9.7 Determination of efficacy of selected antibiotics used in aquaculture

9.7.1 Effects of antibiotics on fish fry health and water quality in cistern

9.7.2 Experimental Procedure

The feeding trails with antibiotic mixed feed were carried out in a static indoor rearing system of the BFRI, Mymensingh consisting a series of rectangular cistern (2500 L each) for 8 weeks. The same aged uniform size of each fish fry were randomly distributed into groups of 100 fish (averaging 1.5g) per cistern. Three selective antibiotics were tested and each had three replications. The fish were individually weighed at the starting of the experiment. A weekly fish weight (fish sampling) was taken to adjust the daily feed ration for the following week. Before using antibiotics all water quality parameters such as temperature, pH, dissolve oxygen and ammonia were recorded though weekly sampling. At the beginning of the experiment, 10 fish were randomly sacrificed for histopathological analysis.

9.7.3 Experimental design

After 5 days of fertilization, Thai koi fry (1.53 ± 0.14 g) were stocked in the cisten with same stocking patterns 100 fish/cistern. Three antibiotics [Renamycin (Reneta), Oxysentin (Novartis) and Aquamycin (ACI)] were applied with pelleted Koi nursery feed (Saudi-Bangla Fish Feed Limited). Three replications were used for each antibiotic and the cisterns were marked as T_1, T_2, and T_3, for Renamycin, Oxysentin and Aquamycin, respectively. Other two cisterns feeding feed without antibiotic were used for control and was marked as T_4. Antibiotic mixed commercial feed was applied in all the cistrans for 60 days except for the control. Water quality parameters (Table 29) and growth parameters of fish were recorded (Table 30).

Table 29 Water quality parameters of cistern under different treatment of antibiotics

Water Quality Parameters	Renamycine		Oxysentin		Aquamycine	
	BT	AT	BT	AT	BT	AT
DO (mg/L)	3.28- 3.52	4.68-6.24	4.12-5.98	4.5-6.10	3.99-5.20	5.1-6.5
pH	6.50-6.82	7.48-7.98	6.58-7.90	7.20-7.45	6.0-7.2	7.12-7.5
Total alkalinity (mg/L)	80- 95	125-140	85-98	98-111	84-96	111-120
Ammonia (mg/L)	0.32-0.58	0.05-0.08	0.09-0.13	0.03-0.06	0.09-0.11	0.03-0.06
Phosphate (mg/L)	0.22-.32	0.28-0.65	0.22-.32	0.28-0.65	0.22-.32	0.28-0.65
Nitrate (mg/L)	0.10-0.24	0.18-0.30	0.10-0.24	0.28-0.55	0.10-0.24	0.18-0.83

* BT= Before Treatment, AT= After Treatment

Table 30 Growth and survival of Thai Koi under different treatment of antibiotics

Growth Parameters	Renamycine	Oxysentin	Aquamycine	Control
Av. Initial wt. (g)	1.50 ± 0.14	1.55 ± 0.15	1.55 ± 0.15	1.53 ± 0.11
Av. Final wt. (g)	8.78 ± 0.35^{a}	8.50 ± 3.31^{b}	8.15 ± 0.27^{c}	6.65 ± 0.29
Av. live weight gain (%)	485.73 ± 7.02^{a}	448.28 ± 8.60^{ab}	434.72 ± 0.28^{c}	334.50 ± 0.24^{c}
Survival rate (%)	92 ± 3.06^{a}	90.00 ± 3.03^{a}	90.23 ± 2.15^{a}	70.15 ± 2.12^{b}
Specific growth rate (%)	2.96 ± 0.55^{a}	$2.84 \pm 0.54^{a\,b}$	2.83 ± 0.55^{b}	2.45 ± 0.08^{c}

9.7.4 Effects of antibiotics on fish fry health and water quality in pond

The follow up trail with antibiotic mixed feed was designed with three treatments having three replicates of each. Twelve (12) nursery ponds (each pond 40 m^2) were used for nursing of fry. Ponds were prepared through sun drying and liming the bottom soil with agricultural lime @ 250 kg/ha and mustard oil cake @ 500 kg/ha. The ponds were filled up with underground water up to a depth of 1 m and inorganic fertilizers of TSP and urea were applied @ 35 kg/h with 3:1 ratio. After 5 days of fertilization, *O. niloticus* fry (ABW: 3.33 g, ABL: 5.53±0.83 cm), were stocked in the ponds with same stocking patterns of 100 fish/pond.

