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Investigations on antibiotics residue and their effects on the growth and survival of *Macrobrachium rosenbergii*

Alok Kumar Hazra[1], Md. Sumon Mia[2]*, Farida Yeasmin[2], Debashis Kumar Mondal[3], Sadhan Chandra Sarker[4], Md. Kamal[1]

[1]Department of Fisheries Technology, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh
[2]Department of Fisheries Management, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh
[3]Scientific Officer, Bangladesh Fisheries Research Institute, Freshwater sub-station, Jessore, Bangladesh
[4]Senior Upazilla Fisheries Officer, Noakhali Sadar, Noakhali, Bangladesh

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***Corresponding Author:**

Md. Sumon Mia
Email: sumon_reod@yahoo.com

ABSTRACT

Studies were conducted on the use of antibiotics especially nitrofuran, chloramphenicol (CAP) and oxytetracycline (OTC) drugs in bagda/galda farms and hatchery and their effects on the growth performance of galda through well-structured questionnaires in Phultala upazila and Rampal upazila. Farmers used pharmaceuticals such as Aquamarine, Oxycentril in both commercially and locally prepared feed whereas few of them use growth hormone in feed. Studies were also done to know the withdrawal periods of AMOZ, AOZ and SEM in galda muscles after feeding of nitrofuran medicated diets for 15 days. On 15th day of feeding, galda samples were analyzed for AMOZ, AOZ and SEM and detected their concentrations at a level of 5.89, 15.98 and 0.44 ppb, respectively. However, accumulation of these metabolites in galda muscles reduced considerably after stopping medicated diets with time. The withdrawal period of AMOZ and AOZ in galda bodies was 10 days whereas in the case of SEM the withdrawal period was 20 days. On the other hand, withdrawal period of CAP and OTC in galda muscle was 20 days and 5 days, respectively. Studies were also conducted on the effects of antibiotics (nitrofurazolidone, CAP and OTC) on the growth and survival of galda and changes in environmental conditions. The weight gain was higher in galda after feeding with antibiotics treated feed compared to those of control samples. The average initial protein content of the PLs (prawn larvae) was 18.21 % which increased to 20.33 % after 90 days of rearing in controlled ponds i.e. diets without using antibiotics. There was little or no marked differences in chemical composition among the galda reared in ponds either using medicated diets or without antibiotics medicated diets. Protein content of the galda muscles increased to in the range of 20.59 to 21.16 % after 90 days of rearing in ponds fed with medicated diets in different treatments. There are also little or no changes in physico-chemical parameters in pond water between controlled pond and antibiotics medicated ponds. Bacterial loads decreased greatly in ponds used antibiotics medicated feeds compared to control pond. The research suggested that to improve the culture and management practices of galda in Bangladesh it is necessary to investigate the generic names of all chemicals used in galda farms to have control over the use of chemicals and antibiotics on legal basis for food safety and sustainable healthy prawn industry.

INTRODUCTION

Prawn culture is of central importance to the fisheries sector in Bangladesh particularly in the contest of export earning. Of all the exportable agro-based primary commodities, prawn is by far the most important which contributes more than 70% of the total export earning. It grew from the early 1970s and contributing about 11% of the total export earning in the mid-1990s (DoF, 1999). Next to garments, prawn and fish product (frozen foods) are the main export items from Bangladesh having increasing potential to explore trade and employment opportunity due to upraising global demands for quality frozen foods. Aquaculture and prawn/fish export recently considered as the most effective tools for employment generation, poverty alleviation and economical growth of the country.

Frozen foods are exported to some 40 countries including EU countries, Canada and Japan from Bangladesh. *Penaeus monodon* and *Machrobrachium rosenbergii* are the two major export species cultured in Bangladesh. The fisheries activities mostly due to the attractive profit returns and rapid involvement of the private sector with a view to exploring the expanding domestic and foreign market of the prawn/fish products. Safe and dependable aqua-food is a recent challenge for sustaining in global seafood markets. In the recent years, EU has rejected several prawn consignments from Bangladesh being the products were found to have metabolites of banned nitrofurans and other health hazardous chemicals contaminants. This incidence undoubtedly affects the image of Bangladesh frozen foods and threatened up growing international export market along with serious economic setback to the concerned exporters.

The presence of nitrofurans and other chemical contaminants in galda/fish product is of great concern to Bangladesh export sector. Nitrofuran and its metabolites detected specially in the giant freshwater prawn, locally known as “galda” (*M. rosenbergii*) during recent years which was suspected to be contaminated mostly from the environment where galda culture is practiced as well as galda feed and feed ingredients, the galda seed source, hatchery hygiene, imported inputs utilization, indigenous inputs, insecticides, chemical and drugs used in aquaculture, agriculture, poultry and dairy industry, soil-

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water quality and relevant other factors. Due to the presence of such contaminants galda aquaculture sector is now facing severe problem in producing frozen food products and fail to preserve required food safety which ultimately leads to lose the competitive export market.

