University Journal of
ZOOLOGY
Rajshahi University

Effects of acetic acid on the total viable counts of microbes and overall acceptability of dressed broiler meat

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Abstract: This study was conducted to evaluate efficacy of acetic acid solution to eliminate pathogens, prevent food deterioration and extend the shelf-life of dressed broiler meat without adversely affecting its quality. A total of 40 broilers were categorized into two groups. Each group was further categorized into two sub-groups: one for intact skin and another for without skin. Each bird of either group viz. comprised of two portions breast and thigh muscle. Acid spray and acid immersion were used. Bacteriological analysis by total viable count (TVC) and sanitary quality determination of dressed broiler by taste panel scores were performed. Acetic acid treatment reduced the initial level of TVC by about 0.5 to 0.724 log colony forming unit (CFU)/gm of meat. Maximum reduction in TVC (0.724) was achieved when acetic acid immersion treatment was given to meat and it was evident that the meat quality of dressed broiler after treatment with acetic acid remains better up to 5 days of storage.

Key words: Broiler meat, decontamination, acetic acid, total viable counts.

Introduction

The recognition of modern concepts about the possibilities for eliminating pathogenic microorganisms from meat has received considerable attention in recent year (Cutter, 1999; Acikgoz et al., 2011). Bacterial contamination of raw processed poultry products continues to be of great concern to consumers and regulatory and health official (Akyurek & Yel, 2011; Al-Kassi & Mohssen, 2009; Anower et al., 2004). Despite the hygiene measures applied during processes ranging from slaughtering to packaging, pathogenic bacteria can gain access to carcasses and proliferate on there. Commercial poultry industry is growing rapidly in Bangladesh. Estimate shows that poultry population is increasing at the rate of 6.5% per year in the country (Haque, 2001). Shelf-life of refrigerated fresh muscle foods is determined mainly by microbiological and physical qualities during storage and handling (Chen and Shelef, 1992). Since the market price of broiler suddenly falls due to unexpected incidents and unforeseen reasons, it is therefore obvious that there occurs problem to sell broiler, as a result, large number of birds necessitate storing for future use. Prolonged rearing of broilers is always unprofitable. In order to obviate the aforesaid problem, if the broilers could be preserved by storing for a certain period of time and sold later, the crisis and anxiety could be overcome, and the poultry farmers could get rid of this loss. Several intervention strategies have been tested and/or adopted for use in eliminating both pathogenic and spoilage bacteria from carcass surfaces. For example, solutions of acetic acid are commonly used by the slaughter industry as antimicrobial spray wash interventions to reduce the microbial load on freshly slaughtered carcasses (Berry and Cutter, 2000). Researchers have shown significant reduction of microbes on fresh meat carcass surfaces after the use of an acetic acid spray (Bacon et al., 1999; Cutter, 1999). The acetic acid is generally recognized as safe substance with no upper limit of daily intake for humans (FAO, 1965). Substantial increases in the occurrence of food poisoning outbreaks and commercial requirements to extend the safe, high quality shelf-life of food have focused attention on decontamination system (Islam et al., 2008; Canibe et al., 2001). The present study is therefore undertaken to determine the effects of acetic acid applied in the raw poultry meat surface and its influence on microbial growth and on meat quality.

Materials and Methods

A total of 40 broilers were collected from local market. The birds were categorized into two groups, A for control and B for acetic acid treatment. Each group was further categorized into two sub-groups: one for broiler with intact skin and another for broiler without skin. Each bird of either group comprised of two portions namely, breast and thigh. After collection of samples bacteriological analysis and sanitary quality determination were performed for the total viable counts (TVC) by using plate count agar medium to find out the microbiological quality of the meat. Group B was subjected to treatment with 0.5% acetic acid solution. Two methods of treatment were employed: spray and immersion, performed separately. Group A was kept as control. Samples from Group B after five minutes of treatment was subjected to bacteriological analysis and sanitary quality determination using the same method and

subsequently kept at 5^0C refrigeration by wrapping with commercially available polyethylene bags. Then at 3rd day and 6th day of storage again bacteriological analysis and organoleptic quality determination were performed.

Five taste panel experts determined the organoleptic quality of broiler meats kept at different storage periods by assessing and giving scores of the sensory attributes. There were five qualities characteristics viz. colour, flavour, juiciness, tenderness and firmness or consistency and overall acceptance are considered for the taste panel judgment. For each of the characteristic, the highest score was given 10 marks and the total score was marked 50. Out of 10 marks, 10 is considered as excellent, 8 as good, 6 as fair, 4 as poor, 2 as half spoiled and 0 for spoiled. The taste panel scores of different attributes of the meats of dressed broilers of Group A and B was determined at 1 day, 3 day, 5 day and 7 day. The procedures of examinations were as per the recommendations of ISO (1995), Rahman (1999) and Uddin *et al.,* (2006).

Results and Discussion

The total microbial load of dressed broiler meat both for de-skinned and intact skinned sample before and after treatment with acetic acid is presented in Table 1. In case of intact skin sample the meat tissue obtained from thigh and breast found to have initial microbial load as log 7.436 and log 7.501 respectively. While in case of without skin broiler meat tissue these values were log 7.368 and log 7.416 respectively. Mohizea *et al.* (1994) observed the initial total viable count (log_{10} cfu/cm^2) which ranged from log 3.8 to 5.5 with a mean of log 4.67. In the present research work however it was found relatively higher in fresh broiler meat tissue. The table further shows the microbial load in meat of dressed broiler meat given treatment with acetic acid which were determined on 0th day (5 min after treatment), 3rd day and 6th day of storage. These counts indicated lower load than that of the control group, indicating the decontamination effect of the organic acid.

Table 1. Total viable counts in the control and acetic acid treated groups of dressed broiler meat at different periods of storage.

Dressed broiler	Treatments			Mean TVC *±SD		
		Region of treatments	Control groups	5 min after treatments	3rd Day	6th day
With intact skin	Acetic acid spray	Thigh	7.436±0.091	7.085±0.07783	7.024±0.0765	7.125±0.0694
		Breast	7.501±0.033	7.132±0.1227	7.076±0.0998	7.193±0.0847
	Acetic acid immersion	Thigh	7.436±0.091	6.901±0.0547	6.712±0.0538	6.896±0.0564
		Breast	7.501±0.033	7.056±.0139	6.861±0.129	7.007±0.0996
Without skin	Acetic acid spray	Thigh	7.368±0.4328	6.997±0.0595	6.896±0.06521	6.993±0.07512
		Breast	7.416±0.01259	7.048±0.0850	6.943±0.0754	7.02±0.06487
	Acetic acid immersion	Thigh	7.368±0.4328	6.851±0.1152	6.733±0.0986	6.78±0.08775
		Breast	7.416±0.01259	6.958±0.06414	6.798±0.06214	6.869±0.06345

* All counts are expressed in logarithms, TVC= total viable counts; SD= standard deviation

These counts indicated lower microbial load in after acetic acid treatment group than that of control group, thus indicating the decontamination effect of acetic acid.

It is evident that in the treated group the TVC were in the highest range on the last day of storage emphasizing the fact that the number of organisms decreased initially due to the antimicrobial effect but on prolonged storage the count gradually increased on the 6th day. This could probably be due to the adaptation of the microbes with the new environment. The result of the study yielded interesting phenomenon to note that the total microbial counts although were found to have increased with the subsequent days of storage, however, the mean TVC on the 6th day of storage were still found to be lower than that of the control, thus indicating the effect of decontamination effect

by organic acid. It is an accepted principle that when the microbial load is determined, it needs to make judgment whether the food is safe or not. Hence microbial criteria are established to help make a valid judgment concerning the safety and keeping quality of a food. The total microbial counts of food products not only reflect handling history, state of decomposition or degree of freshness, they may in some instances reflect the sanitary quality of the foods (Dickens *et al.*, 1992).

The determination of total viable bacteria effectively evaluates the hygienic quality of foods (Anower *et al.*, 2004). In this study, the total counts were considered to indicate the nature of sanitary control measures to be exercised in the production, transport and storage of poultry meat. The same may be valid for foods when it is desired to set a standard to be used as a guide to storage life

(Uddin *et al.*, 2006). However, it is obvious from this research work that the total viable bacterial count as found in dressed birds is not the only criterion that could ensure that the material will be safe consistently; but other quality control tests must be incorporated to make a final judgment for assessing its acceptability and wholesomeness. On the basis of this principle many investigators conducted studies on the incidence of microorganisms associated with dressed poultry (Dickens *et al.*, 1992). The results show that the initial TVC levels were reduced by about 0.50 log CFU/g of meat tissue by acetic acid spray treatment while the initial TVC levels declined by 0.61 to 0.72 log CFU/g of meat tissue after acetic acid immersion treatment was employed. The study revealed an important fact that irrespective of the types of treatments employed, TVC were lowest on the 3rd day of storage. This may be due to the combined effect of acid treatment and cold effect of storage. Woolthuis and Smulders (1985) observed in calf carcasses that the total viable count was reduced by 1 log with similar reductions in Enterobacteriace using 1.25% acid. Bosilevac *et al.* (2006) found that the lactic acid spray treatment reduced the TVC by 1.6 log

CFU/g of meat sample. The present work obtained the reduction of less than 1 log cycle. This may be due to the application or techniques employed for the treatment or may be similar to the observation of Francois (2004) who reported that the decontamination effect of a solution was very much correlated with the pH of that solution and the chicken meat and skin pH variation. Comparing initial microbial load present in meat tissues eventually it can be concluded that acid immersion treatment results more in reduction of total viable counts in meat tissues compared to acid spray treatment regardless of intact skin or de-skinned dressed broilers. Francois (2004) observed that decontaminating chicken skin by immersion in an organic acid solution at 7 °C led to 3.7 decimal reductions in TVC. Similar results were demonstrated by Haque (2007). Anderson and

Taste panel scores of different attributes of the meats of dressed broilers before and after treatment with acetic acid solution and kept for different storage periods is presented in table 2..

Table 2. Taste panel scores of different attributes of the meats of dressed broilers before and after treatment with acetic acid solution and kept for different storage periods.

Dressed broiler	RT	SP	Colour			Flavour			Juiciness			TFC			OA			Total		
			C	T1	T2	C	T1	T2	C	T1	T2	C	T1	T2	C	T1	T2	C	T1	T2
With intact skin	Thigh	1	9	9	10	8	9	10	9	9	9	9	9	9	8	9	9	43	45	47
		3	4	8	9	3	7	9	3	8	8	4	8	8	4	7	8	18	38	41
		5	2	7	7	1	6	6	1	6	6	1	5	6	1	6	6	6	30	31
		7	1	3	3	0	2	2	1	3	3	0	2	2	1	2	2	3	12	12
	Breast	1	9	9	10	8	9	10	9	9	9	9	9	9	8	9	9	43	45	47
		3	5	7	7	3	8	8	3	8	8	4	8	8	3	7	8	18	38	39
		5	2	6	6	1	6	6	1	6	6	1	7	7	1	6	6	6	31	31
		7	1	3	3	0	2	2	0	3	3	0	2	2	1	2	2	2	12	12
Without skin	Thigh	1	9	9	10	8	9	9	9	9	9	9	9	9	8	9	9	43	45	46
		3	4	8	9	3	7	7	3	8	8	4	7	7	3	7	8	17	37	39
		5	1	7	7	1	6	6	1	5	5	1	7	7	1	6	6	5	31	31
		7	0	2	2	0	2	2	0	2	2	1	2	2	1	2	2	2	10	10
	Breast	1	9	9	10	8	9	9	9	9	9	9	9	9	8	9	9	43	45	46
		3	4	7	8	3	7	7	3	8	8	4	8	8	3	7	8	17	37	39
		5	1	6	6	1	6	6	1	6	6	1	6	6	1	6	6	5	31	31
		7	1	3	3	0	3	3	0	3	3	1	2	2	1	2	2	3	13	13

* Sensory characteristics with marks:
Excellent = 10; Good = 8; Fair = 6; Poor = 4; Half spoiled = 2; Spoiled = 0

Legend: RT= Region of treatments; SP=Storage period (days); TFC= Tenderness, firmness and consistency; OA=Overall acceptance; C=Control; T1= Treatment with acetic acid spray; T2= Treatment with acetic acid immersion.

It is evident from the result that the meat quality of dressed broiler after treatment with acetic acid remained better up to 5 days of storage. However, there was no significance difference among the scores achieved by acid spray and immersion treatment of dressed broiler irrespective of

dressed broiler with intact skin and without skin. Thus it becomes apparent that the treated meat quality was found better than the untreated one. Moreover, the shelf-life and keeping quality were not only enhanced but there was an obvious reduction of microbial load. Ricke (2003) used

organic acids to improve the meat and keeping quality of meat. Their findings are similar to the present results.

The present work founds relatively higher microbial load in fresh broiler meat tissue, microbial load in breast muscles was higher than shank muscles, and in dressed broiler with skin was higher than that of dressed broiler without skin. The acetic acid treatment effectively reduces the microbial loads due to its antimicrobial and decontamination effects and emersion treatment was better than its spray. The acetic acid treatment enhances meat quality, the shelf-life and keeping quality of meat in addition to reduction of microbial load.

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Protozoan parasites in a wastewater treatment plant of Bangladesh

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Abstract: Parasitic infection is a global health problem especially in developing countries. Municipal wastewaters always contain cysts of parasitic protozoans at some level. The present study was conducted to detect protozoan parasites in different stages of the treatment plant to check its efficacy. Wastewaters were collected from 3 points of the Pagla Sewage Treatent Plant (PSTP) of Dhaka, Bangladesh, throughout the year, 2007-08 at fortnight intervals. *Giardia* spp., *Entamoeba* spp., *Entamoeba coli*, *Endolimax nana, Idoamoeba butschlii* and *Balantidium coli* were detected at different times in different stages of the treatment plant. Among these *Giardia* and *Entamoeba* spp. were found most frequently than others. Both the prevalence and dominance of protozoan parasites were reduced gradually with the sampling point of the treatment plant which means that the treatment plant was effective in reducing protozoan parasites but not too effective to eliminate them completely.

Key words: Protozoan parasites, Wastewater treatment plant, Dhaka.

Introduction

Waterborne pathogens can be broadly characterised as parasitic protozoa, helminths, bacteria or viruses. Several protozoan infectious agents have been recognized as waterborne pathogens: *Cryptosporidium parvum*, *Giardia lamblia*, *Cyclospora*, *Entamoeba histolytica* (Steiner *et al.*, 1997). To date, there have been at least 325 water associated outbreaks of parasitic protozoan disease documented worldwide (Karanis *et al.* 2007). Most are responsible for opportunistic infections in immunocompromised hosts and belong to the phylum Apicomplexa, with an exception of Microsporidia which are characterised by a unique mode of cellular infection and spore production (Levine *et al.*, 1980). Presently, the role of these emerging protozoan parasites in waterborne infections remains unclear (Marshall *et al.* 1997). Other protozoa, such as *Isospora belli*, *Cyclospora* and *Enterocytozoon bieneusi* have also been implicated in waterborne outbreaks, although the presence of these parasites in water has rarely been documented (Miegeville *et al.*, 2003). The frequent contamination of surface water by *Cryptosporidium* and *Giardia* is well established (; Fayer *et al.* 2004; Yoder *et al.*, 2004).

As documented, most waterborne protozoan parasites are causative agent for gastroenteritis, diarrhoea and others related to cellular or tissue infections (Stuart *et al.* 2003 and Roy *et al.* 2004). *C. parvum* and *G. lamblia* are protozoan parasites causing human gastrointestinal illness worldwide, with *C. parvum* reported to cause approximately 2% and *G. lamblia* between 2%-7% of all diarrhoeal illness in developed countries (Griffiths, 1998). For children under 5 years of age in developing areas and countries, there was a median of 3.2 episods of diarrhoea per childyear. Estimates of mortality revealed that 4.9 children per 1000 per year in developing countries died as a result of diarrhoeal illness in the first 5 years of life. Despite improving trends in mortality rates, diarrhoea accounted for a median of 21% of all deaths of children under 5 years in developing countries like Bangladesh, India, Brazil, Pakistan, Nigeria and including China, being responsible for 2.5 million deaths per year (Kosek *et al.*, 2003).

Sewage wastewater discharges are worldwide risk factors for the introduction of human protozoan enteropathogens into surface waters. The demand for microbiologically safe reclaimed waters grows exponentially owing to the global demographic rise of the population. Improvements in reclaimed water quality by lowering faecal coliform counts are not a sound solution for human protozoan enteropathogens (Hespanhol, 1997). In urban areas the use of treated wastewater can be considered in irrigation of public parks and recreational centres, sports fields, school gardens, landscaped areas, residential gardens, for commercial uses such as car and glass washing, for decorative purposes such as fountains and waterfalls, for dust control and building projects, to combat fires, in industrial and commercial constructions including bathroom flushing (United States Environmental Protection Agecy, 1992).

The possibility of an outbreak of diseases increases when there is a treatment plant malfunction that enables these parasites to penetrate the treatment processes more easily. Sewage treatment plants have the potential to be a source of contamination to our watershed if the treatment processes employed do not sufficiently treat the effluents before being discharged into nearby waterbody (Lim *et al.* 2007).