9.7.5 Experimental Design

Three antibiotics [Renamycin (Reneta), Oxysentin (Novartis), Aquamycin (ACI)] were applied with pelleted Tilapia nursery feed (Saudi-Bangla Fish Feed Limited). Three replications (feeding feed with antibiotic) were used for each antibiotic and the ponds were marked as T_1, T_2, and T_3 for Renamycin, Oxysentin and for Aquamycin. Other three ponds (feeding feed without antibiotic) were used for control and were marked as T_4. All of the above ponds were prepared by drying, liming and fertilization before stocking. Fish was stocked at the rate of 400/m^2. Water quality parameters were measured and recorded before applying feed. Antibiotic mixing feed applied twice a day for 25 days at the rate of 50% body weight. From 26th day to 45 days feed was applied at the rate of 25% body weight and from 46th day to 60 days at the rate of 10% body weight. Feeding rate was reduced to 5% body weight from 61th day to next 90 days. Growth, survivability, water quality and plankton monitoring were done after 15 days interval. Histopathological study was also done before and after the end of experiment.

9.8 Results

Water quality parameters of antibiotic treated ponds were recorded using commercial test kits (HANNA Test kit). Water temperature (^{0}C), pH, dissolved oxygen (mg/L) and total alkalinity were measured every 7 days interval for Talapia and Thai Koi fishes separately. Water quality parameters of antibiotic treated ponds have been presented in the Table 31 & 32. Influence of various antibiotics was tested in experimental ponds. Water quality parameters found to be more or less similar in antibiotic treated ponds when compared to those in the control pond. Microbial load decreased significantly in antibiotic treated ponds as compared to control. Histopathological changes of different organs of fishes like muscle, gill, kidney and liver of treated ponds were observed (Figs. 20-23). No change in structural configuration of muscle, gill, kidney and liver was observed. No disease was noticed in the ponds feeding with antibiotic mixed feed. Fish growth and production were satisfactory in all the antibiotic treated ponds (Table 33).

Table 31 Abundance of plankton (x10^3cells/L) in ponds feeding antibiotic mixed feed

Aquatic Organisms	Renamycine		Oxysentin		Aquamycine	
	BT	**AT**	**BT**	**AT**	**BT**	**AT**
Chironomidae	37.65± 11.11	22 ± 13.1	44.07± 9.98	30.12 ±10.33	23.1.98±9.06	24 ± 3.98
Oligochaeta	25.93± 3.23	10.12 ± 3.12	16.05± 3.04	11.53 ± 3.15	22.35± 24.69	11.,36 ± 2.65
Mollusca	9.51± 2.14	8.23 ± 2.23	6.46 ± 2.56	5.23 ± 2.17	8.10± 1.13	7.11 ± 2.12
Unidentified	34.57± 7.11	36.16 ± 7.40	39.51± 8.06	30.13 ± 7.99	35.19± 9.85	28.33 ± 6.23
Bacillariophyceae	13.54 ± 3.01	10.21 ± 2.11	12.04 ± 4.74	5.14 ± 1.76	11.37 ± 4.86	8.0 ± 2.1
Chlorophyceae	13.79 ± 4.48	8 ± 2.8	19.92 ± 8.24	10.32 ± 2.87	17.95 ± 3.61	12.09 ± 3.65`
Cyanophyceae	10.38 ± 3.67	6 ± 2.1	7.79 ± 1.88	3.97 ± .2.13	10 ± 5.76	6.12 ± 2.56
Euglenophyceae	1.63 ± 0.96	1.0 ± 0.12	2.29 ± 1.18	2.1 ± 1.0	1.5 ± 1.11	1 ± 0.23
Crustacea (Nauplius)	10.79 ± 2.66	6.12 ± 1.34	11.92 ± 3.18	6.12 ± 1.34	14.58 ± 5.33	11 ± 1.2
Bacillariophyceae	8.1± 1.38	3.33 ± 1.0	6.67± 1.89	4.23 ± 1.9	4.1± 1.74	3.21 ± 0.9
Chlorophyceae	22.27± 2.95	15 ± 1.77	2.43 ± 1.68	2 ± 0.2	4.4 ± 1.54	2.0 ± 0.25
Euglenophyceae	12.17 ± 6.72	8.1 ± 2.13	9.97 ± 2.49	5.1 ± 1.37	7.5 ± 3.89	6 ± 1.5
Rotifera	3.73 ± 1.62	2.1 ± 0.2	2.5 ± 1.46	1.44 ± 0.2	2.43 ± 1.49	2.2 ± 0.5
Cladocera	3.9 ± 1.7	2.0 ± 1.0	2.43 ± 1.43	1.55 ± 0.1	2.2 ± 1.01	1.4 ± 0.44