However, it is urgently needed to trace out the source of health hazard contaminations or any other substances intended or expected to be incorporated into feed in galda and detecting their affects on the growth performance for recommending possible preventive measures complying the food safety regulations are the most important national challenges to sustain in the competitive global seafood export business as to increase the revenue earning from Fisheries Sector. There is very little or no research was undertaken in this alarming issue in the past. So, the objectives of the present research was to collect information about the uses of chemicals and antibiotics in galda farms as well as to determine the withdrawal periods of some harmful and banned antibiotics found in bagda/galda. At the same time, determination of growth performance and survivability of galda in grow out pond after using antibiotic medicated feeds were also done for a period of 6 months from April, 2009 to September, 2009 in grow out ponds of Faculty of Fisheries, Bangladesh Agricultural University, Mymensingh, Bangladesh.

MATERIALS AND METHODS

Determination of antibiotic withdrawal period in galda

Galda (*Macrobrachium rosenbergii*) were fed with antibiotic medicated diets for two weeks. After two weeks galda samples were collected at definite time interval to determine the residues and retention time. The catch statistics were maintained based on 9 sampling plan (Table 1). Among all samples, 21 selected samples were analyzed in LC MS/MS machine of the Fish Inspection and Quality Control (FIQC) laboratory, Department of Fisheries (DoF) for detection of nitrofuran residues and chloramphenicol (CAP). On the other hand, 10 samples were analyzed for oxytetracycline (OTC) in LC MS/MS machine of the Bangladesh Council of Scientific and Industrial Research (BCSIR) laboratory.

Table 1. Samples used for Nitrofuran metabolites, OTC and CAP analysis.

No.	Samples
1	Feed containing antibiotic (Nitrofurazolidone, OTC and CAP)
2	Prawn (Before antibiotic treatment)
3	Prawn (Continuous antibiotic feeding)
4	Prawn (1 day after antibiotic feeding stopped)
5	Prawn (5 day after antibiotic feeding stopped)
6	Prawn (10 day after antibiotic feeding stopped)
7	Prawn (20 day after antibiotic feeding stopped)
8	Prawn (30 day after antibiotic feeding stopped)
9	Prawn (45 day after antibiotic feeding stopped)
10	Bottom mud of corresponding pond

Effects of antibiotics on the growth and survival of galda

Experimental site

The experiment was performed in 10 ponds of the Faculty of Fisheries, Bangladesh Agricultural University, Mymensingh. All the ponds were more or less similar in size, depth, basin conformation, bottom-soil type and contour (Table 1). The experimental ponds were numbered as pond-1 to pond-12 for the convenience of the study. Pond 1 and 5 were kept blank. Pond-9 was designated as control (Treatment-I) where feed without any antibiotic was given. Pond 2 and 3 were under treatment-II where feed containing 5g/kg nitrofurazolidone, was used for first two weeks and then continued feeding without using antibiotics for the remaining period. Pond 4 was under treatment-III where feed containing nitrofurazolidone (5g/kg) were applied throughout the study period; ponds 6 and 7 were under treatment-IV where feed containing 5g/kg OTC, was applied for the first two weeks and then continued feeding without using antibiotics until termination of the experiment; pond 8 was under treatment-V where feed containing same antibiotics were used throughout the study period. Ponds 10 and 11 were under treatment- VI where feed containing 12g/kg CAP, was applied for the first two weeks and then continued feeding without using antibiotics until termination period of the experiment. Pond 12 was under treatment-VII where the prawns were fed continuously with feed containing 12g/kg CAP throughout the study period. The ponds were different sizes varying from 32 to 46 sq. meters with depth 2.5 to 4 feet. The pond size was 43 sq. meters for treatment-I, 37 and 37 for treatment-II, 32 sq. meters for treatment-III, 37 and 39 for treatment-IV, 40 sq. meters for treatment-V, 38 and 41 for treatment-VI and 43 sq. meters for treatment-VII (Table 1). The ponds were free from aquatic vegetation, well expose to sunlight and completely free from flooding. The embankment was well protected and covered with grass. Rain water and water from deep tube-well were the main sources of water in the experimental ponds during the study period.