The objective of this research was to identify protozoan parasites in the treatment plant. This study was conducted to provide a quantitative basis for risk assessment studies and development of mitigation strategies, such as improving wastewater treatment efficiency.

Materials and Methods

Samples were collected from three points of the Pagla Sewage Treatent Plant (PSTP) which is situated in the eastern part of Dhaka, the capital of Bangladesh. The three sampling points were: Raw Sample from Grit Chamber, Reservoir Sample from Measuring Chamber and Outlet Sample from Outlet Lagoon. A volume of two liters of sample water from each sample site was collected at fortnight intervals during the period of 13 February, 2007 to 3 March, 2008. All the samples were immediately transferred to the laboratory of International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B) for parasitological examination. All the samples were processed by Formol-Ether Concentration Method (Cheesbrough, 2004) following Light Microscopy to observe and identified with the help of Bench aids of WHO (1994), then counted the number of protozoan parasites. However, the method utilized to detect the protozoan parasites does not differentiate between viable and nonviable organisms. Data were analyzed by using Microsoft Excel and SPSS software and the ecological terms considered in this study were according to Margolis *et al.* (1982) and Bush *et al.* (1997).

Results and Discussion

A total of 72 (= 24 samples X 3 sites) samples were collected from PSTP throughout the year. Six protozoan parasites were recognized distinctly by microscopy and these were: *Giardia* spp., *Entamoeba* spp., *Entamoeba coli*, *Endolimax nana, Idoamoeba butschlii* and *Balantidium coli.* Among these waterborne protozoans *Giardia* spp. and *Entamoeba* spp. were found in most of the samples throughout the year. Other parasites were comparatively less frequent; especially *B. coli* were very seldom observed (Table-I). The mean intensity, abundance, dominance and standard deviation (SD) were determined andh has been presented in the Table-II.

Table-I: Prevalence of protozoan parasites in different sampling point of the PSTP

Parasitic Protozoa	Prevalence (%)		
	Grit Chamber	Measuring Chamber	Outlet Lagoon
Giardia spp.	100	75	20.83
Entamoeba spp.	83.33	83.33	8.33
E. coli	41.67	41.67	16.67
E. nana	37.5	37.5	0
I. butschlii	54.17	33.33	4.17
B. coli	8.33	0	4.17

The mean intensity, abundance and dominance were found higher for *Giardia* spp. than all other protozoan parasites detected. *B. coli* was absent in Measuring Chamber and *E. nana* was absent in the Outlet Lagoon (Table-II). The mean intensity and abundance of identified parasites were very high. The dominance was the highest (44%) for *Giardia* spp. all the time and absent for *B. coli* and *E. nana* were absent some times.

Table-II: Mean intensity, abundance and dominance of protozoan parasites in different sampling points.

Sampling Site	Parasitic Protozoa	No. of contaminated samples	Mean intensity±SD (No. of parasite/L)	Abundance±SD (No. of parasite/L)	Dominance
Grit Chamber	*Giardia* spp.	24	$(2.23\pm1.44)X\ 10^5$	$(2.23\pm1.44)X\ 10^5$	44.03%
	Entamoeba spp.	20	$(1.40\pm0.75)X\ 10^5$	$(1.17\pm0.87)X\ 10^5$	23.05%
	E. coli	10	$(1.40\pm0.70)X\ 10^5$	$(5.83\pm8.30)X\ 10^4$	11.52%
	E. nana	9	$(1.39\pm1.11)X\ 10^5$	$(5.21\pm9.50)X\ 10^4$	10.29%
	I. butschlii	13	$(9.23\pm5.34)X\ 10^4$	$(5.00\pm6.08)X\ 10^4$	9.88%
	B. coli	2	$(7.50\pm3.54)X\ 10^4$	$(6.25\pm22.4)X\ 10^3$	1.23%
Measuring Chamber	*Giardia* spp.	18	$(1.14\pm0.78)X\ 10^5$	$(8.54\pm8.40)X\ 10^4$	33.88%
	Entamoeba spp.	20	$(6.25\pm4.98)X\ 10^4$	$(5.21\pm6.34)X\ 10^4$	20.66%
	E. coli	10	$(9.00\pm4.62)X\ 10^4$	$(3.75\pm5.16)X\ 10^4$	14.88%
	E. nana	9	$(6.67\pm2.50)X\ 10^4$	$(2.50\pm3.61)X\ 10^4$	9.92%
	I. butschlii	8	$(1.56\pm1.24)X\ 10^5$	$(5.21\pm10.2)X\ 10^4$	20.66%
	B. coli	0	0	0	0.00%
Outlet Lagoon	*Giardia* spp.	5	$(7.00\pm2.74)X\ 10^4$	$(1.46\pm3.12)X\ 10^4$	38.89%
	Entamoeba spp.	2	$(1.00\pm0.00)X\ 10^5$	$(8.33\pm28.2)X\ 10^3$	22.22%
	E. coli	4	$(6.25\pm2.50)X\ 10^4$	$(1.04\pm2.54)X\ 10^4$	27.78%
	E. nana	0	0	0	0.00%
	I. butschlii	1	$(5.00\pm0.00)X\ 10^4$	$(2.08\pm10.2)X\ 10^3$	5.56%
	B. coli	1	$(5.00\pm0.00)X\ 10^4$	$(2.08\pm10.2)X\ 10^3$	5.56%

Both the prevalence and dominance of protozoan parasites were reduced gradually which means that the treatment plant was effective in reducing protozoan parasites. However, the plant could not eliminate them. The number of protozoan parasites has been demonstrated by the Figure-I, II and III. A number of assessments on the efficiency of wastewater treatment plants by activated sludge have done by using the bacterial population as the indicator organism.
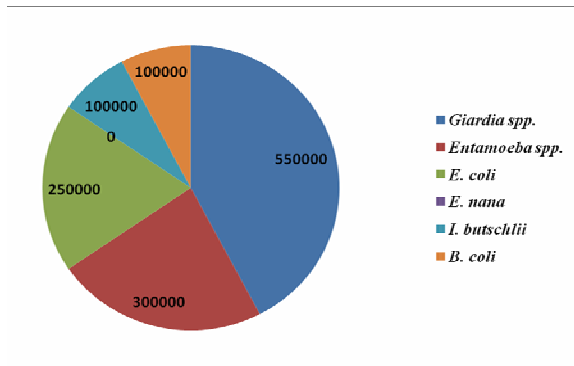
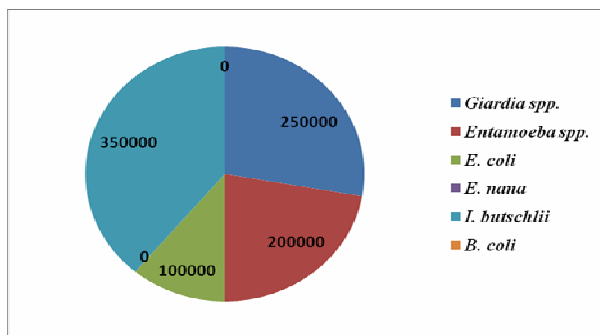Figure-I. The number of protozoan parasites in Grit Chamber (Cysts/Litre) of the PSTP.

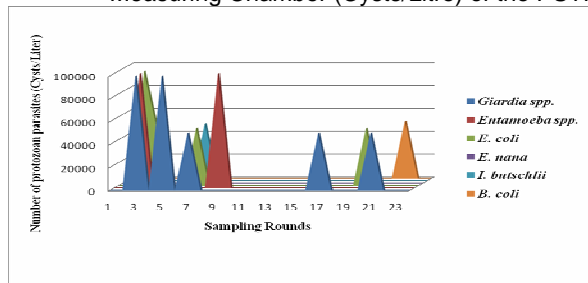Figure-II. The number of protozoan parasites in Measuring Chamber (Cysts/Litre) of the PSTP.

Figure-III. The number of protozoan parasites in Outlet Lagoon (Cysts/Litre) of the PSTP.

Environmental conditions make human beings vulnerable to parasites, and thousands of people in developing countries live under inadequate conditions without proper water supplies and sanitation. Waterborne diseases are common due to the shortage of drinking water, and conditions of storage and manipulation of foods contaminated by human and animal excrement (World Health Organization, 1991). The importance of monitoring and controlling the quality of residual water for reuse in irrigation and aquaculture has been highlighted by the World Health Organization (1989). The contamination of water bodies by discharged treated wastewater may contain emerging pathogens such as *C. parvum* oocysts and *G. lamblia* cysts (Cutolo *et al.,* 2006).

No strong correlation was found for any indicator-pathogen combination. Public health is not adequately protected by simple monitoring schemes based on detection of a single indicator (Harwood *et al.* 2005). The study revealed the occurrence and quantity of various parasitic protozoans in different stages of the treatment of the PSTP. The PSTP covers about 18% of the city population. No previous record concerning sewage treatment plant has been found and hence the present study seems to be very significant to assess the health risk of the Dhaka city. All the identified parasites are endemic in this country where *Giardia* and *Entamoeba* spp. were found most frequently than others. Raw and treated wastewater samples were analyzed for parasites and an array of parasites were detected, namely *Giardia* sp., *E. histolytica*/*dispar*, *E. coli*, *Ascaris* sp., *Enterobius vermicularis*, *Taenia* sp. by Lim *et al.* (2007) in Malaysia.

In the present study the count of parasitic protozoans' cysts varied per litre which is similar to the findings of Lim *et al.* (2007). Other studies conducted in Sweden, Norway and Canada also report the constant detection of *Giardia* in sewage (Ottoson *et al.* 2005). But there was a reduction in the number of cysts *E. nana* which may be due to the treatment of the plant. The results of the present study suggest that the treatment of wastewater promoted a reduction of infectious cysts of parasites but it could not remove all protozoan parasites properly, reflecting a constant risk of infection.

Conclusion

From the study a better understanding of the abundant protozoan parasites and the role of treatment process in removal of these parasites was obtained. As the treated sewage water from the PSTP is directly discharged into Buriganga River which embanks Dhaka city, the present study was designed to assess the Plant's role in meeting the public health criteria. The study discloses the occurrence of a variety of protozoan parasites, which represents a certain range of health risk existing in Dhaka city. The study also provides recommendations to improve the performance of the treatment plants in terms of parasites elimination.

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Biochemical analysis of Five Dried Fish species of Bangladesh

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Abstract: To assess the proximate composition, five dried fish samples of *Mystus vittatus*, *Channa punctatus*, *Chanda nama*, *Corica soborna* and *Trichuirus haumela* were selected. The moisture content ranged from 14.06% to 24.58%, protein varied between 44.08% to 65.65% (moisture basis) and 53.45% to 76.39% (dry matter basis), lipid content of the selected dried fishes ranged from 1.91% to 17.76% (moisture basis) and 2.31% to 21.54% (dry matter basis). Ash content varied from 9.63% to 22.73% (moisture basis) and 11.21% to 28.15% (dry matter basis). The experiment was replicated three times and conducted from February, 2009 to August, 2009. Samples were collected from Sayedpur Upazaila, Nilphamari District, the north-west region of Bangladesh.

Key Words: dried fish, moisture, crude protein, lipid and ash

Introduction

Freshwater fishes play a vital role for animal proteins in the world. Approximately 16 percent of animal proteins consumed by the world's population are derived from fishes, and over one billion people depend on fish as their main source of animal proteins (FAO, 2000). Fishes are easily digestible nature of proteins and are important source of essential minerals. Besides, the dried fishes are also rich in other nutritional components (Nettleton, 1992; Graikoski, 1998; Basu and Gupta, 2004). Laureti (1998) established that dried fishes often are an alternative to fresh fishes in many places. According to DoF (2011) significant amount of dried fishes (approximately 622 mt) were exported and earned 25.06 core taka of foreign currency. But many cases it does not possible to get similar flavor, taste or texture from dried fishes. Saha (2003) reported that in Bangladesh most of the market samples become slightly odourless and some lose the shelf life where rancid and bitter tastes are developed. Nutritional composition also varied in large scale in different dried fish product. Now-a-days consumer wants to know and ensured the nutritional value of the products what they are eating. Although a good number of works on the biochemical composition of fishes in Bangladesh have been done by many researchers viz. Rubbi *et al.* (1987), Mollah *et al.* (1998, 2000), Nurullah *et al.* (2002, 2003), Islam *et al.* (2003), Mazumder *et al.* (2008). But the dried fishes both freshwater and marine species were not focused. So the present investigation was carried out in order to assess the percentage of proximate composition of five fish species through laboratory analysis.

Materials and methods

Five dried fish species eg. tengra (*Mystus vittatus*), taki (*Channa punctatus*), chanda (*Chanda nama*), kachki (*Corica soborna*) and churi (*Trichuirus haumela*) were collected from the local dried fish market of Saidpur upazilla under Nilphamari district district, which is the biggest dry fish market (both wholesale and retail) of the north-west region of Bangladesh. The people of this area prefer dried fishes to even fresh fishes. Collected samples were brought to the Aquaculture Laboratory of the Faculty of Fisheries, Bangladesh Agricultural University, Mymensingh, Bangladesh. The samples were taken to the laboratory, stored in air tight polythene bags at 4°C until biochemical investigations. Proximate composition (percentage of moisture, protein, fat and ash) of the samples was analyzed according to standard Association of Official Analytical Chemists (AOAC, 1980) methods in triplicate. The experiment was conducted during a period from February to August, 2009.

Results and Discussion

Moisture content (%): The highest moisture content was found as 24.58% in *Corica soborna* and the lowest was 14.06% in *T. haumela*. Detailed moisture percentages in other species are shown in Fig. 1. Haque (2004) stated that normally the sun-dried fishes contain an average of 10 to 20% of moisture. Hussain *et al.* (1992) found that the moisture content varied over a wide range from 12.3-54% in *Labeo ghonius*. In the present experiment moisture percentage of the dried fishes were found approximately similar to the referred values. Islam (1982) reported that the moisture content of traditionally dried rui fish was 9.07%. The market samples of sun-dried *Gudusia*

chapra had moisture ranging from 9.61 to 18.64% (Bhattacharyya *et al.*, 1985). Faturoti (1985) showed that the gutted dried and smoked fish samples of African catfish (*Clarias nigrodigitus)* had moisture content as 6.27 to 10.92%. Azam *et al.* (2003) also reported that the range of fourteen selected dried fishes and observed that moisture content ranged from 18.23 to 23.61%.
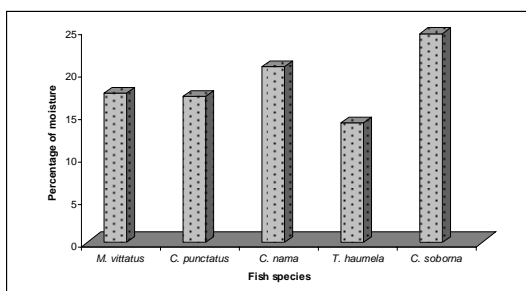

Fig. 1: Moisture content (%) of five dried fish species

Protein content (%): Among the studied five dried fishes, protein content varied between 44.08% (*M. vittatus*) and 65.65% (*T. haumela*) of the moisture basis and 53.45 to 76.39% respectively on dry matter basis. The detailed results (Fig. 2) showed that the value of protein content of all the dried fish species comparable to other parameters of proximate composition. Normally the sun-dried fishes contain 60 to 80% protein (Haque, 2004). Hussain *et al.* (1992) reported that protein content varied widely from 17.2 to 78% in 23 different dried species. Azam *et al.* (2003) found that the protein content varying between 40.69 to 66.52% in fourteen selected dried fish species which were similar to present finding. Traditionally dried rui fish contains 73.26% (Islam, 1982) Faturoti (1985) showed that the gutted dried fish samples of *C. nigrodigitus* had a range of crude protein as 55.02 to 63.05%.
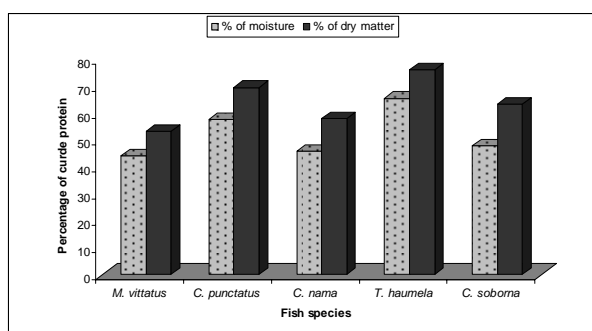

Fig. 2: Crude protein content (%) of five dried fish species

Lipid Content (%): The highest lipid content was found in *M. vittatus* (17.76% based on moisture content and 21.54% on dry matter) and the lowest in

C. punctatus (1.91 and 2.31% based on moisture content and dry matter content respectively) (Fig. 3). Lipid content (%) varied greatly among the dried fish species, which was also reported by worker like Stansby, 1962; Kalamani and Kamasastri, 1998 (3.7-17.8%); Azam *et al.*, 2003 (97.7-26.13%) for other species. Shahiduzzaman *et al.* (2004) reported that the Batashi fish (*Clupisoma atherinoides)* contains 3% lipid. Dried *Rita rita* contains 13.92% lipid (Mollah *et al.*, 1998) and Keshava and Sen (1982) reported that dry fatty fishes contain 7.10% of lipid in average.
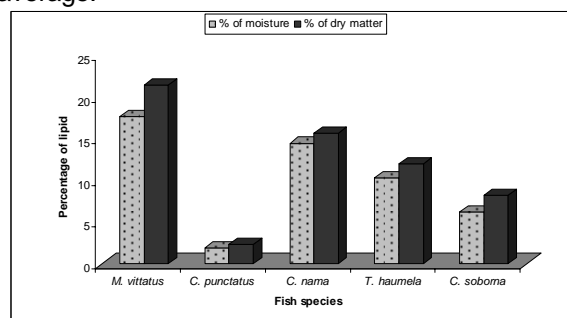

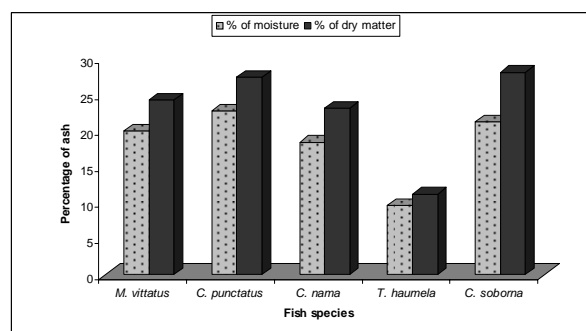**Fig. 3:** Lipid content (%) of five dried fish species

Fig. 4: Ash content (%) of five dried fish species

Ash content (%): The variation of ash content among the studied dried fishes ranged within 9 to 30% on the basis of moisture and dry matter contents. The highest value was found in *C. punctatus* (22.73% against moisture content) and in *C. soborna* (28.15% on dry matter basis), the lowest value was 9.63% on the basis of moisture and 11.21 on the basis of dry matter (Fig. 4). Hussain *et al.* (1992) stated that the ash content varied over a large range 1.4-21.6% in 23 different dried species. Azam *et al.* (2003) found 5.08 to 12.14% of ash content in fourteen dried fishes. Ash content of *Cirrhina reba* was reported to contain 1.7% (Islam *et al.*, 2003).