Table 32 Water quality parameters of ponds feeding antibiotic mixed feed

Parameters	Renamycine		Oxysentin		Aquamycine	
	BT	AT	BT	AT	BT	AT
DO (mg/L)	3.28-3.52	4.68-6.24	4.12-5.98	4.5-6.10	3.99-5.20	5.1-6.5
pH	6.50-6.82	7.48-7.98	6.58-7.90	7.20-7.45	6.0-7.2	7.12-7.5
Total alkalinity (mg/L)	80- 95	125-140	85-98	98-111	84-96	111-120
Ammonia (mg/L)	0.32-0.58	0.05-0.08	0.09-0.13	0.03-0.06	0.09-0.11	0.03-0.06

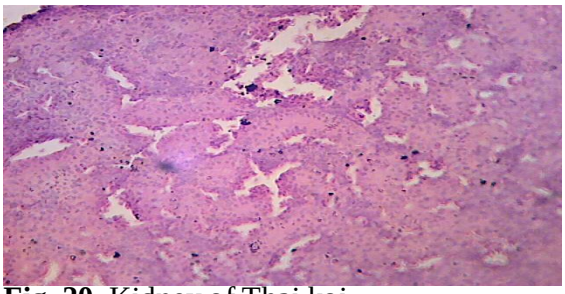

Fig. 20 Kidney of Thai koi

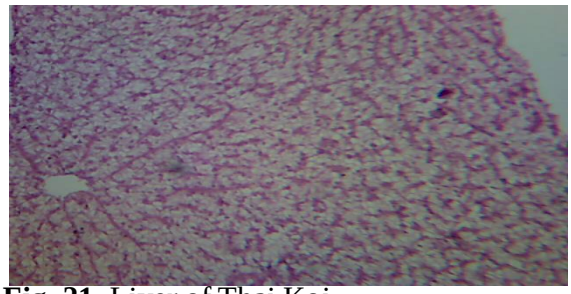

Fig. 21 Liver of Thai Koi

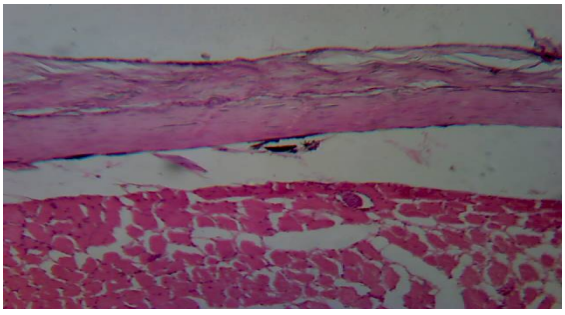

Fig. 22 Muscle of Thai Koi

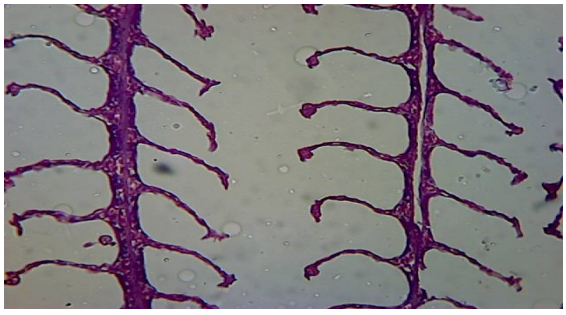

Fig. 23 Gill of Thai Koi

Table 33 Growth and survival Thai Koi in ponds feeding antibiotic mixed feed

Parameters	Renamycine	Oxysentin	Aquamycine	Control
Initial body weight (g)	6.26±0.27	6.26±0.28	6.26±0.23	6.26±0.29
Final weight body (g)	98.30±0. 45[a]	84.45±0. 35[b]	83.10±0.45[b]	73.18 ±0.48[b]
Weight gain (g)	92.04±0.37[a]	78.55±0.25[b]	77.04±0.38[b]	67.53±0.39[c]
Weight gain (%)	1470.28±0.33	1256.80±0.30	232.67±0.32	1168.10±0.38
Specific Growth Rate (SGR) (% day)	2.29±0.35	2.17±0.34	2.16 ±0.36	2.26±0.38
Survival rate (%)	89.25±5.41	88.72±6.09	76.20±4.77	70.34±5.71

9.9 Discussion

Few studies have been conducted with the status of aqua-drugs and chemicals in Bangladesh but no such study was carried out with impact of aqua-drugs and chemicals on health and production of fish. The purpose of this work was to provide current status and impact of aqua-drugs and chemicals in aquaculture activities in Bangladesh. Aquaculture activities became intensified that demanded frequent use of aqua-drugs and chemicals for higher growth and production.

The present study provided the impact of drugs and chemicals on water quality parameters, and fish health and production. In the present study, it was found that most of the farmers used aqua-drugs and chemicals for pond preparation, health management and disease treatment. However, fish disease treatment was the major area where most of the aqua-drugs and chemicals were used. There are many purposes of the use of aqua-drugs and chemicals in aquaculture such as pond preparation, water treatment, health management, enhancement of natural productivity and growth promotion (Subasinghe *et al.