Sample analysis

Ten galda were sampled by using a seine net from each pond in predetermined time interval to assess their growth performance and health condition and for residual analysis of antibiotics. Collected samples were sent to Fish Inspection and Quality Control (FIQC) Laboratory, Department of Fisheries (DoF), Dhaka for analysis of antibiotic residues. Total bacterial count of the galda was also determined and number of bacteria per gram of the sample (CFU/g) was calculated by using the following formula:

$$\mathrm{CFU/g} = \frac{\text{No of colonies on petridish} \times 10 \times \text{dilution factor} \times \text{wt. of total sample soltution}}{\text{Wt. of fish sample (g)}}$$

Water samples were collected from each pond from surface to a depth of 20 cm to determine dissolved oxygen (DO), pH, total alkalinity, ammonia, nitrate-nitrogen, nitrite-nitrogen, and chlorophyll-*a* in Water Quality and Pond Dynamics Laboratory of the Faculty of Fisheries, BAU. Temperature and transparency were observed on the spot. Proximate composition of galda samples such as crude protein, lipid, ash, moisture and carbohydrate content of prawn muscle were analyzed according to AOAC method to evaluate the effects of different antibiotics on the composition of muscle of finally harvested galda.

Table 1. Length, wide and area of experimental ponds.

POND	Length (m)	Width (m)	Area (sq.m)	Area (App.) (sq.m)	Total No. of seed (2 galda/sq.m)	Given no of seed
POND-1	6.9	5.4	37.26	37	74	100
POND-2	6.4	5.7	36.48	37	74	100
POND-3	6.2	6	37.2	37	74	100
POND-4	7.7	4.1	31.57	32	64	100
POND-5	7.6	6.1	46.36	46	92	100
POND-6	6.4	6	38.4	38	76	100
POND-7	6.9	6	41.4	41	82	100
POND-8	7.9	5.4	42.66	43	86	100
POND-9	7.1	6.1	43.31	43	86	100
POND-10	6.1	6.1	37.21	37	74	100
POND-11	6.4	6.1	39.04	39	78	100
POND-12	7.6	5.2	39.52	40	80	100
TOTAL				Area=470 sq m.	Galda = 940	Galda = 1200

The total length (cm) and weight (g) of individual galda were recorded separately pond wise during stocking period and final harvesting period to estimate the growth performance of galda after feeding trials. The growth rates (by weight) of the galda were calculated by the following formula:

Weight gain = Mean final galda weight – Mean initial galda weight

$$\%\ \text{weight gain} = \frac{\text{Mean final wt.} - \text{Mean initial wt.}}{\text{Mean initial wt.}} \times 100$$

$$\text{Average daily gain (g)} = \frac{\text{Mean final wt.} - \text{Mean initial wt.}}{\text{T2} - \text{T1}} \times 100$$

% Specific growth rate (per day) ((Brown, 1957)

$$\%\ \text{Specific growth rate (per day)} = \frac{\text{Loge W2} - \text{Loge W1}}{\text{T2} - \text{T1}} \times 100$$

Where
W_1= Initial live galda body weight (g) at time T_1 (day), W_2= Final live galda body weight (g) at time T_2 (day)

Estimation of survival rate (%)

The survival rate of galda for each treatment was estimated on the basis of the number of galda harvested at the end of the experiment using the following formula:

$$\text{Survival rate (\%)} = \frac{\text{No. of actual galda survived}}{\text{No. of actual galda stocked}} \times 100$$

Statistical Analysis

The data obtained in the experiment were recorded and analyzed by using ANOVA. The mean values compared using Duncan's Multiple Ranged Test (DMRT) as post-hoc test using SPSS (Statistical Package for Social Science, version 11.5) statistical software (SPSS mc; Chicago. USA). Significant differences were determined among treatments at the 5 % level ($P < 0.05$).

RESULTS AND DISCUSSION

Antibiotic withdrawal period in galda

The detection of nitrofuran metabolites AMOZ, AOZ and SEM were done in prawn muscles after feeding of nitrofuran medicated diets. The prawn samples were fed nitrofuran medicated feeds for 15 days and then analyzed the residues. AMOZ, AOZ and SEM in the prawn muscles detected were 5.8, 15.98 and 0.44 ppb, respectively. The level of these metabolites in prawn muscles reduced considerably after stopping medicated diets with time. However, after 30 days of stopping treatment AMOZ, AOZ and SEM residues were not detected in prawn muscles. The results obtained from the present study indicated that the withdrawal period of AMOZ and AOZ in shrimp body was 10 days whereas in the case of SEM the withdrawal period was 20 days. In a study by Coopera and Kennedy (2005) depletion half lives over the 6 week period were: AOZ 3.8 weeks, SEM 3.8 weeks, AMOZ 2.4 weeks and AHD 1.9 weeks. AHD exhibits the lowest concentrations (0.1 mg/kg) remaining after 6 weeks withdrawal) and the shortest half life, suggesting this is the least stable of the nitrofuran metabolites in vivo. By contrast, AMOZ concentrations are remarkably high, 14 mg/kg remaining in retina after 6 weeks. AOZ and SEM demonstrate similar concentrations and depletion characteristics after 6 weeks. In another study four tissue-bound metabolites, AOZ (furazolidone), AMOZ (furaltadone), AHD (nitrofurantoin) and SEM (nitrofurazone) were measurable for a period of six weeks post-treatment in all tissues and residue concentrations in the three tissues after six weeks withdrawal were 10-40 μg/kg AOZ, 10-60 μg/kg AMOZ, 4-7 μg/kg AHD and 40-250 μg/kg SEM (Keeffe *et al*., 2005). However the withdrawal periods of the metabolites are very short in fish muscle compared to the animal muscle.