Conclusion

In tropical country like Bangladesh where relative humidity is always high there is a chance of moisture uptake from the environment by dried fish. In many cases moisture content depends on species variation. It is well known that traditional drying of fishes is often

done in the open field or on the sand. As a result there are chances of contamination with sands and other particles which ultimately increase ash content. However, the results of the present investigation states that the percentage of protein is quite satisfactory in the selected fish species and *M. vattatus* and *C. nama* also contain lipids. From these results, it can be concluded that dried fish, both freshwater and marine can provide satisfactory nutrition to the nation.

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Assessment of the production performance and economic efficiencies of available chicken breeds (*Gallus domesticus* L.) in Rajshahi, Bangladesh

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Abstract: Production performance and economic efficiencies of broiler of Cobb 500, cockerel of ISA Brown, Fayoumi, and RIR (Rhode Island Red) and *Sonali* (derived from RIR♂ × Fayoumi♀) available in Rajshahi were investigated. Identical care and management practices were provided to chickens of all genetic groups reared for meat and egg production. Performance of four meat purpose chickens *viz.*, Cobb 500, ISA Brown, Fayoumi and *Sonali* were evaluated in terms of such important parameters as initial body weight (IBW), 5-wk rearing period (RP), achieved body weight (ABW), feed intake (FI), body weight gain (BWG) and feed conversion ratio (FCR). Performance of three egg purpose chickens *viz.* Fayoumi, RIR and *Sonali* included weight of day-old chick (WDOC), growth rate (GR), death rate (DR), fertility (FR), hatchability (HT), first laying age (FLA) and monthly egg production (MEP). Economic efficiency parameters *viz.*, total cost (TC), gross return (GRR), net return (NR) and cost-benefit ratio (CBR) were calculated for both types. In terms of FI, FCR and BWG values, broiler of Cobb 500 was the best preferred and cockerel of ISA Brown the least preferred chicken. Conversely, in terms of the CBR values for meat producers, the cockerel of ISA White (1.58) was the best and the broiler of Cobb 500 (1.15) the worst. Taking the FLA and MEP into account, RIR topped the list (19.1 wks and 23 eggs per month) whereas Cobb 500 ranked at the bottom (25.2 wks and 16 eggs per month). CBR for egg productivity, on the other hand, was highest in *Sonali* (1.11) followed by RIR and Fayoumi (1.10 each) and Cobb 500 (1.09). As regards the meat productivity, significant correlations existed between TC and NR for all chickens except *Sonali*, which exhibited a negative correlation between the traits. Negative and non-significant associations prevailed for egg productivity in all the chickens. Although broiler of Cobb 500 was found to be the most popular for meat and RIR for egg, the cockerel of ISA Brown was the chicken that earned the maximum CBR.

Key words: Production performance, chicken breeds, meat and egg producers, economic efficiency, cost-benefit ratio

Introduction

Bangladesh is an agro-based developing country in the Southeast Asian region and livestock especially poultry is a promising sector for employment generation and poverty reduction in this country (GOB, 1999). The contribution of poultry to the total animal protein was about 22 to 27 percent in the country (Ahmed & Haque, 1990). About 89% of the rural households that rear livestock were also found to rear poultry (BBS, 1996). Poultry meat and eggs are used chiefly as human food and poultry meat alone contributed 29% of the total meat production in Bangladesh (BBS, 2001). FAO (2003) estimated the status of poultry production in the country to be 140 million chickens and 13 million ducks. Studies on productive and reproductive performance of chickens under intensive management (Islam *et al.*, 2003) and a comparative assessment of hatchability of different strains of broiler parent stock (Mamum, 2005) provide important guidelines for the poultry enterprise in the country.

Islam (2005) described the comparative performance of the broiler parent stocks of Fayoumi and studied their fertility and hatchability, while Rashid *et al.* (2005) studied the reproductive and meat yield parameters of crossbred chickens. Further reports on the production performance of Fayoumi (Khan *et al.*, 2006), commercial broiler (Nahar *et al.*, 2007) and the hybrid

Sonali (Sarkar, 2007) verified that these breeds are suitable for the environment of Bangladesh. Quantitative data on the White Leghorn (WLH), Rhode Island Red (RIR) and indigenous chicken revealed that RIR was the superior breed where the egg was heavier in size and the indigenous breed was famous for its flavour and meat quality (Islam & Nahar, 2008).

The most popular poultry genetic resources of the country include chicken, duck and pigeon (Faruque *et al.*, 2009) and broiler is a specially preferred breed for commercialization in Bangladesh (Islam *et al.*, 2010). From the current situation of small-scale production units, it has become essential to get some clear-cut conception on financial statement of poultry production scenario in the country. So, the present study was designed to evaluate the production performance and economic efficiencies of different genetic group of chickens available in Rajshahi. These findings would be valuable to the policy makers and extension workers in order to guide policies towards increasing efficiency of the poultry enterprise in Bangladesh.

Materials and Methods

Experimental design: Day-old chicks of Cobb 500, ISA Brown and Fayoumi were procured from the hatchery of Index Group, Rajendrapur, Gazipur and were reared at Basit Poultry and Hatchery, Tetulia, Darusa of Rajshahi District. For obtaining day-old *Sonali* chicks, RIR cocks

and Fayoumi hens were reared at the ratio of 1:6, crossed and eggs were incubated for hatching at the Regional Poultry Farm, Rajabarihat, Rajshahi. Stratified random sampling technique was followed to collect data on meat and egg production performance of the chicken breeds under study.

Selection of chicken breeds: Four meat producers (Cobb 500, ISA Brown, Fayoumi and *Sonali*) and three egg producers (Fayoumi, RIR and *Sonali*) were considered for this study. Cobb 500 is the parental broiler stock in Bangladesh which is raised specifically for meat production. It was introduced in the sub-continent from USA during 1930s and became a dominant breed now-a-days. Before the development of commercial poultry breeds, the males were slaughtered for meat and the female were kept for egg production. The standard body weight of cock is 2.3-3.0kg and hen 1.5-2.0kg. The cockerels of ISA Brown are characterized by their white plumages. Average weight of the chicks is 0.55kg. Fayoumi, originated in Egypt and has been present in the West since 1940, is a light-weight fowl which has upright tail and forward breast and neck. Fayoumi cock is around 2kg and hen 1.6kg, which produces about 200 eggs per year. The RIR originated from New England of the USA by hybridization of Leghorn (Malay wild fowl) and Asian local variety. RIR cocks weigh approximately 4kg, hens 3kg, cockerels 3.4kg and pullets 2.5kg. The hybrid *Sonali* is derived from the cross between RIR cock and Fayoumi hen. The average body weight of the cock is 2.5kg and hen is 2kg. This breed is popular for its light weight, body colour and taste resembling that of indigenous chicken.

Selection of study area: A total of six private farms situated in the urban, semi-urban and rural areas of Rajshahi District were selected randomly from six Upazillas *viz.,* Boalia, Godagari, Motihar, Mohonpur, Poba and Rajpara. The average flock size of each farm was 500 chickens. All the five breeds had considerable customer demand either for meat or egg characteristics and are available in the selected farms and local markets. Data were collected during the period of July 2009 and June 2010.

Poultry management system: The poultry management of the selected farms varied according to prevailing weather conditions of the locality and system of poultry rearing. However, poultry keepers practice three general systems (Prasad, 2000) that include (i) free-range system, (ii) semi-intensive system and (iii) intensive systems *viz.* cage or battery system and deep litter system. However, the cage system is the latest invention for the poultry entrepreneurs. In this system, the birds are confined in a cage just large enough to permit very limited movement and allow the bird to stand and sit comfortably. The usual floor space of the cage is 14 by 16 inches and the height is 17 inches. The floor is made with strong galvanized wire and a tray is fixed underneath the floor for collection of droppings. The feeder and waterier remain outside of the cage. Cage system can be used for birds of all age groups and in all climate conditions while, the deep litter system

is widely used for scientific and successful poultry farming in all over the world. Here the poultry birds are kept in large pen up to 250 birds each in a house, whose floor is covered with dry litter up to a depth of 20 to 30 cm. Rice husk, saw dust, dried leaf, chopped straw and ground nutshells, depending upon their availability, can be used as litter materials. Since deep litter resembles dry compost, ammonia gases come out of this litter so it needs to be cleaned regularly.

Parameters studied: Meat production performance of the chickens were investigated in terms of several important production parameters (Jull, 1952) such as initial body weight (IBW in g), 5-wk rearing period (RP in wks), achieved body weight (ABW in g), feed intake (FI in g), body weight gain (BWG in g) and feed conversion ratio (FCR). Egg productivity was evaluated, according to Ketelaere *et al.* (2002), in terms of weight of day-old chick (WDOC in g), growth rate (GR in g), death rate (DR in %), fertility (FR in %), hatchability (HT in %), first laying age (FLA in wks), and monthly egg production (MEP in numbers). The economic efficiencies (all in Tk.) for the rearing of meat and egg purpose chickens were evaluated following Nair & Ghadoliya (2000) and Alabi & Aruna (2005) by estimating total cost (TC), gross return (GRR), net return (NR) and cost-benefit ratio (CBR). Expenses involved for dead birds were also included in TC, whereas NR was calculated on the basis of live weights of the birds ready for selling.

Statistical analyses: For computing analysis of variance (ANOVA), a completely randomized design involving 5 chicken breeds, each with three replications was used. Treatment means for each parameter were compared using the least significant difference (LSD) tests. Moreover, co-efficient of correlation (r) values between the economic efficiency parameters excepting CBR were also calculated for both meat and egg productivity. SPSS version 11.0 for Windows was used for the statistical analyses.

Results and Discussion

Meat productivity: Results clearly demonstrate that broiler of Cobb 500 attained the highest BWG as well as FCR (Table 1, Fig. 1) compared to the rest of the breeds whereas Fayoumi showed the lowest value for BWG and ISA Brown for FCR. However, a similar trend was reflected in terms of FI where Cobb 500 showed the highest and ISA Brown the lowest. The gain in body weight was statistically significant between the breeds ($F_{3,\ 11}$=7.96; P<0.001), although insignificant difference in BWG existed between *Sonali* and ISA Brown.

The present findings are logical as rearing temperatures and genotypes influenced the productive performance of broilers chickens (Aengwanich, 2007). The supplementary feed like blood meal not only increased the growth performance of broiler chicks but also enhanced the growth and feed conversion efficiencies significantly (Shahidullah *et al.*, 2008; Habib *et al.*, 2009).

Table 1. Production performance parameters of the chicken breeds in Rajshahi reared for meat.

Breeds	IBW	ABW	FI	BWG
Cobb 500	41.5±0.85[a]	1390.4±5.24[a]	2347.7±8.86[b]	1348.9±4.86[a]
ISA Brown	32.2±1.47[c]	417.9±3.78[c]	1177.9±15.04[c]	385.7±5.24[c]
Fayoumi	32.1±1.55[c]	359.5±7.44[d]	1405.7±10.06[b]	327.4±6.78[d]
Sonali	33.3±2.36[c]	407.5±23.17[c]	1395.3±8.56[b]	374.2±24.86[c]

Rearing period of 5 wks for all breeds; values are mean±SD of three replicates; IBW= initial body weight (g); ABW= achieved body weight (g); FI= feed intake (g); BWG= body weight gain (g); different superscripts for a parameter in the same column differ significantly by LSD (P<0.05).
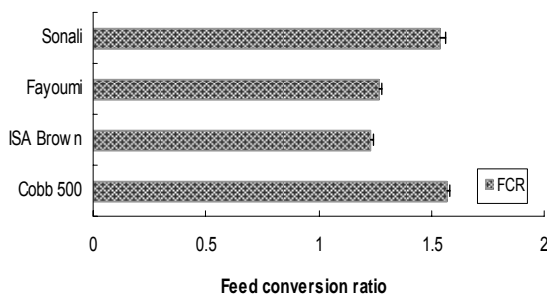

Fig. 1 Feed conversion ratio (FCR) for different chicken breeds reared for meat production in Rajshahi.

Moreover, Paul (2010) observed that multivitamin and enzyme supplementation significantly increased body weight in broiler chickens and the deficiency of vitamin C was related to meat yield reduction of broilers in a hot humid environment (Ali *et al.*, 2010). Under intensive management system, on the other hand, chicken genotypes showed distinct physical variations for both qualitative and quantitative traits (Faruque *et al.*, 2010). Growth parameters were also significantly affected by genotypes where male breeds exhibited higher average body weights than the female breeds and on average, hybrids consumed more feed compared to the purebreds (Ilori *et al.*, 2010). The present variations in meat productivity parameters were likely due to the differences in genetic make-up and feeding and management practices of the chicken breeds under study.

Egg productivity: In terms of GR, RIR showed the highest value whereas *Sonali* exhibited the highest DR and HT values (Table 2). With respect to FLA, a highly significant difference existed between the chicken breeds ($F_{3, 11}$ = 14.74; P<0.001) but *Sonali* and Fayoumi showed almost similar FLA characteristics. Data on overall egg productivity parameters revealed that RIR had the shortest FLA but the highest MEP while Fayoumi attained the highest FLA and *Sonali* the lowest MEP (Table 2, Fig 2). These inconsistencies in egg productivity might be due to genetic make-up and housing system (Aernia *et al.*, 2005) as they also affect laying performance in poultry breeds (Sonaiya, 2009; Wang *et al.*, 2009; Banga *et al.*, 2010). Stocking density has been shown to influence egg production and performance of broiler breeder hens as higher density produced fewer eggs (Mtileni *et al.*, 2007). In addition, egg storage periods may affect fertility and hatchability in poultry chicken species (Babiker & Musharaf, 2008; Caglayan *et al.*, 2009), and feed restrictions (Sarica *et al.*, 2009) season, flock age and disease-vaccination conditions (Turkyilmaz *et al.*, 2010; Holt *et al.*, 2011) were also found to interact growing and laying performance in chickens. Recent studies have demonstrated that although maternal dietary protein level had no effect on hatchability and growth rate (Kingori *et al.*, 2010), but age (Bekele *et al.*, 2010) and phenotype (Haunshi *et al.*, 2011) affected production performances of laying hens. These crucial parameters need to be taken into account for commercial poultry egg productivity in the country.

Table 2. Production performance parameters of the chicken breeds in Rajshahi reared for eggs.