* 1996). Experiments were conducted by applying different aqua-drugs and chemicals for maintaining water quality, disease free and high fish production.

Geotox, Geolite gold, Mega-geo plus and Miracol lime mila were widely used by farmers in study area. Most farmers use Geolite gold whose active ingredients were SiO_2, Al_2O_3, Fe_2O_3, TiO_2 and MgO. Faruk *et al.* (2008) mentioned that Geotox, JV Zeolite, Timsen, Green Zeolite, Pontox plus, Zeolite, Zeo care, Mega Zeo, Bis Zeolite, Bio-Tuff, Well Zeolite and Aqua zet were found in Mymensingh region.

9.9.1 Physical parameters

Water transparency increased as a result of application of drugs in ponds. Water transparency was 28.40±2.16 cm and 31.27±3.06 cm before and after application of Timsen, respectively. Before using of Geofresh, Geoprime, and Geotox water transparency was 27.73±1.75 cm, 27.47±2.39 cm, 28.67±2.23 cm while after treatment it was 29.53±1.88 cm, 29.20±2.76 cm, 30.07±2.52 cm respectively. Among the drugs applied, Timsen was found suitable to increase water transparency. Other three drugs provided more or less same results in changing water transparency. Water transparency was increased due to application of drugs that was found suitable for aquaculture.

After using drugs water temperature became reduced. Before and after using Timsen, water temperature was 28.41±3.36°C and 28.25±3.38 °C respectively. Before and after using Geofresh, Geoprime and Geotox water temperature was 28.48 ± 2.77°C, 27.78 ± 2.09°C, 28.29 ± 2.98°C and 28.38 ± 2.73°C 27.71 ± 2.07°C, 28.27 ± 2.96°C, respectively. Swann (2009) described the suitable ranges of water quality parameters for aquaculture as 24 to 32°C. The author observed that Timsen and other three drugs were able to maintain water temperature at 28.41 ± 3.36°C and that is suitable to fish culture.