The results on the retention of CAP (CAP) and OTC (OTC) in prawn body after feeding of medicated diets are shown in Table 2. After analysis in LC-MS/MS, CAP parent drugs detected in feeds was 568.45 ppb and OTC 1810.0 ppb. However, the result obtained from the present study indicated that withdrawal period of CAP and OTC in prawn muscle is 20 days and 5 days, respectively. Weifen *et al*., 2004 reported in shrimp muscle the elimination half-life of CAP was 10.04 h, the elimination half-life of OTC was 16.12 h. Extrapolation of the data indicates that it would be passed after a 139.7 h (95% CI = 132.0 – 144.4 h), 30.6 h (95% CI = 27.2 – 33.1 h), 90.3 h (95% CI = 87.9 – 92.5 h) withdrawal period for CAP, SMZ and OTC in shrimp muscle, respectively.

Table 2. Detection of Nitrofuran metabolites, CAP and OTC in muscle of galda fed with antibiotics.

Test Parameter	MRPL	Feed (ppb.)	Antibiotic	After fed (ppb)				
			Before fed (ppb)	day 0	day 1	day 5	days 10	20 days
AMOZ	<1.00	--	--	5.89	1.57	1.04	1.14	--
AOZ	<1.00	--	--	15.98	11.82	7.68	6.02	--
AHD	<1.00	--	--	--	--	--	--	--
SEM	<1.00	--	--	0.44	0.39	0.37	0.47	0.07
CAP	<0.30	586.45	--	27.92	21.04	14.49	17.94	13.28
OTC	<100	1810.0	--	5070.0	1390.0	1190.0	--	--

MRPL- Minimum Required Performance Limit

Table 3. Proximate composition of galda under different treatments and stages.

Proximate composition	PL	Normal galda	Nitrofurazolidone treated galda	OTC treated galda	CAP treated galda
Protein (%)	18.21	20.33	21.16	20.59	21.03
Lipid (%)	0.74	2.08	2.03	1.84	2.01
Ash (%)	0.88	0.91	1.04	0.89	0.92
Moisture (%)	79.1	75.85	74.93	75.86	75.24
CHO	1.07	0.83	0.84	0.82	0.80

CHO = Carbohydrate

Proximate composition

There were little or no appreciable changes in nutritional composition among the galda samples after feeding antibiotic containing feeds (Table 3). Antibiotics treated feeds have little or no positive effect on the increasing of body muscle composition.

Effect on water quality

There were no significant changes in water temperature of ponds treated with different antibiotics. Water transparency was significantly decreased in Treatment –III and Treatment-VII but increased in Treatment –II and Treatment –VI. The DO, pH, NH_3-N, NO_3-N were almost unchanged but NO_2-N was changed (decreased) significantly during Treatment–III and Treatment-V. The alkalinity was significantly increased in Treatment-VI and VII compared to control. Chlorophyll-*a* was significantly increased during Treatment –II to V.

Growth performance of galda under different treatment condition

The growth performance and survival of galda reared in ponds under different treatments were different. The growth performance is more or less similar in galda fed with feeds containing CAP and OTC continuously for 90 days of rearing (Figure 1 to 3). In treatment-IV the final weight gain of galda was 18.68 to 21.48g when they were feed the feed containing OTC for two weeks. The growth performance was higher in treatment V when galda samples were fed with oxytetracycleine treated feed continuously where the body weight of the samples increased more to in a range 18.54 to 25.52g. More or less similar results were also obtained from treatments VI and VII when galda were fed with CAP treated feed for two weeks and continuously, respectively. The results clearly indicated that an antibiotic in feeds acts as growth promoter in galda culture, particularly nitofurans. The results obtained from the present study were in agreement with those reported by Tidwell *et al*., (1993) and Daniels *et al*., (1995) for shrimp.

The percentage of increase in weight of prawn in different treatments ranged from 571.67 to 786.95 % in Control (without antibiotic feeds) pond (Treatment-I), 1007.67 to 1056.13 % in samples fed two weeks nitrofurazolidone treated feeds (Treatment-II) and 1362.46 to 1436.40 % in samples feeding continuous nitrofurazolidone treated feeds (Treatment-III). Similar weight gain was also observed in other treatments. Variation of percentage of increase in weight (%) under different treatments throughout the experiment period is shown in Table 5.