Breeds	WDOC	GR	DR	FR	HT	FLA
Fayoumi	32.6±1.06[c]	11.7±0.04[c]	1.68±0.20[d]	91.2±0.64[a]	82.9±0.40[b]	22.4±0.20[b]
RIR	33.8±1.34[b]	13.8±0.10[b]	2.82±0.15[c]	89.5±2.65[b]	83.1±1.66[b]	19.1±0.33[c]
Sonali	33.8±1.86[b]	13.7±0.10[b]	3.82±0.15[a]	91.2±0.30[a]	83.3±1.41[b]	21.1±0.61[b]

Rearing period of 72 wks for all breeds; values are mean±SD of three replicates; WDOC= weight of day-old chick (g); GR= growth rate (%); DR= death rate (%); FR= fertility (%); HT= hatchability (%); FLA= first laying age (wk); different superscripts for a parameter in the same column differ significantly by LSD (P<0.05).
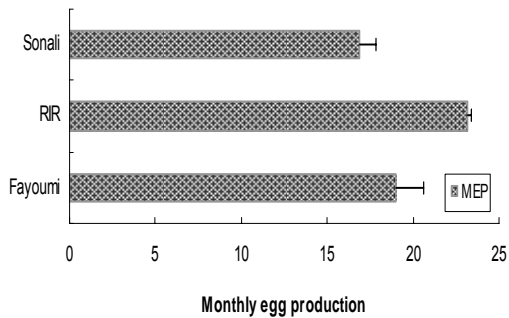
Fig. 2 Monthly egg production (MEP) of three chicken breeds reared for egg production in Rajshahi.

Economic efficiencies for meat producers: Table 3 shows the economics of meat productivity by different chicken breeds in the study area. For about a 5-wk meat purpose rearing, results specify that TC was the highest in Cobb 500 followed by Fayoumi, ISA Brown and *Sonali* whereas NR was found to be the highest in ISA Brown followed by *Sonali*, Fayoumi and Cobb 500. As a result, ISA Brown gained the highest CBR and Cobb 500 the lowest. As regards the meat productivity, therefore, significant correlations existed between TC and NR for all chickens except *Sonali*, which exhibited a negative correlation between the traits.

Table 3. Economic efficiency of chicken breeds per bird reared for meat production and their correlation values

Breeds	TC	GRR	NR	CBR	Co-efficient of correlation values (r)		
					TC *vs.* GRR	TC *vs.* NR	GRR *vs.* NR
Cobb 500	83.4±1.67[a]	97.3±4.32[b]	13.9±2.81[d]	1.17[e]	0.939*	0.850ns	0.979*
ISA Brown	49.2±3.35[b]	77.9±2.66[a]	28.8±0.85[a]	1.58[a]	0.985**	-0.851ns	-0.751ns
Fayoumi	45.8±1.85[c]	61.0±1.38[b]	15.2±3.22[b]	1.33[c]	-0.987ns	-0.997ns	0.995***
RIR	48.6±0.52[b]	63.0±2.30[b]	14.4±2.32[c]	1.29[d]	0.068ns	-0.155ns	0.974*
Sonali	41.9±2.64[c]	64.2±3.99[c]	22.3±6.63[b]	1.54[b]	-0.997ns	-0.998ns	0.999***

Values of the cost items are mean±SD of three replicates; cost items included the cost (in Tk.) of chick, feed, litter, vitamins, vaccine, labour, electricity and miscellaneous expenses; TC= total cost; GRR= gross return; NR= net return; CBR= cost-benefit ratio; different superscripts for a parameter in the same column differ significantly by LSD (P<0.05); ns= not significant; *= P<0.05; **= P<0.01 and ***= P<0.001.

Economic efficiencies for egg producers: For a 72-wk egg purpose rearing, TC was the highest in RIR the lowest in *Sonali*, consequently, NR was the highest in RIR and the lowest Fayoumi (Table 4). However, it was interesting to note that CBR became the highest in *Sonali* and identical in RIR and Fayoumi. In addition, negative and non-significant associations prevailed for egg productivity in all the chickens, suggesting that egg purpose rearing of chicken breeds was not profitable in the study area. Although broiler of Cobb 500 was found to be the most popular for meat and RIR for egg, the cockerel of ISA Brown was the chicken that earned the maximum CBR.

Table 4. Economic efficiency of chicken breeds per bird reared for egg production and their correlation values

Breeds	TC	GRR	NR	CBR	Co-efficient of correlation values (r)		
					TC *vs.* GRR	TC *vs.* NR	GRR *vs.* NR
Fayoumi	1062.4±42.76[a]	1166.1±16.92[b]	103.7±40.14[a]	1.10[b]	0.348ns	-0.919ns	0.051ns
RIR	1122.4±14.2[c]	1243.3±35.44[a]	120.9±48.66[a]	1.10[b]	-0.904ns	-0.950ns	0.992***
Sonali	1060.9±32.16[a]	1175.4±36.32[b]	114.5±43.96[a]	1.11[a]	0.180ns	-0.583ns	0.694ns

Values of the cost items are mean±SD of three replicates; cost items included the cost (in Tk.) of chick, feed, litter, vitamins, vaccine, labour, electricity and miscellaneous expenses; TC= total cost; GRR= gross return; NR= net return; CBR= cost-benefit ratio; different superscripts for a parameter in the same column differ significantly by LSD (P<0.05); ns= not significant; *= P<0.05; **= P<0.01 and ***= P<0.001.

In agreement with the observations of Gordon *et al.* (2009), it became evident from the present results that profits associated with lighter hens like ISA Brown and *Sonali* were higher than those associated with heavy hens such as RIR and Cobb 500 and the increased profitability of lighter hens was largely due to their improved feed conversion, because lighter hens produced predominantly larger or extra large eggs. Broiler farming with Cobb 500 was reported profitable because of lower investment, less space requirement, utilization of family

labour and quick returns as examined by Islam *et al.* (2010). This contradicts with the present findings because cockerel of ISA Brown was found to be earning the maximum CBR. Substantial technical, allocative and economic inefficiency for egg purpose rearing of the poultry breeds might be the cause of loosing concern and/or marginal profits compared to that for meat purpose rearing which lend support to the findings of Begum *et al.* (2010). The present results therefore emphasize the need for adequate knowledge on poultry productivity and profitability to the government policy makers, producers and marketers, thus suggesting for an integrated approach to genetically improved chicken breeds and strict bio-security poultry farming in the country.

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Selection strategies considering varietal differences with respect to egg characters of mulberry silkworm, *Bombyx mori* L.

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Abstract: The genotypic variability and correlation coefficients were studied in 27 indigenous multivoltine varieties of mulberry silkworm, *B. mori* L. for six egg characters. The genotype was highly significant for all the characters under studied. Least difference between genotypic and phenotypic coefficient of variation were observed. Majority of the characters showed high heritability comparing with dead egg percentage. Furthermore Unfertilized egg percentage showed high genetic advance together with high heritability but HP and EW showed high heritability and low genetic advance. These indicate the importance of additive and non-additive gene effect of these characters respectively. Total number of egg laid by female, hatching percentage, and blue egg percentage showed both positive and negative significant correlation to each other at both the phenotypic and genotypic levels. The results suggest that these characters specially total number of egg laid by female, blue egg percentage and unfertilized egg percentage appeared as importance in selection programme for genetic gain in *B. mori.*

Key words: genetic variability, correlation, *B. mori.*

Introduction

Silkworms are well known industrial insects which produce natural silk. More than 3000 silkworm strains are available all over the world due to various ongoing breeding programmes (Rao *et. al.,* 2006; Thangavelu *et. al.,* 2003; Nagaraju, 2002). These are univoltine, bivoltine and multivoltine. In tropical countries like Bangladesh, multivoltine silkworm strains play important role in the production of silk. There are about fifty indigenous multivoltine varieties maintained at the Germplasm Bank of Bangladesh Sericulture Research and Training Institute (BSRTI), Rajshahi. These varieties being highly resistant to diseases were very much popular among the farmers of Bangladesh. But the qualitative and quantitative traits of these varieties including silk yield and quality of silk fibre are very poor. To overcome these difficulties and to boost up silk production in the country, high yielding developed varieties of silkworm were introduced in Bangladesh for commercial rearing.

Genetic variability is a prerequisite for an effective selection of any economically important plant and animal species and a critical survey of genetic variability is essential aiming at developing high yielding varieties (Akanda *et. al.,* 1998). In silkworm breeding numerous traits are considered as important for improving them to increase the profit of silk producers and other sections of sericulture industry. Reproductive traits are considered important by egg producers. These traits jointly or singly influence the egg production (Reza & Rahman, 2005; Zhao *et al.,* 2007; Ahsan & Rahman, 2008). A great diversity of polyvoltine silkworm *Bombyx mori* L. must exist globally, by considering the fact that a large number of silkworm breeds are already evolved by selection or cross breeding and also some of the tropical countries allowed individual farmers to produce silkworm eggs (Kumaresan *et al.,* 2007). The selection of best genotypes depends on a of characters (Ahsan and Rahman*,* 2010). Therefore, a clear understanding and knowledge of association and contribution of various reproductive components is essential for any selection programme aimed at egg production.

Hence, the present study was undertaken to estimate the genetic variation for six important egg characters and correlations among them at phenotypic and genotypic levels for designing suitable breeding programmes.

Materials and methods

The materials for this experiment comprised of twenty seven indigenous multivoltine varieties of mulberry silkworm, *B. mori.* These parents were obtained from the Germplasm Bank maintained at the Bangladesh Sericulture Research and Training Institute (BSRTI), Rajshahi. The names of the varieties are:

1. Nistari	2. Nistri (M)	3. Nistri (P)	4. Nistri (KP)M
5. Nistri (ISK)	6. Nistri (O)	7. Nistri (L)	8. Nistri (IL)
9. Nistri (K)P	10. Nistri (DC)	11. BSR-3(M)	12. BSR-3(P)
13. BSR-10(M)	14. BSRI-801	15. BSRI-802	16. BSRI-83/1
17. BSRI-83/2	18. BSRI-83/3	19. BSRI-85/1	20. BSRI-85/2
21. BSRI-85/3	22. Urboshi-1	23. Urboshi-2	24. Urboshi-3
25. Urboshi-4	26. M_2P_2	27. O_5	

The eggs of F_1 hybrids were brushed (3dfls for each genotype) in a randomized design with three replications each. The rearing was conducted in the rearing house No. 2 of the BSRTI, Rajshahi. Scientific technology of silkworm rearing was followed according to Krishnaswami (1978) and Rahman (1983). Data recorded for this study

were: total number of eggs laid per female (TEL), hatching percentage (HP), blue egg percentage (BEP), unfertilized egg percentage (UEP), dead egg percentage (DEP) and egg weight (EW) . The collected data were analyzed successively for estimating genetic variability, heritability and genetic advance according to the formula described by Burton & De Vane (1953), Burton (1952), Hanson *et. al.* (1956), Lush (1949) and Johnson *et al.* (1955). The phenotypic and genotypic correlations were calculated according to the formula as follows:

$$r\ (x_1 x_2) = \frac{COV(x_1 x_2)}{\sqrt{V(x_1)V(x_2)}}$$

Results and discussion

Overall range, mean with standard error, mean square and component of variations for different characters are presented in Table 2. The mean square in the table showed that variations among genotypes were highly significant (P<0.001) for all the characters studied indicating that the varieties possess a wide range of genetic diversity and these could be suitable for breeding purposes. Ahsan *et al.* (2000) reported that varietal differences with respect to egg, larval and cocoon characters were found in *B. mori*. Similar results on varietal diversity have also been substantiated by the findings of Reza & Rahman (1996) and Ahsan & Rahman (2008). Phenotypic variances ($\sigma^2 p$) were generally greater than their corresponding genotypic ($\sigma^2 g$) or environmental variances ($\sigma^2 e$) in all the cases. All the characters showed least difference between genotypic ($\sigma^2 g$) and phenotypic coefficient ($\sigma^2 p$) of variation suggesting less influence of environment on these characters but they are under strong genotypic control (Table 1).

Table 1. Range, mean with SE, mean square and components of variance of different characters of hybrids of silkworm, *B. mori* L.

Characters	Range	Mean	SE	MS	P	G	E
TEL	300.67-519.33	418.77	3.41	11279.72***	3772.03	3753.85	18.18
HP	71.68-83.98	77.96	0.73	30.51***	10.71	9.90	0.81
BEP	5.62-14.85	9.56	0.42	16.85***	5.79	5.53	0.27
UEP	0-13.86	5.69	0.61	36.68***	12.60	12.04	0.56
DEP	0-6.43	3.05	1.61	7.13***	2.96	2.09	0.88
EW	0.31-0.79	0.439	0.003	0.016***	0.005	0.005	.000015

*** Significant at 0.1% level, P = Phenotypic variance, G = Genotypic variance, E = Environmental variance.

The genetic parameters, phenotypic (CV_p), genotypic (CV_g) and environmental (CV_e) co-efficient of variability, heritability (H), genetic advance (GA) and genetic advance as percentage of mean (GA%) were estimated and presented in Table 2. The highest phenotypic and genotypic co-efficient of variation was observed in UEP and the lowest in HP whereas the highest environmental co-efficient of variation was recorded in DEP and the lowest in EW. The inconsistent results of environmental co-efficient of variation for different characters indicated that the genotypic-environment interactions had a great influence on these characters. The significance of genotypic environment interaction in *B. mori* has also been recognized. In the present experiment the highest heritability was obtained for EW followed by TEL, UEP, BEP, HP and the lowest for DEP. Unfertilized egg percentage (UEP), BEP and EW showed very high heritability together with high phenotypic (CV_p) and genotypic (CV_g) coefficient of variability. Ahsan *et al.*, (2010) reported high heritability together with high phenotypic and genotypic coefficient of variability of filament length and estimated cocoon yield per 100 dfls. The results of Ahsan & Rahman (2008) is similar with the present findings who reported a high heritability coupled with high phenotypic and genotypic coefficient of variability for total eggs laid by female.

The estimates of genetic advance expressed as percentage of mean showed a wide range from 7.99 for HP to 122.85 for UEP. Unfertilized egg percentage (UEP) expressed the highest genetic advance together with high heritability. It indicated the importance of additive gene effects of these characters (Rahman, 1984; Reza *et. al.,* 1993, Hasan *et al.,* 2011). It also indicated a wide rage of genetic diversity which could be used in a breeding programme and phenotypic selections of these characters would be effective. Rao (1997) reported that the characters such as, single shell weight (in bivoltine) and single cocoon weight, single shell weight and filament length (in

multivoltine) showed high heritability with high genetic advance.

High heritability does not always give high genetic advance as was indicated by Johnson *et al.,* (1955). High heritability but relatively low genetic advance was observed for the characters such as HP and EW. It suggested limited scope for

manipulation of these characters. These could be due to non-additive gene action which includes dominance and epitasis (Ahsan & Rahman, 2008). In such situations, progeny testing and recurrent selection might be helpful to improve these traits (Rahman, 1984; Rao, 1997; Ahsan & Rahman, 2010; Hasan *et al.,* 2011).

Table 2. Phenotypic (CVp), genotypic (CVg), and environmental (CVe) coefficient of variation, heritability (h^2), genetic advance (GA) and genetic advance as percentage of mean (GA%) for different characters of hybrids of silkworm, *B. mori* L.

Characters	CVp	CVg	CVe	h^2	GA	GA%
TEL	14.67	14.63	1.02	99.52	125.91	30.07
HP	4.20	4.04	1.15	92.46	6.23	7.99
BEP	25.18	24.59	5.42	95.37	4.73	49.48
UEP	62.39	60.98	13.19	95.53	6.99	122.85
DEP	56.41	47.40	30.58	70.61	2.50	81.96
EW	16.39	16.38	0.87	99.81	0.15	34.17

The phenotypic (r_p) and genotypic (r_g) correlations between all pairs of characters studied in this investigation analysed have been shown in Table 3. In the present study TEL, HP, DEP and EW showed positive correlations to each others. Of these correlations TEL, HP and HP, EW pairs associations were significant at phenotypic and genotypic levels. Significant positive correlation of BEP was found with UEP at both the levels. But the characters TEL, HP, DEP and EW showed negative correlations with BEP and UEP. Majority of these correlations were significant except BEP, DEP and UEP, EW pair associations at both the levels and UEP, DEP only at genotypic level. Ozdzenska & Kremky (1987) reported both positive and negative correlations between different component characters. They reported high positive correlations between survival rate and cocoon yield, and a negative correlation between hatchability and number of eggs per

gram in *B. mori.* Singh *et. al.* (1994) reported the same results between shell weight and fecundity. Chatterjee & Pradeep (2003) investigated the relationship between yield potential and molecular markers in silkworm. Similar results on different characters of silkworm were also reported by Mistri and Jayaswal (1992), Singh *et al.* (1994), Ahsan & Rahman (2008), and Ahsan & Rahman (2010).

In general, genotypic correlation coefficients (r_g) were greater in magnitude compared to those of phenotypic correlations (r_p). These low phenotypic correlations could be due to a modifying effect of environments of the association of characters at genotypic level (Rahman, 1984). Similar results were also reported by Siddiqui *et al.,* (1992) in *Antheraea mylitta* and Ahsan & Rahman (2008 & 2010) in *B. mori.*

Table 3. Phenotypic (r_p) and genotypic (r_g) correlation coefficients between all pairs of characters of hybrids of silkworm, *B. mori* L.