9.9.2 Chemical parameters

Water pH, DO, phosphate and Nitrate have increased due to application of drugs. Before and after using Timsen pH was 6.93±0.25 and 8.12±0.58, respectively while before and after using Geofresh, Geoprime, and Geotox water pH was 6.62 ± 0.34, 6.49 ± 0.19, 6.42 ± 0.17 and 7.71 ± 0.31, 7.64 ± 0.14, 7.56 ± 0.18 respectively. DO was 3.72 ± 0.33 mg/L and 4.56 ± 0.29 mg/L before and after application of Timsen, respectively. Before and after using Geofresh, Geoprime, and Geotox DO was 3.44 ± 0.28, 3.30 ± 0.16, 3.33 ± 0.26 and 4.14 ± 0.29, 4.07 ± 0.17, 4.06 ± 0.26 mg/L, respectively. Total Alkalinity was 95.00 ± 8.84 and 119.40 ± 9.67 mg/L before and after application of Timsen, respectively. Before and after using Geofresh, Geoprime, and Geotox water Total Alkalinity was 94.07±6.56, 93.33±8.01, 92.47±6.32 and 115.73±11.13 , 115.33± 6.97 , 110.33±9.19 mg/L, respectively.

9.9.3 Fish production

Fish production was considered as an indicator in determining efficacy of water quality treatment drugs applied in culture pond. Four drugs were applied in different fish culture ponds to determine efficacy of water quality treatment drugs. Among the water quality treatment drugs applied, Timsen provided the highest fish production. Net fish production was 1955.64 ± 24.28 kg/ha/60 days with Timsen that was higher than the other three drugs applied.

The present study provided higher fish production in drugs treated ponds while Khaled & Mamun (1996) achieved production of Tilapia as 339.39 kg/ha, 600.00 kg/ha and 624.24 kg/ha over a period of three months in three earthen ponds by using two prepared and one

commercial diets. Hussain *et al.* 2000 reported Tilapia production as 125-140 kg/m^2 in cage system. Nahid *et al*. reported tilapia production as 13832kg/ha/ya. Production of Thai pangas in the control pond at BAU was higher (7328.16 kg/acre) than in the treated ponds (6400.08 kg/acre) which disagrees to this experiment. Production of Thai Koi in the treated pond at BAU was higher (1471.92 kg/acre) than that of control ponds (1296.00 kg/acre) which agrees to the present study.

In the present study survival rate was 85.00±1.73% which is online with the works of Sayeed *et al.* (2008). They reported survival rates of Thai pangas was 94 to 97% in nine earthen chemicals treated ponds with a period of 11 months. Islam (2009) studied survival rates were 70.4, 80.75 and 72.25% for native koi, Thai koi and other two hybrids, respectively. Khan *et al* 2011 reported that application of Geoprime was suitable for fish production that's disagrees with the present investigation as this experiment Timsen show the supremacy over other drugs in producing fish. Faruk *et al.* (2008) mentioned the name of several aqua-drugs used in aquaculture but did not mentioned the name of the best drug. In this experiment Timsen showed the best performance for producing fish among Geo fresh, Geoprime and Geotox.

9.9.4 Histopathology

Histopathological study was done to know the impacts of water quality treatment drugs on different organ of fishes. In this study water quality treatment dugs did not produce any remarkable changes to the different organs such as liver, kidney, gill and muscle of fish.

Samsuzzaman *et al.* (2011) reported that in the chemical treated pond, different organs such as liver, kidney, gill and muscle of fishes had remarkable pathological changes like necrosis, hemorrhage, vacuum, pyknosis, necrosis, hypertrophy and partial loss of some parts. It was also observed that loss of epidermis, necrosis, vacuum, haemorrhage and pyknosis were noticed in the fishes of ponds where aqua-drugs and chemicals were applied. Some important pathological changes such as haemorrhage, necrotic hepatocytes, pyknotic cells and vacuum were recorded in the liver of chemical treated fishes. Anderson *et al.* (2005) reviewed that malachite green is readily absorbed by fish

tissue and is metabolically reduced to leucomalachite green (LMG) which is lipohilic and can be stored in edible fish tissues for extended periods of time.