Table 4. Water quality parameters of different treatments (Mean ± SD, Minimum and Maximum).

Parameters		Control	Nitrofurazolidone Treated Ponds		OTC Treated Ponds		CAP Treated Ponds	
		Treatment I	Treatment II	Treatment III	Treatment IV	Treatment V	Treatment VI	Treatment VII
Water temperature (°C)		30.04 ± 1.046	30.41 ± 1.017	30.18 ± 0.541	30.71 ± 1.263	30.25 ± 0.753	30.84 ± 1.310	30.32 ± 0.723
Transparency (cm)		45.00 ± 7.360	50.65 ± 4.665*	38.15 ± 9.797*	43.66 ± 9.297	45.38 ± 8.940	56.50 ± 9.516	38.38±14.841*
DO	Morning	5.66 ± 0.142	5.37 ± 0.210	5.29 ± 0.137	5.65 ± 0.212	5.56 ± 0.204	5.92 ± 0.283	5.39 ± 0.225
(mg/l)	Evening	7.16 ± 0.148	6.77 ± 0.210	6.89 ± 0.244	7.02 ± 0.251	7.09 ± 0.156	7.34 ± 0.275	7.06 ± 0.331
pH		7.75 ± 0.165	7.77 ± 0.101	7.69 ± 0.100	7.63 ± 0.064	7.82 ± 0.133	7.63 ± 0.107	7.99 ± 0.158
Alkalinity		97.38 ± 8.996	98.31 ± 7.028	100.69 ±8.654	99.15 ±12.844	97.69 ± 11.309	92.92±15.157	105.77 ±15.93*
NH_3-N (mg/l)		0.24 ± 0.044	0.23 ± 0.030	0.25 ± 0.020	0.25 ± 0.050	0.23 ± 0.029	0.25 ± 0.040	0.23 ± 0.023
NO_3-N (mg/l)		0.12 ± 0.010	0.12 ± 0.015	0.14 ± 0.023	0.12 ± 0.012	0.14 ± 0.029	0.12 ± 0.022	0.13 ± 0.018
NO_2-N (mg/l)		0.024 ± 0.005	0.023 ± 0.004	0.020 ± 0.003*	0.023 ± 0.005	0.020 ± 0.003*	0.024 ± 0.005	0.025 ± 0.005
Chlorophyll-*a*		79.91 ± 2.872	96.76 ± 9.503*	96.04 ± 1.284*	105.9 ± 4.058*	102.06 ± 9.181	76.76 ± 2.520	77.71 ± 8.019

*p<0.05

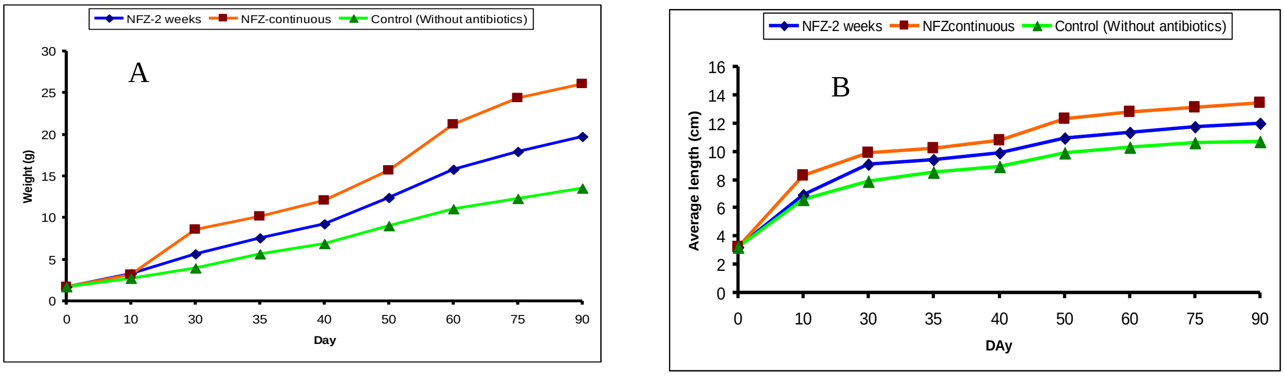

Figure 1. Variation in Average weight in g (A) and average length in cm (B) in treated ponds compared with control pond during the study period.