Variables		TEL	HP	BEP	UEP	DEP
HP	r_p	0.7423***				
	r_g	0.7785***				
BEP	r_p	-0.4851**	-0.8568***			
	r_g	-0.4993**	-0.8572***			
UEP	r_p	-0.7497***	-0.7601***	0.3971*		
	r_g	-0.7732***	-0.7698***	0.3924*		
DEP	r_p	0.1739	0.0057	-0.0835	-.2274	
	r_g	0.1982	0.134	-0.184	-0.3375*	
EW	r_p	0.0752	0.368*	-0.4777**	-0.1408	0.0356
	r_g	0.0754	0.3853*	-0.4916**	-0.1457	0.0468

Genetic parameters and characters association of the present study revealed that these characters had the inherent association to each other specially the characters, TEL, HP, BEP and UEP,

and thus implying prime importance to include them in selection programmes for genetic gain in *B. mori.*

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Efficacy of two inducing agents PG and DoM+SGnRH in the induced breeding of the major carp, kalibaus (*Labeo calbasu*)

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Abstract: An experiment on the induced breeding of the endangered fish, *Labeo calbasu* (Hamilton-Buchanam) was conducted in the Fish Seed Multiplication Farm,Rajshahi to know the efficacy of two inducing agents (PG and DoM+SGnRH). Three breeding trials of each inducing agent were performed. A total of 24 females weighing from 1.5 kg to 2 kg were given an initial and a resolving dose of 1.5 mg and 6 mg PG extract per kg body weight respectively as treatment-1. On the other hand, a total of 24 females weighing from 1.5 kg to 2 kg were given a single dose of 12 mg DoM + SGnRH/kg as treatment-2. In case of treatment-1, 12 males weighing from 1.5 kg to 1.95 kg were administered a single dose of 1.5 mg PG/kg body weight during resolving dose of female. In treatment-2, 12 males weighing from 1.5 kg to 1.8 kg were administered 3 mg DoM+SGnRH /kg body weight during initial dose of females. In treatment-1, the time interval between initial and resolving dose was 5 hours and ovulation occurred in all the injected females within 6 hours after resolving dose. Ovulation occurred within 6 to 8 hours after the injection of inducing agents for treatment-2. The mean rates of ovulation, fertilization and hatching were 100%, 77.36% and 74.5% respectively in treatment-1. On the contrary, the mean rates of ovulation, fertilization and hatching were 83.33%, 63.83% and 59.66% in treatment-2. Hatchery produced fry were reared in nursery pond for 40 days. In nursery pond. Flour, oil cake and wheat bran were applied as nursery feeds. Both the inducing agents were effective in respect of overall breeding performance. But the best results were obtained with PG although in case of DoM+SGnRH complete breeding takes place within short time with less labour and cost than that of PG.

Key words: *Labeo calbasu*,pituitary extract,ovaprim, induced breeding.

Introduction

In Bangladesh culture of carps is a very old practice. In China, carp culture is being practised since long time ago. The carp culture improves the social and economic status of farmers by adopting new scientific technology for breeding (Nandeesha and Rao, 1989).

The major problem in carp culture is the non-availability of quality fish seed.In early years fish seeds were collected from rivers by cloth happas. But this technique was unsafe as with the collection of carp seeds, some seeds of predatory fishes would have also been collected .Chaudhary and Alihunhi (1957) for the first time successfully carried out the spawning of major carps with induced breeding by pituitary extracts. According to Ahmed (1945), Alikunhi *et al.* (1960), Chaudhnri, 1960), Ali (1967), Sinha (1971) and Haque (1975), Alam *et. al.* (1999), Bhuiyan *et. al.* (2008), the PG influences the spawning of carp fishes. Such findings were observed by Lin (1965) and Tang *et al.* 1963) in Chinese carps.

In Bangladesh, the successful induced spawning was first done by Ali (1967) in carps through hypophysation. Other workers who worked on the induced breeding of carps include Haque, 1975; Islam & Chawdhury, 1976; Alam, 1983 and Ahmed, 1983. DoM+SGnRH was used, as a substitute for pituitary gland but it could not get the success as it was thought. The present investigation was conducted to know the comparative efficacy of the pituitary gland (PG) and Domperidome (Dom) + gonadoreleasing hprmone (SGnRH) on the carp, *Labeo calbasu*

Material and Methods

The present study was conducted with a view to knowing the comparative efficacy of the breeding performance of *Labeo calbasu* with two inducing agents PG hormone and DoM+SGnRH. Nursery management of this species was also conducted.

Brood stock rearing was conducted in the ponds of Fish Seed Multiplication Farm,Rajshahi.

For induced breeding of *Labeo calbasu* brood fishes were collected from the brood fishes rearing pond of the hatchery complex at 1:1 ratio. In this experiment three breeding trials with PG hormone and three breeding trials with DoM+SGnRH were performed, respectively as Treatment 1 and Treatment 2 to find out the comparative outcomes of these two inducing agents. Each trial was conducted by using 8 pairs of brood fishes. The dehydrated carp pituitary glands (PG) and DoM+SGnRH were collected from the local market in preserved condition in air tight vials used as an inducing agents. An electronic balance (College HP-TC 11, China) was used to weigh the required amount of PG and DoM+SGnRH by using the following formula-

Weight of inducing agent (mg) = $\dfrac{W_t\text{-}P_t}{1000}$

Where,

W_t represents total body weight (g) of all the fishes to be injected and

P_t represents the rate in mg inducing agents (PG and DoM+SGnRH) to be injected per kg body weight under a particular treatment.

The weighed PG was transferred to a tissue homogenizer and thoroughly crushed. The crushed PG was then diluted by distilled water and was centrifuged by a centrifuge machine for precipitation. The following ratio of inducing agents and water were maintained in order to prepare extracts. In treatment 1 the ratio of PG and water was 1 mg: 2.5 ml.In case of treatment 2 the ratio of DoM+SGnRH and water was 1mg:2ml

In case of treatment-1, the females were injected with 1.5mg PG/kg body weight as initial dose and 6 mg PG/kg body weight as resolving dose. On the other hand, males were administered at the rate of 1.5 mg PG/kg body weight. On the contrary in case of treatment-2, the females and males were injected DoM+SGnRH at the rate of 12 mg/kg body weight and 3 mg/kg body weight respectively. For determination of fertilization and hatching rates approximately 100 eggs were placed in plastic bowls of

1.25 liter capacity with three replications each having water flow from porous PVC pipe and outlet facility. At first the number of fertilized and unfertilized eggs of each bowl was counted with naked eyes. After approximately 18-30 h of fertilization, it was observed that hatching was almost complete and the number of hatchlings in each bowl was counted.

The following breeding parameters were recorded:

$$\text{Ovulation rate (\%)} = \frac{\text{No. of fish ovulated}}{\text{Total no. of female fish injected}} \times 100$$

Fertilization rate (%)

$$= \frac{\text{No. of fertilized eggs}}{\text{Total no. of eggs (fertilized+unfertilized)}} \times 100$$

$$\text{Hatching rate (\%)} = \frac{\text{No. of eggs hatched}}{\text{Total no. of fertilized eggs}} \times 100$$

Table-1. Treatment-1 with PG for male and female brood fishes of *Labeo calbasu* in three breeding trials.

Trials	Pairs of brood fishes	Weight of brood fishes (kg)	Initial dose of PG (mg/kg body weight of fishes)	Time interval (hours)	Resolving dose of PG (mg/kg body weight of fishes)	Ovulation (hours)
Trial-1	8	Female=1.7±0.2 Male=1.65±0.1	Female=1.5	5.30	Female=6 Male=1.5	6.30
Trial-2	8	Female=1.8±0.1 Male=1.6±0.1	Female=1.5	5.15	Female=6 Male=1.5	6.15
Trial-3	8	Female=1.8±0.2 Male=1.75±0.2	Female=1.5	5	Female=6 Male=1.5	6

Table-2: Treatment-2 with DoM+SGnRH for male and female brood fishes of *Labeo calbasu* in three breeding trials

Trials	Pairs of brood fishes	Weight of brood fishes (kg)	Dose of DoM+SGnRH (mg/kg body weight of fishes)	Ovulation (hours)
Trial-1	8	Female=1.7±0.2 Male=1.6±0.1	Female= 12 Male=3	8
Trial-2	8	Female=1.8±0.1 Male=1.65±0.2	Female= 12 Male=3	6
Trial-3	8	Female=1.8±0.15 Male=1.6±0.1	Female= 12 Male=3	7

After administration of the inducing agents, the brood fishes were again released in the rectangular tank.

Collection of fertilized eggs and transferring to hatching tank
The fishes were removed from the rectangular tank when the ovulation was completed. Stripping was not required because the fertilization occurred in the circular tank. The fertilized eggs were collected from the outlet of circular tank with a net where the eggs came with water flow. The fertilized eggs were transferred into mini circular hatching tank with sufficient care. The mini circular tank was previously filled with filtered pond water to minimize the temperature difference and environmental shock. Continuous flow of water was maintained for aeration.

Ovulation rate
The average ovulation rate of the three breeding trials in case of treatment-1 and treatment-2 are shown respectively in Table-3 and Table-4. In Treatment-1, mean ovulation rate was (100%) in all the three trials. On the contrary, in

Results
After preparation of inducing agent extracts, it was injected to brood fishes. The fishes were caught carefully by scoop net and kept in sponge. Inducing agents was then injected near the pectoral fin base. The amount of PG and DoM + SGnRH solution for each fish was determined before according to the body weight of the brood fishes.

Doses of inducing agents for male and female broods
In this experiment, similar doses of PG extract were applied in different breeding trials as treatment-1 and similar doses of DoM+SGnRH were applied in different breeding trials as treatment-2. The doses of both solution (PG and DoM+SGnRH) are demonstrated in the following tables:

treatment-2, the mean ovulation rate was (83.34%) recorded in the three trials. The highest ovulation rate (100%) was found in all the trials of treatment-1. The lowest ovulation rate (75%) was found in trial-I for Treatment-2. The graphical presentation of ovulation rate for both treatments are shown in Fig.1.
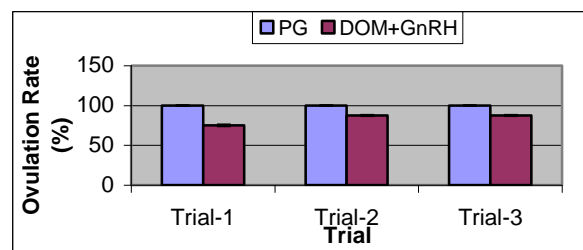

Fig-1. Ovulation rate (%) of *Labeo calbasu* in different breeding trials

Fertilization rate

In case of treatment-1 and Treatment-2 the average fertilization rate of three trials are shown respectively in Table 3 and Table 4. In treatment-1 the mean fertilization rate was 77.34% recorded in the three trials. On the other hand, in case of treatment-2 the mean rate of fertilization was 63.83 recorded in the three trials. The highest fertilization rate was 80% recorded in trials 3 followed by trial-1 and trial-2 (75% and 77%, respectively) for treatment-1. The lowest fertilization rate was 62.5% recorded in trial-2 for treatment-2. Graphical presentation of fertilization rate for both treatments are shown in Fig-2.
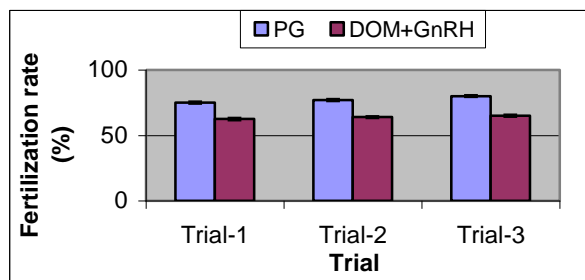
Fig.2. Fertilization rate (%) of *Labeo calbasu* in different breeding trials

Hatching rate

During the experimentation of *Labeo calbasu* the average hatching rate of three breeding trials for both treatments are shown in the Table 3 and 4. The mean rate of fertilization in treatment-1 was recorded 74.5% in three trials and the mean rate of fertilization in treatment-2 was recorded 59.66%. The highest hatching rate (76%) was found in Trial-3 of treatment-1. The lowest hatching rate was 58% recorded in trial-1 for treatment-2. Graphical presentation of hatching rate for both treatments are shown in Fig-3.
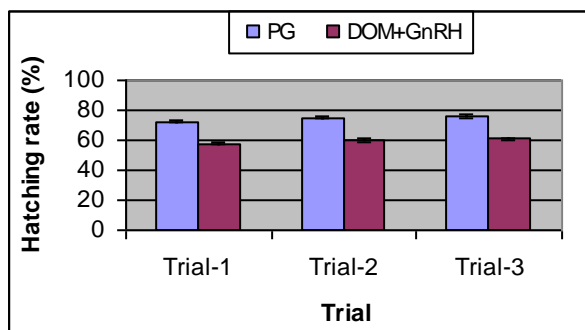

Fig-3. Hatching rate (%) *Labeo calbasu* in different breeding trials

Table-3: Breeding performance of kalibaus (*Labeo calbasu*) with different doses of PG hormone in different breeding trials.

Trials	Ovulation rate (%)	Fertilization rate (%)	Hatching rate (%)
Trial-1	100 ± 0.0	75 ± 0.73	72.5 ± 0.56
Trial-2	100 ± 0.0	77 ± 0.73	75 ± 0.73
Trial-3	100 ± 0.0	77.36 ± 0.59	74.50 ± 0.73

Table-4: Breeding performance of kalibaus (*Labeo calbasu*) with different doses of DoM+SGnRH in different breeding trials.

Trials	Ovulation rate (%)	Fertilization rate (%)	Hatching rate (%)
Trial-1	75 ± 0.73	62.5 ± 0.84	58 ± 0.98
Trial-2	87.5 ± 0.35	64 ± 0.56	60 ± 1.38
Trial-3	83.33 ± 0.35	63.83 ± 0.70	59.66 ± 0.43

First feeding: Although the hatchlings of *Labeo calbasu* retained yolk sack upto 72 h after hatching the larvae started first feeding from 45-55 h of post-hatching at ambient temperature of 26.5-29°C. Boiled egg-yolk was provided as first food for the hatchings.

Discussion

Brood stock management is one of the major aspects in induced breeding of any fish species. In hatchery management, maintenance of brood fishes for the development of modern aquaculture activities has become one of the most important concepts.

Proper care of brood stock is very important for assuring good production of eggs, fry and fingerlings (Robert *et al.*, 1982). The daily and seasonal rates of feeding of brood stock diets have direct effects on fecundity and egg size (Jones and Bromage, 1987, Bromage and Cumaranatunga, 1988). After successful completion of brood stock management with balanced feed comprising adequate amount of protein, lipid, and carbohydrate, especially enriched with vitamin-E the fish *Labeo calbasu* attained gonadal maturity in late April. In the present study, it was found that *Labeo calbasu* bred in April and August. The peak breeding season was May and June and it continued till August. According to Jhingran (1982) the breeding season of the Indian major carps generally start from April and continues to August, with an optimum period between May and June. Ibrahim *et. al.* (1968) reported that temperature ranging from 26.5° to 35.0°C was appropriate for spawning of major carps. Breeding of *Labeo calbasu* was performed at an ambient temperature of 26.5° to 31.1°C.Use of feeds with vitamin mineral premix might have some positive effect for the maturation of fishes. Hoque (1990) reported that diets containing 1% vitamin premix showed better result in all aspects viz., selectively, spawning success, fertilization and hatching rate. Spawning performance of the reared broods indicated that the spawners might have been at their optimal breeding condition. This might be due to good management practices of brood stock which prolomged their breeding season in artificial condition.

In this experiment the induced breeding trials of *Labeo calbasu* were done with pituitary gland (PG) extract and DoM + SGnRH in inducing of *Labeo calbasu*. They achieved good fertilization and hatching rate (73.05% and 60.43%, respectively) by using an initial and a resolving dose of 2.0-3.0 and 5.0-7.0 mg/PG/Kg body weight for female. In this experiment best fertilization and hatching rate (80% and 76% respectively) were gained by using PG in trial-3 for treatment-1. In this study Trial-3 showed best result due to brood fishes which were fully matured. The result in consideration of fertilization and hatching rate was lowest in Trial-1 for the synthetic hormone (DoM+SGnRH). The ovulation occurred in all the injected females within 5.65 to 7.36 hrs after resolving dose to the females at the temperature from 27 to 31°C. But in this experiment ovulation occurred within 6 to 6.30 hrs after resolving dose to females at the temperature from 27 to 31°C.The success of entire operation of induced breeding depends largely on the proper selection of brood fishes. Accomplishment of successful spawning depends on selection of suitable recipient fish at the proper stages of ovarian development and creation of congenial spawning conditions.

The present study was conducted to compare the effects of two inducing agents on the induced breading of *Labeo calbasu*. Three breeding trials were done with PG hormone as treatment-1 and three breeding trials were done with

DOM+GnRH as treatment-2. Brood fishes were collected from the river. The collected broods were reared in the pond, providing special diet up to their maturation. After rearing for certain period the gravid fishes were selected for induced breeding trial. Total 16 broods (8 female and 8 male) were used in each breeding trial for breeding. In case of Treatment-1, the females were injected 1.5mg PG/kg body weight as initial dose and 6 mg PG/kg body weight as resolving dose. On the other hand, males were administered at the rate of 1.5 mg PG/kg body weight. On the contrary in case of Treatment-2, the females and males were injected DOM+GnRH at the rate of 12 mg/kg body weight and 3 mg/kg body weight respectively. In treatment-1 gave the better result in terms of ovulation, fertilizaiton and hatching rate compared to treatment-2. In case of treatment-I, better results were obtained by trial-3 among other trials. From the present study it can be said that, both the inducing agents were effective in respect of overall breeding performance. Although best results were obtained with PG hormone but the entire induced breeding technique could be performed within short time with less labour and cost in case of synthetic agent (DOM+GnRH) than that of PG hormone.