Haemopoeitic necrosis, hemorrhages, vacuolation in haemopoeitic cell were common pathological changes in kidney of chemical treated fishes. Ahmed *et al.* (2009) also found similar result for freshwater eel in winter season which was occurred due to antibiotic treatment. Ahmed *et al.* (2007) found necrosis, pyknosis, inflammation, hypertrophy, hyperplasia and missing of gill lamellae in *Anabas testudineus* during the month of December and January in. The author disagreed with the findings of Ahmed *et al.* (2007) as the author did not found any remarkable changes in the organ of fishes by using water quality treatment drugs during the month of April-June. Aqua-drugs and chemical treated fish exhibited pathological changes in gills which include hypertrophy, haemorrhage, missing of secondary gill lamellae and necrosis. In this study gill had no remarkable change due to treatment with aquadrugs and chemicals. Liver had highly necrotic hepatocytes, pyknotic and inflammatory cell during the months of December and January (Roy *et al.* 2006).

9.10 Recommendations

Due to large demand of fish protein, it is the appropriate to extend the production of fish. Now a day's farmers are interested to produce more fish by culturing fish with high stocking densities. For this reason farmer apply aqua drugs and chemicals to protect fish from any disease. Among the drugs tested, Timsen produced the best results in all aspects including improvement of fish health and water quality. Other three drugs such as Geo-tox, Geo-fresh, and Geo-prime produced similar results in maintaining and producing fish in ponds.

Histopathological study does not show any negative changes on the organ of GIFT. These drugs may be used in aquaculture to control production of ammonia in ponds.

Among the antibiotics tested, Renamycine showed the best performance in all aspects including improvement of fish health. Other two antibiotics (Oxysentin and Aquamycine) have similar actions in improving fish health.

9.11 Training organized

Within the project period four training courses have been completed in 2011-2012 and 2012-2013. Each training course was consisting of 3-day long program on "Impact of Aqua drugs on fish culture practices in Bangladesh". One hundred twenty farmers (including women and unemployed youths) participated in the training course. During the training course, the trainee farmers were provided with the hands-on knowledge on improved fish culture and protocol of appropriate drug management techniques. Most of the trainees felt encouraged on the improved fish culture technique and expressed their interest of adopting it in their own farms. The trainees requested to the authority in organizing more training programmes with upgraded knowledge and technology of fish culture and application of aqua-drugs and chemicals. Details of completed training under the project have been furnished in Table 34.

Table 34 Details of training conducted during the project period

Details	Year 2011-2012	Year 2012-2013
Topic(s)	Impact of Aquadrugs on Fish culture	Impact of Aquadrugs on Fish culture
No. of trainings	02	02
No. of trainees	60	60
Duration	06 days (02 to 04 May & 25 to-27 May)	06 days (11 to 1 3 May & 18 to 20 June)

9.12 Field days

Under the project one field day was organized in 2012-2013. Fish farmers including women and unemployed youths, and twenty office staff, GO and NGO representatives, have been participated on the occasion. After the inaugural session the participants were provided with the hands-on knowledge on improved fish culture and and the protocol of appropriate drug management techniques. Most of the participants felt encouraged on the drug management technique both in pond and in fish hatchery. The participants were divided into groups to identify the problems and prospects of application of aqua-drugs and chemicals in a focus group discussion (FGD) session. After group discussion open discussion was done on the problems identified from the group discussion. The

participants have shown their keen interest about the ongoing Aqua-drug Research activities. Details of field days have been shown below.

Venue **:** Farmer's field (Haluaghat, Mymensingh)
No. of field day **:** 01
No. of participants : 50
Duration **:** From 09:00 to 17:00 hours