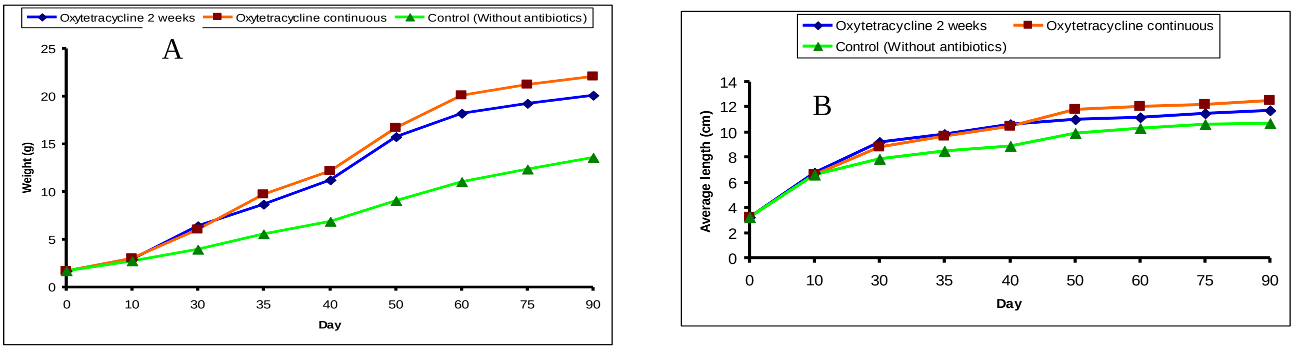

Figure 2 Variation in Average weight in g (A) and average length in cm (B) in OTC treated ponds compared with control pond during the study period.

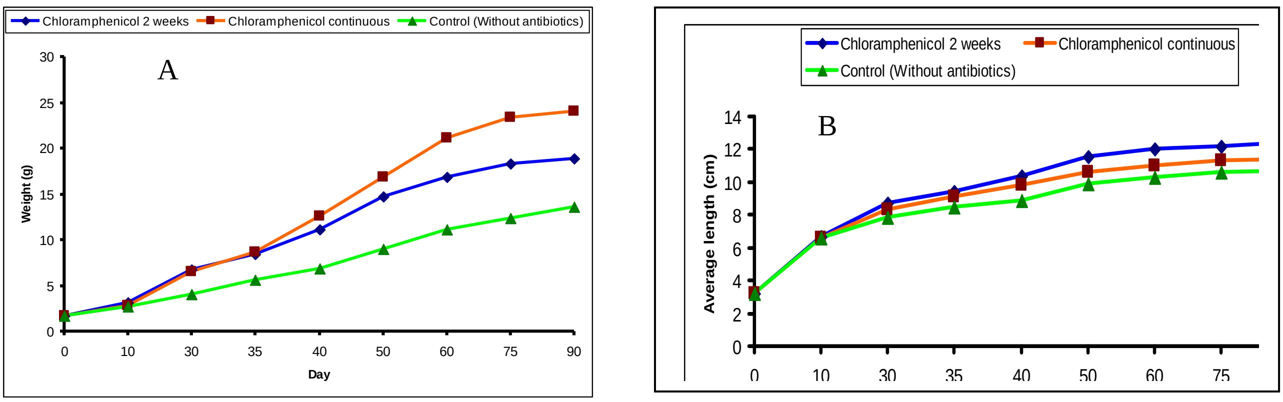

Figure 3. Variation in Average weight in g (A) and average length in cm (B) in CAP treated ponds compared with control pond during the study period.

The mean specific growth rates (SGR) of PL (prawn larva) of freshwater prawn were 2.28± 0.035 (%), 2.70± 0.009 (%), 3.01± 0.012 (%), 2.72± 0.07 (%), 2.82± 0.071 (%), 2.65± 0.049 (%) and 2.92± 0.086 (%) in T-I, T-II, T-III, T-IV, T-V, T-VI and T-VII, respectively (Table 5).

The specific growth rate (SGR % day^{-1}) of prawn in the present study ranged from 2.11 to 3.53 % day^{-1}. Kumar *et al*., (2000) reported the specific growth rate of 2.04 % Day^{-1} in *M. rosenbergii* monoculture for 180 days with fertilization and monitoring of water quality. The specific growth rate of the present study was slightly higher than the above study but much lower than the study of Indulkar and Belsare (2003) who reported the specific growth rate of 9.45 + 0.1 % day^{-1} in monoculture of prawn with five live or inert food organisms.