A second experiment was conducted to know the nursery management technique of *Labeo calbasu* in the nursery pond for a period of 60 days. The fry were stocked at the rate of 30g/deci. Wheat bran, mustered oil cake, flour were given as nursery feeds. The mean size of the fry was 2 cm during harvesting.

At present, concerns have been expressed for its conservation through habitat improvement and attempts have been made towards the culture of the fish in the pond. The seed production technology of the species is the prerequisite of development of culture technique. With this end in view, to develop the induced breeding, nursery management of *Labeo calbasu* the present study was undertaken. From the available references along with the present investigation on the induced breeding of the fish comparatively higher percentages of fertilization and hatching were achieved from PG hormone. This can be considered as an effective means for commercial seed production in the hatcheries since cost and quality of hormone is on of the important key factors for a hatchery manger. The results indicated that the hatchery operators can easily breed, rear and supply requisite quantities of *Labeo calbasu* seeds to meet up the demand of the fish farmers. The present study can throw a light for further research on the improvement of induced breeding technique of the fish concerning the hormone application, nursery management techniques of *Labeo calbasu* as well as optimization of the environmental parameters.

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Production of small and big fishes of selected ponds

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Abstract: To study the big and Small Indigenous Species (SIS) of fish production, 15 ponds of Rajshahi University campus were selected. The pond production was ranged from 326.16 to 2187.40 kg/ha. The ratio of SIS and big fish production was calculated maximum as 1 : 0.10 (by number) and 1 : 7.46 (by weight). Majority of these SIS fish are Self Recruiting Species (SRS), because they were not stocked in the studied ponds. The F/C ratio was obtained as 4.583 in average.

Key Words: SIS, Production.

Introduction

Bangladesh is a country having vast water bodies with high production potential of fish which are very much nutritionally enriched. Fish is one of the most important sources of animal protein and has been widely accepted as a good source of protein and other elements for the maintenance of health body (Andrew, 2001) at low cost.

The Small Indigenous Species (SIS) of fish in Bangladesh are generally considered to be those which grow to a length of approximately 5-15 cm at maturity (Felts *et al.*, 1996). Detailed biological information of these fishes are not presently available except few publications as stated by Hossain and Afroze (1991).

The SIS fishes have short life cycle and can grow in all types of inland waterbodies. Because of overfishing and reduced waterbodies a number of small fishes are now under the threat of extinction. In Bangladesh 143 freshwater fish species are categorized as small indigenous fish. In the past, these fishes were abundant in the rivers, beels, canals, streams and ponds. Both natural and man made catastrophes caused degradation of the aquatic environment, and reduction of many wet lands and water areas of Bangladesh have resulted in the disappearance of many suitable habitats for floodplain, riverine and brackish water small indigenous fish species. Many of these valuable small indigenous fish species have been threatened or endangered. Indeed, some are already on the verge of extinction. So, there is presently an urgent need to conserve and to increase the production of the SIS fishes through proper management of the waterbodies of Bangladesh. Side by side these species should be introduced in the farming systems of country.

Conservation of SIS can also help to control mosquito larvae. In the past, some of the small fishes were regarded as weed fishes and eradicated from the fish ponds using pesticides. But recently these species have been considered as an important source of essential macro and micro-nutrients, which can play an important role in the elimination of malnutrition in Bangladesh (Ahmed *et al.*, 1997; Thilsted *et al.*, 1997; Hossain, 1997; Hossain *et al.*, 2003; Wahab *et al.*, 2003 and Faculty of Fisheries, BAU, 2008).

The swamps are one of major source of small indigenous fish species in which it grow without care and without culture. These fishes enter in the swamps with the river water or flood water from the different sources, grow and reproduce there, so why is called as Self Recruiting Species (SRS).

The present research describes the production of SIS and big fishes from selected waterbodies. Where SIS fishes were not cultured they were naturally introduced.

Materials and Methods
Study of the habitats

Selected water bodies: For the present research, ponds of different types and swamps were selected. These waterbodies are situated within the Rajshahi University (RU) campus.

Total 15 ponds were selected.

Fish production
The total harvested weight of fishes of individual water bodies was recorded. Fishing of the swamps was done by cast net (larger fishes); and the smaller fishes were caught by hand after dewatering. Bamboo traps like Kholsoon and Dhiar were used to trap fish.

The ponds were harvested using encircled gill net and cast net. The miniponds were dewatered and fishing was done by hand. Total weight of individual species of bigger fishes were recorded.

However, fish diversity of the swamps was noted, with recording the total length (cm) and total weight (g) of the individuals of each species.

Calculation of F/C ratio
The ratio between the carnivorous and the other fishes (forage) (F/C ratio) were determined following Swingle's (1950) simple formula, as below:

$$\text{F/C ratio} = \frac{\text{Weight of the forage fish (F)}}{\text{Weight of the carnivore fish (C)}}$$

Results and Observations
Description of the ponds
Location: All the study ponds are situated scatteredly within the Rajshahi University campus. These ponds were formed gradually in man made dugout cavities made for different purposes

Pond type: Among the studied 15 ponds, in 10 water stand for 9 months, in five for 7 months. Other nine

ponds are perennial. Pond no. 11 is not exactly a pond. It is a narrow natural canal.

Soil condition: The condition of the bottom soil of ponds are very important for fish culture. Among the 15 study ponds bottom of all ponds were sandy-muddy.

Water supply: The source of water of these ponds are rainwater, drain water, sewerage water. However, during the flood the flood water from rivers and canals also enter in these swamps.

Water area: The water area of these swamps ranged from 45.50m^3 to 1144 m^3

Water colour: Water colour of most of the ponds was found to be greenish.

Total production

Total production by weight of SIS (*C. fasciatus, A. testudineus, C. nama, C. ranga, P. ticto, P. gonionotus, C. reba, O. mossambicus, E. danricus, C. punctatus, N. nandas, A. mola, G. giuris, M. tengara,* Prawn) and big (*L. rohita, L. calbasu, C. cirrhosus, H. molitrix, C. carpio, A. nobilis, C. striatus, C. catla*) fishes from individual pond are presented in Tables 1 and 2. Among the ponds, maximum production 135.863 g/m^3 was recorded from pond no. 10, and minimum production was calculated as 16.31 g/m^3 in pond no. 13 (Table 2). The estimated weight produced per hectare of these ponds were ranged from 326.16 kg/ha to 2187.40 kg/ha in pond no. 13 and 10 respectively.

The ratio of production of SIS and big fishes in ponds were calculated as the minimum 1 : 0.02 in pond no. 6 (by number) and 1 : 0.48 in pond no. 14 (by weight); the maximum ratios are 1:0.13 (by number) in pond no. 11 and 1:7.46 (by weight) in the same pond, though the production rate of this pond (actually a canal) is too less compared to other ponds.

Ratio between carnivorous and forage fish (F/C ratio)

As production of the ponds which are not stocked or managed scientifically, and the fish population can be designated as "self recruiting species" (SRS), the ratio between the carnivorous and non-carnivorous (forage) fishes was determined to find out fish population these ponds (no. 1-15) are either balanced as unbalanced.

The F/C ratio of the fish population of individual 15 ponds are presented in Table 3. The results show that the population of pond no. 2 and 14 are unbalanced having F/C ratio as 1 : 2.79 and 0.913 respectively (Table 3). The population of pond no. 2 was unbalanced due to higher production of forage fish than carnivore fish, and in other cases the production of carnivore fish was more than that of the forage fish. However, the total population of the ponds showed an average balanced value (Table 3).

Table-1. Pondwise production of SIS and big fishes by number and by weight in the studied ponds

Pond No.	Total no. of small fishes	Total weight of small fishes (g)	Total no. of big fishes	Total weight of big fishes (g)	Ratio of SIS : big fish	
					by no.	by weight
1	463	4547.60	31	8974.00	1 : 0.07	1 : 2.17
2	318	1234.40	16	7745.00	1 : 0.05	1 : 6.27
3	524	3162.00	26	10380.00	1 : 0.05	1 : 3.28
4	478	3646.00	26	11352.00	1 : 0.05	1 : 3.11
5	496	2994.80	25	13697.00	1 : 0.05	1 : 4.57
6	761	3071.30	12	6015.00	1 : 0.02	1 : 2.22
7	566	3111.00	21	9500.00	1 : 0.04	1 : 3.05
8	488	3827.00	28	11016.00	1 : 0.06	1 : 2.88
9	565	4650.07	30	10892.00	1 : 0.06	1 : 2.79
10	848	7391.00	33	11600.00	1 : 0.04	1 : 1.57
11	62	636.00	8	4746.00	1 : 0.13	1 : 7.46
12	868	8489.00	78	49382.00	1 : 0.09	1 : 5.82
13	324	3244.20	32	5660.00	1 : 0.10	1 : 1.74
14	265	1116.11	15	535.00	1 : 0.06	1 : 0.48
15	158	1664.60	16	1185.00	1 : 0.10	1 : 0.71

Table-2 The average fish production per unit area of different ponds

Pond No.	Area			Production		
	Area (m²)	Water area	Total wt. of	Weight/m²	Weight/m³ (g)	Weight per
1	82.22	124.97	13521.60	164.4563	108.1988	1644.56
2	82.03	124.69	8979.40	109.4648	72.0138	1094.65
3	86.64	139.48	13542.00	156.3019	97.0892	1563.02
4	87.42	131.13	14998.00	171.5626	114.3750	1715.63
5	89.30	133.95	16691.80	186.9183	124.6122	1869.18
6	85.32	128.83	9086.30	106.4967	70.5294	1064.97
7	86.82	140.65	12611.00	145.2545	89.6623	1452.55
8	81.78	122.67	14843.00	181.4991	120.9994	1814.99
9	88.35	132.53	15542.70	175.9219	117.2768	1759.22
10	86.82	139.78	18991.00	218.7399	135.8635	2187.40
11	74.989	44.99	5382.00	71.7705	119.6266	717.71
12	880.00	1144.00	57871.00	65.7625	50.5865	657.63
13	273.00	546.00	8904.20	32.6161	16.3081	326.16
14	50.00	45.50	1651.11	33.0222	36.2881	330.22
15	572.00	572.00	2849.60	4.9818	4.9818	49.82

Table-3 . Forage fish and carnivorous fish production in ponds and F/C ratio

Sl. No.	Forage fish		Carnivorous fish		Value of F/C ratio	Balanced (B) or Unbalanced (U) production	Average F/C ratio in ponds (n=15)
	No.	Wt (g)	No.	Wt (g)			
1	179	9952.8	351	3568.8	2.788	B	
2	141	8221	193	758.4	10.897	U	
3	206	10880	344	2662	4.087	B	
4	236	12120	268	2878	4.211	B	
5	226	14520	295	2171.8	6.685	B	
6	442	7220	331	1866.3	3.868	B	
7	205	10192	382	2419	4.213	B	4.583
8	293	12388	223	2455	5.046	B	(Balanced)
9	272	12394	323	3148.7	3.936	B	
10	457	13574	424	5417	2.505	B	
11	45	4882	25	500	9.764	B	
12	441	50240	505	7631	6.583	B	
13	162	6115	194	2789.2	2.192	B	
14	89	788.11	191	863	0.913	U	
15	96	1469	78	1380.6	1.064	B	

Discussion

The present results showed that as a habit the small waterbodies with rich aquatic vegetation and permitted to be flooded, can yield or produce a satisfactory quantity (by number and by weight) of a variety of both indigenous and introduced a number of SIS fishes which were naturally introduced, are listed as highly nutritious and containing vitamins and minerals (Roos *et al.*, 2003). Among the SIS fishes, mola fish (is also a SRS) have been successfully introduced in the commercial fish farms in carp polyculture system (Roos *et al.*, 2003). Alam (2009) suggested that the featherback *N. notopterus* can be cultured with carps without affecting the production.

Swingle (1950) suggested that in the balanced population the F/C ratio will range from 1.4 to 10. Among the 15 experimental ponds, 13 showed balanced population. The average F/C ratio of the ponds was determined as 4.583 which is a balanced population. The unbalanced population is that one which unable to produce accepted amount of fish year after year (Swingle, 1950). When the carnivorous fish is dominant in the water body the F/C ratio is unbalanced, and as a result the fish production is less. So, the entry of the carnivorous fishes in any fish culture area is likely to affect the fish production (Hasan, 1983).

In Bangladesh total annual fish production from pond and ditch was 2839 kg/ha from July 2007-June 2008 (DoF, 2007-2008). The fish production in our country is comparatively less than other countries. Jhingran (1977) noted the all time Indian record fish production was 9,056 kg/ha/yr. A maximum fish production from freshwater ponds of China was reported as 7.5 tons/ha/yr (Lin, 1982). Lakshmanan et al. (1971) reported the average production to be 3,062 kg/ha/yr, while Sinha et al. (1973) found 3232.30 kg/ha production in six months. Davis et al. (1983) recorded fish yields ranging from 1980 to 3,820 kg/ha/yr. Miah et al. (1993) obtained a gross production of 3,670 kg/ha from polyculture of Indian major carps and Chinese carp. Uddin et al. (1994) obtained a production of 2,019 to 3,415.60 kg/ha/yr and Mazid et al. (1997) found a gross production ranging from 2545.50 to 3687.80 kg/ha/11 month from carp polyculture system.

Production of SIS fishes both in diversity and biomass, were found comparatively higher in small unmanaged ponds than in large stocked fish ponds. Total production of ponds in 9 months period was obtained maximum as 2187.40 kg/ha Total SIS production for ponds was ranged from 0.636 to 7.391 kg. Most of the ponds were found to be a balanced one, from their F/C ratio of fish biomass.

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Breeding biology of coppersmith barbet, *Megalaima haemacephala* (Müller, 1776)

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Abstract: Breeding biology of the Coppersmith barbet, *Megalaima haemacephala* (Müller, 1776) was carried out between February, 2006 and January, 2007 at Sharawardy Uddyan, Ramna Park, Curzon Hall and National Botanical Garden. The breeding season started from December and ended in June. In total 20 nests were observed, of which 10 nests were studied in details in four study areas. The coppersmith barbet mostly preferred to make holes on the branches of koroi (*Albizzia procera*) for nesting. Egg laying started on 15th February in the study areas. Average height of nests from the ground was 9.7m and average depth and diameter of the holes was 29.20cm and 4.46cm respectively. New holes were constructed yearly or the old one was reused. Both the sexes took part in incubation of eggs, brooding and feeding to the nestlings. A total of 30 eggs were laid in 10 nests. Clutch size varied from 2 – 4 eggs (average: 3 eggs). Among them, 20 (66.67%) eggs were hatched and the rest 10 (33.33%) were unhatched and lost. Average incubation period was 14 days. The male and the female incubated the eggs for an average of 27.44 minutes/ hours and 32.56 minutes/ hours, respectively. Average number of nestlings (brood size) per nest was 2. Out of 20 nestlings, 16 left their nests successively. The breeding success was 53.33% in relation to the number of eggs laid and 80% in relation to nestlings hatched. The average weight of eggs and nestlings was 3.59g and 9.33g, respectively. The main causes of loss of the eggs and nestlings were human interference, predation and ectoparasitic infections. Insects and fruits were fed to the nestlings by their parents.

Keywords: Ecology, breeding, nesting, frugivore, Coppersmith barbet.

Introduction

The coppersmith barbet, *Megalaima haemacephala* is locally known as 'Choto Basantha Bauri' (Piciformes: Megalaimidae). A total of 80 species of barbets belonged to 11 genera occurs throughout the world, of which 4 species recorded in Bangladesh (IUCN, 2000). It is usually seen alone, in pairs or sometimes in mixed feeding parties, foraging in fruiting trees, especially banyan, peepul and other wild fig trees. Barbets chiefly feed on figs, drupes and berries and sometimes prey moths and flying termites. The coppersmith barbet is common and widely distributed all over Bangladesh. This species occurs in India, Pakistan, Nepal, Bhutan, and Sri-Lanka from the plains and foothills up to 2000 m (Ali & Ripley, 1970; Fletcher & Inglis, 1936; Smythies, 1953; Whistler, 1963). The bird is resident of Bangladesh (Hussain, 1979) and distributed across the country (Khan, 1987; Sarker & Sarker, 1988). Some works have been done on the distribution, ecology and breeding biology of other species of birds (Ali & Ripley, 1983; Harvey, 1990; Jaman *et al.*, 1997; Jaman & Sahreen, 2004; Marshall, 1877; Ray, 1992; Sarker, 1987; Zacharias & Gaston, 1982).

