animal Health, Paris.

[15] Sakai M (1998) Current research status of fish immunostimulants. Aquaculture 172, 63–92.

[16] Samuel M, Lam TJ, Sin YM (1996) Effect of laminaran [β (1-3)-D-glucan] on the protective immunity of blue gourami, Trichogaster trichopterus against Aeromonas hydrophila. Fish Shellfish Immunol 6: 443-454.

[17] Santhosh kumar S, Sivakumar S, Vimal S, Abdul Majeed S, Taju G, Haribabu P, Uma A, Sahul Hameed AS, Biochemical changes and tissue distribution of Enterocytozoon hepatopenaei (EHP) in naturally and experimentally EHP-infected whiteleg shrimp, Litopenaeus vannamei (Boone, 1931), in India. J Fish Dis. 2017; 40(4):529-539.

[18] .Schock TB, Duke J, Goodson A, Weldon D, Brunson J, Leffler JW, Bearden DW, Evaluation of Pacific white shrimp (Litopenaeus vannamei) health during a superintensive aquaculture growout using NMR-based metabolomics. PLoS One. 2013;8(3):e59521.

[19] Jäger T, Hembach N, Elpers C, Wieland A, Alexander J, Hiller C, Krauter G, Schwartz T, Reduction of Antibiotic Resistant Bacteria During Conventional and Advanced Wastewater Treatment, and the Disseminated Loads Released to the Environment. Front Microbiol. 2018; 9:2599..

[20] Uyaguari M, Key P, Moore J, Jackson K, Scott G Acute effects of the antibiotic oxytetracycline on the bacterial community of the grass shrimp, Palaemonetes pugio. Environ Toxicol Chem. 2009; 28(12):2715-24.

[21] Tu HT, Silvestre F, Phuong NT, Kestemont P Effects of pesticides and antibiotics on penaeid shrimp with special emphases on behavioral and biomarker responses. Environ Toxicol Chem. 2010 ;29(4):929-38.

[22] Landsman A, St-Pierre B, Rosales-Leija M, Brown M, Gibbons W, Impact of Aquaculture Practices on Intestinal Bacterial Profiles of Pacific Whiteleg Shrimp *Litopenaeus vannamei*. Microorganisms. 2019;7(4). pii: E93.

[23] Sobha K, V Rao, Ratna K and Mahendra Kumar Verma, An Investigation into phytochemical constituents, antioxidant, antibacterial and anti-cataract activity of *Alternanthera sessilis*, a predominant wild leafy vegetable of South India, Bio-catalysis and Agricultural Biotechnology, 2017; 10; 197-203

[24] Verma MK and Pulicherla KK, Broad substrate affinity and catalytic diversity of fibrinolytic enzyme from Pheretima posthumous—Purification and molecular characterization study, International Journal of Biological Macromolecules. 2017, 95:1011-1021

[25] Verma MK and Pulicherla KK, Enzyme promiscuity in Earthworm serine protease- Substrate versatility and therapeutic potential, Amino Acid. 2016; 48(4); 941-948

[26] Verma MK, Xavier F, Verma MK, Sobha K. Evaluation of the Cytotoxic and Antitumor activity of Serine Proteases Isolated and Purified from the Indian Earthworm *Pheretmia posthuma*, Asian Pac J. Trop. Biomed. 2013; 3 (11): 896-901.

[27] Verma MK and Verma MK, Earthworm- a potential source for stable and potent antimicrobial compounds- isolation and purification study, 2012, Int J Pharm Pharm Sci, 4; 4, 540-543

[28] K Sobha, N Lalitha, N Pavani, R Praharshini, D Pradeep, Verma MK, Statistical optimization of Factors influencing the activity and the kinetics of partially purified Lipase produced from Aspergillus japonicus (MTCC 1975) cultured in protein enriched medium, International Journal of ChemTech Research, 2014, 6,11,4817-4831

[29] Verma MK and Sobha K, Antioxidant and Anti-Inflammatory Properties of Autolysed Extract of the Indian Earthworm Pheretima posthuma after Preliminary Purification - An In Vitro Study, RJPBCS, 2013, 4, 4, 888-892

[30] Kulkarni P, Olson ND, Raspanti GA, Rosenberg Goldstein RE, Gibbs SG, Sapkota A, Sapkota AR, Antibiotic Concentrations Decrease during Wastewater Treatment but Persist at Low Levels in Reclaimed Water. Int J Environ Res Public Health. 2017; 14(6). pii: E668.

[31] Biao X, Zhuhong D, Xiaorong W, Impact of the intensive shrimp farming on the water quality of the adjacent coastal creeks from Eastern China., Mar Pollut Bull. 2004 ;48(5-6):543-53

[32] Barraza-Guardado RH, Arreola-Lizárraga JA, Miranda-Baeza A, Juárez-García M, Juvera-Hoyos A, Casillas-Hernández R, Enhancing Eco-efficiency in Shrimp Farming through Interconnected Ponds. Biomed Res Int. 2015; 2015:873748.

[33] Berry SE, Simon CJ, Foote AR, Jerry DR, Wade NM, Evaluation of baseline haemolymph biochemistry, volume and total body energetic to determine an accurate condition index in the black tiger shrimp, *Penaeus monodon*. Comp Biochem Physiol B Biochem Mol Biol. 2019; 228:1-9.