The survival rates of galda in all treatments were estimated separately from the harvesting data at the end of the experiment. After a rearing of 90 days, the survival rate (mean values) of stocked galda were found to be ranged from 57.27± 5.85, 70.91± 2.33, 87.27± 3.71, 70.91±2.98, 80.91±4.37, 68.64±4.12 and 79.09±3.28 in treatments I, II, III, IV, V, VI and VII, respectively. The highest survival rate (87.27%) of galda was found in treatment-III and the lowest (57.27%) in treatment-I. The mean survival rates of PL of freshwater prawn were 57.27% in T-I, 70.91% in T-II, 87.27% in T-III, 70.91% in T-IV, 80.91% in T-V, 68064% in T-VI and 79.09% in T-VII. The results obtained from the present study on survival rate was more or less in agreement with D'Abramo *et al*., (1995) who reported 54.3 to 89.9 % survival of galda after rearing of 135 to 142 days. Chand (2000) stated that survival rate of freshwater prawn in ponds ranged from 49 to 54 %. Hossain *et al*., (2000) reported that the survival of the prawns ranged between 46.6 to 66.6 % in galda monoculture in earthen ponds. The result of the above studies more or less agreed with the present study. The survival rate of galda (70.74-82.41%) recorded by Haroon *et al*., (1989) was lower than the survival rate obtained in the present study. The high percentage of survival obtained in all treatments except treatment-I suggest that such factors as healthy fish, effect of antibiotics and favorable ecological condition etc. were important in influencing survival.

Table 5. Comparison of yield parameters (Mean ± SD) of galda (*M. rosenbergii*) in different treatments.

Growth parameter / Treatment		Mean initial weight (g)	Mean initial length (cm)	Mean final weight (g)	Mean final length (cm)	Average weight gain (g)	% weight gain	Specific Growth Rate (% d^{-1})	Survival (%)
Control	Treatment (I)	1.74 ± 0.00	3.20 ± 0.00	13.56 ± 3.49	10.7 ± 0.42	11.82 ± 3.35	679.31 ±107.64	2.28 ± 0.035	57.27 ± 5.85
N-Treated Ponds	Treatment (II)	1.74 ± 0.00	3.20 ± 0.00	19.70 ± 0.57	12.00 ± 0.15	17.96 ± 0.80	1031.90 ±24.23	2.70 ± 0.009	70.91 ± 2.33
	Treatment (III)	1.74 ± 0.00	3.20 ± 0.00	26.09 ± 3.43	13.4 ± 0.30	24.35 ± 1.27	1399.43 ±36.97	3.01 ± 0.012	87.27 ± 3.71
O-Treated Ponds	Treatment (IV)	1.74 ± 0.00	3.20 ± 0.00	20.08 ± 1.40	11.7 ± 0.231	18.34 ± 0.76	1054.02 ±30.55	2.72 ± 0.07	70.91 ± 2.98
	Treatment (V)	1.74 ± 0.00	3.20 ± 0.00	22.03 ± 3.49	12.5 ± 0.296	20.29 ± 1.92	1166.09 ±41.34	2.82 ± 0.71	80.91 ± 4.37
C-Treated Ponds	Treatment (VI)	1.74 ± 0.00	3.20 ± 0.00	18.85 ± 1.93	12.4 ± 0.169	17.11 ± 1.83	983.33 ± 21.69	2.65 ± 0.049	68.64 ± 4.12
	Treatment (VII)	1.74 ± 0.00	3.20 ± 0.00	24.01 ± 3.12	11.4 ± 0.337	22.27 ± 2.44	1279.89 ±80.64	2.92 ± 0.086	79.09 ± 3.28

N = Nitrofurazolidone; O = OTC; C = CAP

Table 6. Comparison of total bacterial load in galda after different treatments.

Treatments		Initial Bacterial Load (CFU/g)	Final Bacterial Load (CFU/g)
Control	Treatment I	16.6×10^6	27.3×10^6
Nitrofurazolidone Treated Ponds	Treatment II	25.3×10^6	9.95×10^6
	Treatment III	34.4×10^6	2.6×10^6
OTC Treated Ponds	Treatment IV	19.1×10^6	12.65×10^6
	Treatment V	28.6×10^6	2.9×10^6
CAP Treated Ponds	Treatment VI	31.6×10^6	10.1×10^6
	Treatment VII	22.6×10^6	5.7×10^6

Average bacterial load (CFU/g)

Positive effects of antibiotic upon some identical bacteria were found. *E. coli* bacteria reduced significantly after treated with nitrofurazolidone, with the increasing of time and in some cases this bacterium was not found but when nitrofurazolidone was stopped this bacterium appears in the galda's body. Similarly OTC has shown positive effect on *Staphylococcus* sp. and CAP shown positive effect on *Staphylococcus sp. and Streptococcus sp* (Table 6). Our result is similar to Tendencia *et al*. (2007) who were reported that bacteria from prawn ponds have been reported to be resistant to OTC, furazolidone, oxolinic acid and CAP. Luminous *vibrios* and *Staphylococcus* from prawn larvae and ponds were resistant to erythromycin, kanamycin, OTC, Penicillin, streptomycin, sulfadiazine and CAP. *E. coli,* Aeromonas sp. from fish, prawns, and their rearing water was resistant to streptomycin, nitrofuran and sulphamethoxazole.