So far as we know, no any research work on the breeding biology of barbets of Bangladesh was done. This bird helps in dispersal of seeds and act as an ecological indicator of the habitat. Therefore, a detail research on the breeding biology of Coppersmith barbet was conducted. This study may help in the conservation of this important frugivore bird.

Materials and Methods

The study was carried out from February 2006 to January 2007. In total 10 nests were studied in order to collect data on the breeding behavior of coppersmith barbet. The nesting sites were visited on alternate morning and afternoon during the study period. A portable hide was used for closer observation of nest making, incubation activities, feeding of nestling by parents and fledging activities of young birds. Chopper (a butcher's knife having a large square blade) was used in finding the nest of coppersmith barbet, because they made some nests in deep hole. Each nest was marked by marker pen. Wooden ladder was used for climbing the nesting trees in order to measure nests, eggs and nestlings. Height of the nest from the ground and nesting territories were measured by 100m plastic tape. Behavioral activities (construction of nest, feeding, preening, gasping, scratching, stretching, roosting, puffing, shaking, etc.) of barbets were observed with the help of a pair of binocular and camera. Spring balance, polythene bags and ropes were used to weigh nests, eggs and nestlings. Watch was used for determining the time for incubating and non-incubating time and feeding visit. Measurement of holes, nestling and young were taken by scale, fine tipped divider, steel tape and slide calipers. The eggs were marked as 1, 2, 3, 4, etc. by Chinese ink to determine the incubation period and hatching intervals. The eggs were laid at early morning and the marking on eggs was done on

the following morning. Nestlings were also marked after hatching with different coloured rubber band. The measurement and weights were taken daily at a fixed time and the records were kept in tabular form with dates. Factors affecting in the damage of eggs and nestlings were also recorded. Mist nets and gloves were used to catch the nestlings and young birds. Activities of young birds before and after leaving the nest were also recorded.

Study area: The study area was at Sharawardy Uddyan, Ramna Park, Curzon Hall and National Botanical Garden. The Dhaka district lies between 24°W latitude and 90° E longitudes. It is situated at a height of about 7.62m above the sea level. **Sharawardy Uddyan:** This park acquires an area of about 65 acres. There are more than 64 species of plants in the park. **Ramna Park:** This park is situated in the heart of Dhaka city. It is about 85 acres. **Curzon Hall: C**urzon Hall is situated at the south-eastern side of the Dhaka University campus. It is about 60 acres and there is a big pond in the middle of this study site. **National Botanical Garden: NBG** situated at the east side of Mirpur Zoo and 20km away from the central point of Dhaka. This garden is about 208 acres. There are 28200 trees under 255 species and 8400 bushy trees under 310 species present.

Results and Discussion

Breeding season: The first breeding activities of coppersmith barbet (*Megalaima haemacephala*) was observed to start from early December and continued until last June. The peak breeding time was in March when temperature ranged from 18°C - 31°C, humidity from 70% - 87% and average rainfall 49 mm. Ali & Ripley (1968 - 1974) noted that this bird bred from November to June, peak in February in Indian subcontinent.

Pair formation: We found that pairing of barbet was occurred mainly in December and continued up to mid March. Pair formation is the first step of breeding biology (Welty, 1975). Coppersmith barbet is monogamy bird and formed pair in December when pair was seen closely sat together. Ali & Ripley (1968 - 1974) noted that barbet formed pair from November to February.

Nest site selection: Both the male and the female selected the nesting site. For making the nest Coppersmith barbet preferred dead or old branches of trees. Ali & Ripley (1968 - 1974) mentioned that the nest of bird is excavated in a dead or decaying softwood branch. Sometimes, bird left the nesting site after site selection was made. Bird took 7 to 10 days to choose the nest site.

Nesting: It was observed that a pair of Coppersmith barbet made two or three identical holes in the nesting branch, of which only one hole was used for nesting. Rest of the holes could be made for camouflage to protect from predators or enemies. The height of the nest from the ground ranged from 6 – 13 m (average: 9.7 ± 1.59 m). We marked 20 nests in 4 species of trees in four study sites. The highest number of nest was 12 (60%) in the branches of koroi (*Albizia* sp.). The highest number of nests was 7 (35%) in Ramna park area (Fig. 1). All the nests were more or less similar in size and shape. They made cup shaped nest with the lining of soft grasses and feathers inside the hole, where the eggs were placed. Ali & Ripley (1983) reported that nest's hole of this bird was long (25 – 80 cm) and ending in a slightly widened chamber. It was found that the average length, breadth and depth of nests were 29.20 ± 2.74 cm, 4.46 ± 0.55 cm and 6.31 ± 0.47 cm, respectively.
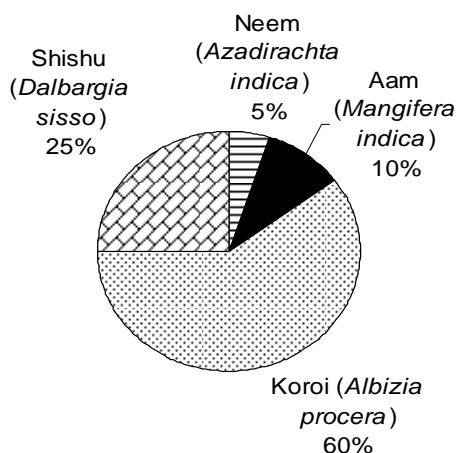

Fig. 1. Percentage of trees used for nesting by Coppersmith barbet.

Territory and habitat: Both sexes of coppersmith barbet were always alert to defend the nest and territory against any other birds which crossed the territory boundary or flew away over the nesting trees. They frequently moved but called not so loudly, because they are very cleaver and exhibit peaceful behavior. They were slightly aggressive during breeding season. If any man who happened to climbed at nesting trees, the birds sat on branches near the nest, and looked here and there very sharply.

Mating: Mating took place on the branches of trees, usually in the morning and late afternoon. Bird mated within few second. Before mating the male and the female sat closely together. At that moment the male scratched the female's forehead for several times.

Table 1: The measurement of eggs and incubation period of Coppersmith barbet

Nest No.	Date of egg laying	Weight(gm)	Length (mm)	Breadth (mm)	Date of hatching	Incubation period (days)
1	8th March	3.2	25.00	17.50	22nd March	14
	9th March	3.3	25.30	17.25	23rd March	14
	10th March	3.3	24.90	17.20	23rd March	13
2	12th March	3.8	25.15	17.45	26th March	14
	13th March	3.9	25.30	17.40	28th March	15
	15th March	3.8	25.25	17.20		
3	8th March	3.0	25.00	17.15	23rd March	15
	9th March	3.1	25.15	17.20		
	11th March	3.2	25.50	17.45	25th March	14
	12th March	3.4	25.35	17.25	25th March	13
4	21th March	4.1	24.10	16.90	5th April	15
	22th March	3.8	24.56	16.80		
	24th March	4.0	24.55	16.70		
5	14th March	3.4	24.34	17.25	27th March	13
	15th March	3.7	25.14	17.30		
	16th March	3.4	25.15	17.80		
6	24th March	3.4	24.67	17.65		
	25th March	3.6	25.00	16.70		
	26th March	3.5	24.25	17.50		
7	11th April	3.5	24.20	16.50	26th April	15
	12th April	3.3	25.50	16.50		
	13th April	3.5	25.40	16.40	28th April	15
8	14th April	3.5	25.60	17.55	28th April	14
	15th April	3.4	25.45	16.50	29th April	14
	16th April	3.4	25.20	16.90	30th April	14
9	25th April	3.7	24.35	16.85	8th May	13
	26th April	3.8	25.50	17.70	9th May	13
	27th April	3.6	25.30	17.55	10th May	13
10	15th February	4.1	25.25	17.50	3rd March	16
	17th February	4.2	-	17.60	5th March	16
Average		3.56±0.31	24.98±0.43	17.17±0.40		14±0.99

Egg laying time and laying sequence: The first date of laying eggs was observed on 15th February and the last on 27th April (Table 1). Egg laying normally started as soon as the nests were completed. Eggs laid regularly one after another and also in successive days with few exceptions having gap of one day. Welty (1975) defined laying as "the deposition of the eggs in the act of position itself and various attendant circumstances". The peak period of egg laying was found in March (Table 1 and Fig. 2). Ali & Ripley (1970) reported the peak period of egg laying in February.

Clutch size: A total of 10 clutches of eggs were studied. Thomson (1964) defined 'clutch size' as "the complete set of eggs laid by one female which were brooded simultaneously". The clutch size of this species varied from 2 - 4 eggs with an average of 3 eggs. Only one clutch of a pair of birds was recorded in one breeding season.
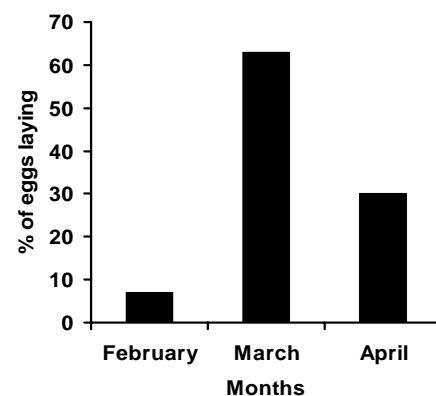
Fig. 2. Egg laying of Coppersmith barbet in successive month.

Measurements and weight of eggs: Eggs were varied in size, colour, dimension and weight even within the same clutch. The length and breadth of 30 eggs were ranged from 24.90mm to 25.50mm (average: 24.98 ± 0.43mm) and 17.15mm to 17.50mm (average: 17.17 ± 0.40mm), respectively

(Table 1). The weight of 30 eggs were varied from 3.0 to 4.2g (average: 3.56 ± 0.31g) (Table 1). It was found that the weight of the eggs gradually decreased as the incubation continued. But the decreased rate was very high in some eggs, might be due to the infertility of eggs.

Incubation: Incubation started as soon as the first egg was laid and continued up to the hatching of last egg. The incubation period of 20 eggs varied from 13 to 16 days (average: 14 ± 0.99 days). The rest of the eggs (10 eggs) were not hatched (Table 1). The incubation behavior was observed in three different nests. In total 36 hours were spent for data collection, of which the male and the female incubated the eggs for an average of 24.44 minutes and 32.56 minutes/hrs, respectively. Out of 20 eggs, 5 (25%) were incubated in 15 days, 7 (35%) in 14 days, 6 (30%) in 13 days and 2 (10%) were incubated in 16 days (Table 1).

Loss and infertility of eggs: Out of 30 eggs laid in 10 nests, 10 (33.33%) eggs were lost due to various factors). It was found that some eggs failed to hatch in some nests. It was probably due to the infertility of eggs. The infertile eggs were not removed by the birds from the nest till 5th day after usual incubation period was over.

Loss of nestlings and Nest sanitation: Out of 20 nestlings hatched, we recorded 4 (20%) nestlings were lost due to human interferences, predation by animals, disease and unknown causes. Barbet was very much careful about their nest sanitation. They always removed any foreign objects, which were harmful for their nestlings. After hatching of each nestling the broken parts of the eggs shell were removed by the parents. Both sexes shared the task of nest sanitation.

Breeding success: Out of 30 eggs, 20 (66.67%) eggs were hatched and 16 (53.33%) nestlings were successfully grown up and fledged. Therefore, we found 53.33% of breeding success in relation to eggs laid and 80% in relation to nestlings hatched.

Dispersal: The young bird left the nests when parents perch on the branch of trees near the nest. After getting food either they returned to the nest or move from one branch to another of the nesting tree. When parents came with the food, the young were called softly (Ki- Ki-Ki……), because they are very gentle bird. At that moment they were jerking their wings and came near the opening of the hole to obtain food from parents. After 2 to 3 days when the young were learned to fly freely then left the nestling tree and never came back. The fledging period (time for young to grow feathers necessary for fly) was about four weeks. Out of 20 nestlings the maximum young fledged in May. Both parents fed the young. After dispersal of the young, they started to lead an independent life.

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High hatching success of saltwater crocodile (*Crocodylus porosus*) in a commercial Crocodile Farm of Bangladesh

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Abstract: An extensive study was conducted from March 2007 to February 2012 on hatching success of saltwater crocodile (*Crocodylus porosus*) in the Reptiles Farm Ltd. (RFL) located at Hatiber village of Uthura union under Bhaluka upazila in Mymensingh. The study was mainly based on direct field observation and some previous data collected by farm's technicians. A special type of incubator having 98-100% moisture and 31-33^{0}C temperature was maintained to improve the hatching success. Yearly hatching success in captivity was 95.8%, 95.15%, 97.44%, 96.03% and 94.53% in 2007 through 2011, respectively. The average rate of hatching success in RFL was 95.8 ± 1.09%. Hundred percent hatching success was found in 29 out of 56 clutches. Clutch size varied from 19 to 68 eggs. Unhatched eggs were 4.19%, of which most of the embryos died before hatching. The average time required for incubation was 79 ± 3, 79.5 ± 4.5, 80 ± 4, 80.5 ± 4.5 and 78.5 ± 3.5 days in the above mentioned period. Compared to the wild habitat, captive environment in controlled weather and predation might improve hatching rates. This study suggests that conservation of this endangered species is possible by captive breeding and reintroduction program.

Keywords: Clutch size, incubation, hatching, captive breeding, saltwater crocodile.

Introduction

Reptiles Farm Ltd. (RFL) is the first commercial saltwater crocodile *(Crocodylus porosus)* farm in the Indian subcontinent, located in the Mymensingh district, Bangladesh (24°26′52.38″ N and 90°15′50.94″ E). The aim of the farm is to produce crocodile commercially while protecting and promoting wild crocodile population in Bangladesh. Crocodile farming is a new concept for the entrepreneurs of Bangladesh as this industry has never existed in Bangladesh. The geophysical and climatic condition of Bangladesh is suitable for crocodile farming as it is the historical living place of saltwater crocodile. Farming of this reptilian species has been spreading all over the world because of high demand for its skin, meat, bones and usage in ecotourism. During the last decade international demand for crocodile skin increased tremendously which resulted in lucrative crocodile farming to the entrepreneurs of the world (Magnusson, 1984; Cox & Rahman, 1994).

Since saltwater crocodiles have commercial importance and a critically endangered species in Bangladesh, the main target of the farm is to make an effective breeding success in captivity that may help to establish a successful commercial crocodile farm as well as may help in reintroduction of this species in nature. The ability of individual crocodile to mate successfully and produce viable offspring in captivity is a significant indicator as to how effectively husbandry and management practices are being engaged within a facility (Elsey *et al.,* 1994). The capacity of RFL was to incubate 5000 eggs at a season. In

captivity, mating, egg laying, and the more or less synchronized hatching dates can be controlled (Trutnau & Sommerlad, 2006). Although, almost all crocodilian species can breed in captivity, some seems to be particularly suited for keeping and breeding in the farms. Breeding in the farm contributes to conserve the species and also allowed to legally raise animals that are listed in appending 1 and 2 of CITES for commercial use (Trutnau & Sommerlad, 2006). Therefore, current study was conducted on the breeding activities of saltwater crocodile (*Crocodylus porosus*) in a commercial crocodile farm to reveal the factors affecting hatching rates in captivity.

Materials and Methods

Study Area: Reptiles Farm Ltd. (RFL) situated in Hatiber village of Uthura union under Bhaluka upazila in Mymensingh. It is spread over 13.4 acres of land and located on 24°26′52.38″ Northern altitude and 90°15′50.94″ Eastern longitude (Fig 1).

Study was conducted between March 2007 and February 2012 in the Reptiles Farm Ltd (RFL). The observation was started early in the morning and was continued still afternoon. The study was mainly based on direct field observation and some previous data collected by the farm technicians for their computer record. Weather data has been collected from the weather recording devices located at the farm.