The farmers used various chemicals and antibiotics in galda farms for prevention of disease. The generic names of those chemicals are now known. They also used various insecticides when the same ponds are used for paddy culture. All the antibiotics used in this present studies were found growth promoters. Bacterial loads reduced considerably in ponds where antibiotic medicated feeds were used. The withdrawal period of nitrofuran metabolites were 10 days for AOZ and AMOZ and 20 days for SEM. The withdrawal period of CAP was 20-25days whereas tetracycline was 5-7 days.

Table 7. Comparison of identified bacterial species in galda (M. rosenbergii) under different treatments.

Days	Control	Nitrofurazolidone Treated Ponds		OTC Treated Ponds		CAP Treated Ponds	
	Treatment I	Treatment II	Treatment III	Treatment IV	Treatment V	Treatment VI	Treatment VII
10	*Bacillus, E. coli Pseudomonas Streptococcus, Staphylococcus*	*Bacillus Streptococcus, Staphylococcus*	*Pseudomonas Staphylococcus Bacillus*	*Bacillus, E. coli Streptococcus Staphylococcus*	*Staphylococcus Bacillus, E. coli Streptococcus*	*Pseudomonas Bacillus Staphylococcus*	*E. coil, Bacillus Streptococcus Staphylococcus*
30	*Bacillus, E. coli Streptococcus Staphylococcus*	*Aeromonas Bacillus, E. coli*	*Aeromonas Bacillus*	*Bacillus Pseudomonas*	*Pseudomonas E. coli*	*Bacillus E. coli Aeromonas*	*Pseudomonas*
35	*Bacillus Aeromonas Pseudomonas*	*Pseudomonas Staphylococcus*	*Bacillus E. coli Aeromonas*	*E. coli Aeromonas Streptococcus*	*Pseudomonas Streptococcus E. coli*	*Bacillus E. coli Aeromonas*	*Pseudomonas Bacillus Aeromonas*
40	*Bacillus Staphylococcus Streptococcus*	*Staphylococcus E. coli Aeromonas*	*Bacillus Pseudomonas Bacillus*	*E. coli, Bacillus Streptococcus*	*Pseudomonas Bacillus*	*E. coli, Bacillus Streptococcus*	*Pseudomonas Bacillus, E. coli*
50	*Staphylococcus Aeromonas Bacillus, E. coli*	*Bacillus, E. coli Aeromonas*	*Aeromonas Pseudomonas*	*Streptococcus Pseudomonas*	*Bacillus, E. coli Aeromonas*	*Pseudomonas Bacillus, E. coli Staphylococcus*	*E. coli, Bacillus Streptococcus*
60	*E. coli Pseudomonas Staphylococcus*	*Pseudomonas Bacillus Staphylococcus*	*Streptococcus Staphylococcus*	*Pseudomonas Staphylococcus*	*Streptococcus Pseudomonas Bacillus, E. coli*	*Pseudomonas Bacillus Staphylococcus*	*Bacillus E. coli Aeromonas*
75	*Staphylococcus Bacillus Pseudomonas*	*E. coli, Bacillus Streptococcus Staphylococcus*	*Pseudomonas Bacillus E. coli*	*Bacillus Aeromonas Pseudomonas*	*E. coli, Bacillus Streptococcus*	*E. coli, Bacillus Streptococcus Staphylococcus*	*Bacillus Aeromonas*
90	*Bacillus, E. coli taphylococcus Aeromonas*	*Staphylococcus Bacillus Pseudomonas*	*Bacillus E. coli Aeromonas*	*Staphylococcus Pseudomonas Bacillus, E. coli*	*Streptococcus Pseudomonas E. coli*	*Bacillus, E. coli Aeromonas Staphylococcus*	*Pseudomonas Bacillus, E. coli Aeromonas*

The present survey provided very useful information on the use of antibiotics especially nitrofuran, CAP and OTC drugs in galda/bagda farms and its detailed information about pharmaceuticals, growth promoter/hormone, poultry dropping/waste and chemicals used in hatchery and farm, during handling and transportation of galda in special regard to food safety in Phultala upazila of Khulna district and Rampal upazila of Bagerhat. Different types of commercial feeds were used in galda farms in Phultala and Rampal Upazila.

However, food safety is an essential factor for ever-growing global prawn farming and processing industries. More research support is needed to improve the cultural and management practices of galda culture in Bangladesh which is a promising segment for the national economy. It is necessary to investigate the generic names of all chemicals used in galda and bagda farms to have control over the use of chemicals and antibiotics on legal basis as well as all the feeds used for galda, bagda and poultry should be analyzed for antibiotics in metabolites and parent forms for food safety. The knowledge about harmful effect of antibiotic on prawn growth and survival is discovered which will help to develop the management measures for healthy prawn industry through sustainable production and export of prawn.

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