We observed female breeders along with the technicians during egg laying time when and whether the breeders laying eggs in the ponds. Eggs were collected within 24 hours or immediately after egg laying. Female crocodiles

had showed less aggressiveness for exhaustion. Angle of collected eggs from the mound nest was maintained and numbered with pencil markers. Collected eggs were cleaned up and placed on plastic tray on moisture soil and maintained the collected orientation of the eggs in the nest. One tray was for one clutch and a collected time of the clutch was recorded. Once eggs were collected, the top orientation of the egg in the nest was marked and placed in the incubator in approximately the same position to prevent embryo mortality (Hutton & Webb, 1994; Ojeda *et al.*, 1998). A special type of incubator is on the farming area, where 98-100% moistures and 31-33^{0}C temperature was maintained for the better success of hatching. We used incubation trays because it allowed easy access to the eggs in order to remove infertile eggs and may reduce the danger of rotting and contamination. Easy air access to the incubator room also prevents overheating from the metabolic heat produced by the embryo with the banding pattern on the eggs. Eggs which were showed banding pattern kept as fertile and which were not selected as infertile.
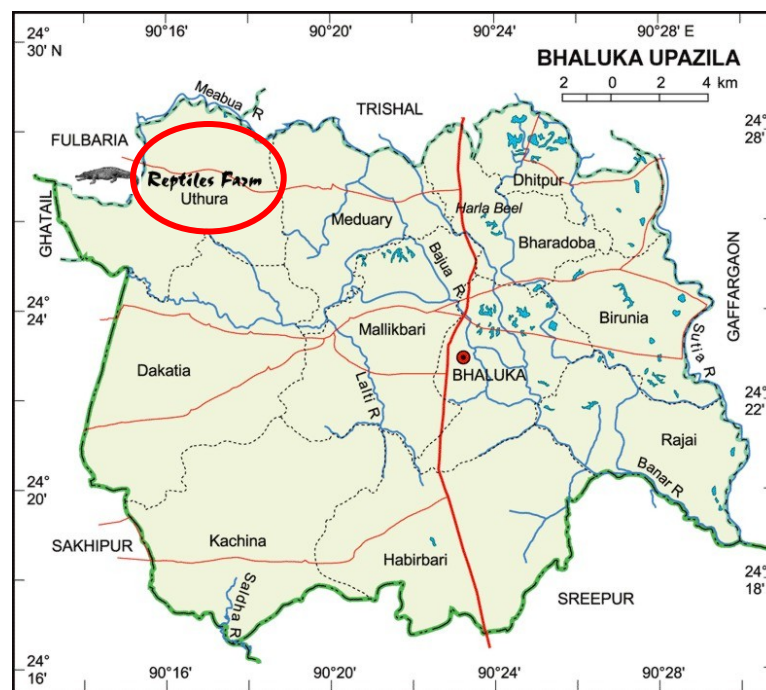

Fig. 1. Location map of the study area.

The temperature and humidity data was collected from the thermometer and hygrometer attached within the incubator. Door of the incubator was opened for 30 minutes at the morning and 30 minutes at the evening in order to maintain oxygen level. In constant temperature, 75-85 days were required for hatching in the incubator. Banded and damaged eggs were recorded on regular basis. Non-banding eggs were sorted out within 48 hours after placing in the incubator.

Results and Discussion

Monsoon is the mating season for the crocodiles. Afterward crocodile started to make nest and egg laying. A total of 2371 eggs were collected from 56 clutches from 2007 to 2011 at the Reptiles Farm Ltd. (RFL) where the male and the female breeders had mated successfully. The clutch size varied from 19 to 68 eggs. The number of banded eggs varied from 1 to 60 per clutch. Of the total eggs, we found 1768 (74.5%) banding eggs, of which 1694 (95.8%) eggs were hatched successfully in the incubator.

The average time required for incubation was more or less similar in each year (78.5±3.5 – 80.5± 4.5 days, Table 1). Incubation took place from 80 to 90 days, although the period of incubation might be greatly extended according to the weather conditions (Whitaker *et al.,* 1981). Required time for hatching in the incubator was comparatively less because of controlled temperature, humidity and fresh oxygen circulation on the regular basis (Hutton & Webb, 1994; Ojeda *et al.,* 1998). We maintained very high level of humidity to avoid dehydration of eggs. Dehydration of eggs results in malformed hatchlings or premature death of the embryos. The incubation temperature not only determines the sex of hatchlings but also maintains body temperature of crocodiles in their

later life (Huchzermeyer, 2003). A lack of fluid inside the eggs suggests that an egg is infertile or that the embryo died at an early stage. As the embryo grows, blood vessels and blood can be distinguished inside fertile eggs. Embryos may die in their eggs for a variety of reasons. Possibilities include wrong incubation temperature, low relative air humidity, poor nutrition, lack of oxygen, nutrients, water in the eggs and mechanical damages (Trutnau & Sommerlad, 2006).

Table 1. Incubation period of eggs in the incubator of the farm from 2007 to 2011

Year	No. of clutches	No. of banded eggs	Days required		
			Maximum	Minimum	Average ±sd
2007	6	143	82	76	79.0±3.0
2008	9	289	84	75	79.5±4.5
2009	15	431	84	76	80.0±4.0
2010	13	429	83	77	80.5±4.5
2011	13	476	82	75	78.5±3.5

The total number of banded eggs and eggs hatched had been gradually increased from 2007 to 2011 (143 – 476 eggs and 137 – 450 nestlings, respectively, Fig. 2). We found 100% hatching success in 29 clutches out of 56 clutches studied and these are 4 clutches out of 13 clutches in 2011, 8 out of 13 in 2010, 10 out of 15 in 2009, 4 out of 9 in 2008 and 3 out of 6 in 2007. Hatching success was 95.8 % in 2007, 95.15% in 2008, 97.44% in 2009, 96.03% in 2010 and 94.53% in 2011. The average hatching success was 95.8 ± 1.09%. Average unhatching rate in the farm was 4.2% where embryo died before hatching.
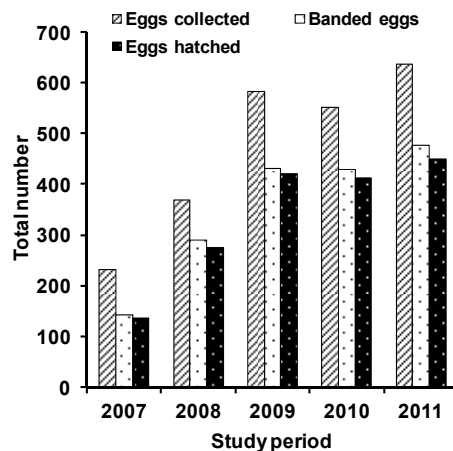

Fig. 2. Yearly hatching records of the eggs of saltwater crocodile in captivity (2007 - 2011).

In the wild, survivorship can be typically as low as 1% reported by Mayer et al. (1998), whereas survivorship of hatchlings hatched from eggs in captivity in our study was significantly high (95.8 ± 1.09%). It was reported that 70% hatching success was found in *C. porosus* when provided with ideal conditions (Mayer et al., 1998). The hatchlings were kept in mild disinfectant water in a basin and the basin was kept in the incubator for acclimatization of the hatchlings with prevailing temperature and humidity. The time required for hatching of banded eggs varied from 1 to 3 days. Hatchlings were placed in a dark, nurturing pen with shallow and hot water at 30 - 33°C until their body temperature increases to 35°C. Temperatures above 36°C or below 28°C have been found to increase significantly stress levels and mortality in hatchlings and should be avoided (Turton et al., 1994). Tanks can be concrete or plastic with simple and effective drainage and a constant warm temperature (Elsey et al., 1994; Mayer et al., 1998).

Conclusion

Keeping and breeding of crocodile in the captivity is an effective protective measure. Breeding also makes possible to commercially exploit crocodiles legally within the framework of CITES. We found that control of air temperature and air humidity can improves hatching rates. We also observed that protection from predators and providing provisioning food may increase survival rate of young crocodiles.

Acknowledgement

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Anotated checklist of birds of Rajshahi University campus: An update

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Abstract: The result of bird watching at Rajshahi University (RU) campus during a period from March 2008 to July 2012 is presented in this article. A total of 159 species of birds under 102 genera, 36 families and 13 orders were observed. Highest number of species (76) and genera (41) are passerines, of which the highest number of species belongs to Corvidae family. Among the non-passerines (83 species) maximum number of species is under the family Ardeidae. Three species remained unidentified. Among the birds of RU campus 121 species are residents of Bangladesh; 38 species are migratory, of which 29 are winter visitors, 2 are summer visitors and 7 are passage migrants. Maximum of the bird species are habitants of the open woods.

Key words: Birds, Rajshahi University, habitat.

Introduction

The University of Rajshahi (RU) the second largest university of Bangladesh, was established in 1953 with a land of 743 acres. The university campus is renowned for her natural beauty, and was named as *Motihar Green*. Though a number of infrastructures have been constructed for the faculties, offices and residents, which altered the ecological condition of the campus, but still it conserves the diverse natural habitats to a large extent.

RU campus is inhabited by quite a good number of wild animals among which the avifauna is the most significant and rich in regard to its diversity (unpublished reports). Wildlife scientists have published reports on the wildlife and avifauna of Bangladesh, and different areas of the country. Most of these literature are Hussain (1969, 1975, 2008), Harvey (1990), Khan (2008b), Khan (1982, 2008a), Sarker & Sarker (1986), Siddique *et al* (2008). Karmakar *et al.* (2010) published a report on the birds of Joypurhat District; Sarker *et al* (2000a,b, 2001) reported the avifauna of St. Martin's island, Bagkhali of Cox's Bazar and Moheshkhali island respectively. The birdsof two urban sites of Dhaka city was published by Sarker *et al* (2009), and the birds of Chittagong University campus was published by Ahsan and Khanom in 2005.

In 1976, for the first time a list of birds of the RU campus was published by Haque, who identified 76 species from the campus. At that time the university authority put on a ban on the wildlife hunting and poaching within the campus. The population of the wildlife including the birds became satisfactory until the late 1990's. A group of peoples residing the adjacent area of the campus are killing the wild reptiles and mammals, and poaching the water birds, which could not be stopped. Moreover, for the development of the infrastructures of the university, the natural habitats are being destroyed. As a result the amphibian, reptilian and mammalian populations of the campus have been decreased.

In 2008, a list of birds of the RU campus was prepared, where number of the species was found as 85. Which depicted that compared to other wildlife fauna, the avifauna is quite rich (unpublished report). To update the bird list of the RU campus field study on bird watching was started since 2008. In this paper the updated list of birds up to July 2012 is presented.

Methodology

The present report is prepared based on field studies. Bird watching and some conservation efforts were conducted during a period form March 2008 to Juy 2012. The field studies were based on bird watching, and for such studies the bird watchers using the following instruments were used for bird watching and photographing:

a) Digital Cameras: Still Camera; Video Camera
b) A pair of binoculars.

Bird watching: During bird watching the birds were photographed. Habitats of those birds were also studied. Call or song and morphs of birds which vary with the season, bird's age and reproductive phases, were also recorded and identified species wise.

In the present work bird watching was designed in two categories; (a) routine bird watching, (b) casual bird watching.

Routine bird watching: A routine bird watching at RU campus was carried throughout the study period, and it is still continuing. Except in rainy morning, a regular visit was made to different areas of the campus, for 2-4 hours. In summer days bird watching started around 5.30 am and in the winter it was started from 6.30 am. Some times short visits were made for an hour to a definite site at 11.30 am or at 2 pm and at 5 pm.

During each visit a definite area of around one kilometre was selected. While walking, keen observation was made for every movement of branches and leaves of trees, moving objects nearby the water bodies and birds call. Every section of the habitats was screened in search of bird by keen watching. No study was carried at night.

Casual bird watching: While walking in the RU campus at any time looking for birds was continued randomly. Whenever a bird's call, or flight, or movements in the bushes and trees or besides the water bodies, roosting on trees or electric wires, were observed, and photographs were taken wherever possible.

Bird identification and habitat study
All the bird species presented in this report were photographed during the study period. The birds were then identified from their images and with the help of published literature, *viz.*, Baker (1922-30), Ali (1961, 2002), Ali & Ripley (1968-1972), Khan R (2008b), Grewal *et al.* (1993), Siddique *et al.* (2008), and also using the documents from the internet. Not only the bird species, but their habitats were also confirmed from the literature published in different books and journals.

Habitats: Habitats of the RU campus were categorized as garden (G), cropland (CL), light forest having moderately high trees and bushes underneath (LF), open woodland which includes trees of different height, bushes and vines on the trees, varieties of herbs and grasses underneath (OW), grassland (GL), wet land which includes all types of water bodies (WL) and human habitation, the buildings (HH).

Most of the agricultural lands are situated at the eastern and north-western parts of the campus. Faculty, administration buildings and most of the residents are present at the central and western part. All the roads and the infrastructures are surrounded by bushes and trees. The birds were seen to live and forage at the above mentioned niches of the campus. Continuous bird watching at the campus makes it possible to chalk out the route and definite sites of the birds of the campus.

Results and Discussion
During the study period of 52 months 159 species of birds of 13 orders, 36 families and 102 genera from the Rajshahi University campus were recorded (Table 1). Among the recorded birds one duck, one Bee-eater and a parakeet remained unidentified. Total 83 and 76 species of non-passerine and passerine species were recorded respectively.

Table 1. List of birds of Rajshahi University Campus

Order/Family	Scientific Name	English Name	Bengali Name	Sl. no. of species
1. Anseriformes 1) Anatidae (Ducks and Geese)	*Dendrocygna bicolor* (Vieillot, 1816)	Large Whistling-Duck	Boro Sarali	1
	Dendrocygna javanica (Horsfield, 1821)	Lesser Whistling-Duck	Choto Sarali	2
	Aythya fuligula Linnaeus, 1758	Tufted Pochard	Choto Dubalu	3
	Nettapus coromandelianus, Gmelin, 1789	Cotton Pygmy-Goose	Dhola Balihansh	4
	-	Duck (unidentified)	-	5
2. Turniciformes 2) Turnicidae	*Turnix suscitator* (Gmelin, 1789)	Barred Buttonquail	Dagee Nataboter	6
3. Piciformes 3) Picidae (Woodpeckers)	*Dinopium benghalense* (Linnaeus, 1758)	Lesser Golden-backed Woodpecker	Kaththokra	7
	Dinopium javanense (Ljungh, 1797)	Common Golden-backed Woodpecker	Bormee Kaththokra	8
	Dendrocopos macei (Vieillot, 1818)	Fulvous-breasted Woodpecker, Pied Woodpecker	Pakra Kaththokra	9
	Picus xanthopygaeus (Gray & Gray, 1847)	Streak-throated Woodpecker	Doragola Kaththokra	10
4) Megalaimidae (Barbets)	*Megalaima asiatica* (Latham, 1790)	Blue Throated Barbet	Neelgola Bashanta Bouri	11

Order/Family	Scientific Name	English Name	Bengali Name	Sl. no. of species
	Megalaima haemacephala (Muller, 1776)	Coppersmith Barbet	Shekra, Bhogiroth	12
	Megalaima lineata (Vieillot, 1816)	Lineated Barbet	Dagee Boshonto	13
4. Upupiformes 5) Upupidae	*Upupa epops* Linnaeus, 1758	Common, Hoopoe	Hoodhood	14
5. Coraciiformes 6) Coraciidae (Rollers)	*Coracias bengalensis* (Linnaeus, 1758)	Indian Roller	Nilkantha	15
7) Alcedinidae (Kingfishers)	*Alcedo atthis* (Linnaeus, 1758)	Common Kingfisher/ Small Blue ingfisher	Chhoto Machranga	16
	Halcyon smyrnensis (Linnaeus, 1758)	White-breasted Kingfisher	Sadagola Machranga	17
8) Dacelonidae	*Pelargopsis capensis* (Linnaeus, 1766)	Stork-Billed Kingfisher	Meghau	18
9) Cerylidae	*Ceryle rudis* (Linnaeus, 1758)	Lesser Pied Kingfisher	Pakra Machranga	19
10) Meropidae (Bee-eaters)	*Merops orientalis* Latham, 1802	Green Bee-eater	Shobuj Suichora, Banspata	20
	Merops philippinus (Linnaeus, 1766)	Blue tailed Bee-eater	Neellej Suichora	21
	Meropes leschenaultia Vieillot, 1817	Chestnut-headed Bee-eater	Patkile-matha Suichora	22
	Merops spp.(unidentified)	Bee-eater	-	23
6. Cuculiformes 11) Cuculidae (Cuckoos)				24
	Eudynamys scolopaceus(Linnaeus, 1758)	Asian Koel /Indian Koel	Kokil	
	Clamator jacobinus (Boddaert, 1783)	Pied-crested Cuckoo	Pakra Papia	25
	Hierococcyx varius (Vahl, 1797)	Common Hawk Cuckoo	Pati Chokhgalo	26
	Hierococcyx nisicolor Horsfield, 1821	Hodgson's Hawk Cuckoo	Hodgsoni Chokhgalo	27
	Cuculus micropterus Gould, 1837	Indian Cuckoo	Bou-kotha-kao	28
	Cacomantis passerinus (Scopoli, 1786)	Indian Plaintive Cuckoo	Karun Papia	29
12) Centropodidae (Coucals)	*Centropus sinensis* (Stephens, 1815)	Greater Cuocal	Kanakua, Boro Kubo	30
	Centropus bengalensis (Gmelin, 1788)	Lesser Coucal	Chhoto Kubo	31
7. Psittaciformes 13) Psittacidae	*Psittacula krameri* (Scopoli, 1769)	Roseringed Parakeet	Konthi Tiya	32
	Psittacula eupatria (Hodgson, 1836)	Large Indian Parakeet	Chandona Tiya	33
	Psittacula cyanocephala Linnaeus, 1758	Plum-headed Parakeet	Lalmatha Tiya	34
	Psittacula sp.	-	-	35
8. Apodiformes 14) Apodidae	*Cypsiurus balasiensis* Gray, 1829	Asian Palm- Swift	Tal Batashi	36
	Apus affinis (Gray, 1830)	House Swipt	Ghar Barashi	37
9. Strigiformes 15) Tytonidae	*Tyto alba* (Scopoli, 1769)	Barn Owl	Laksmi pencha	38
16) Strigidae	*Athene brama* (Temminck, 1821)	Spotted Owlet	Kutare pencha	39

Order/Family	Scientific Name	English Name	Bengali Name	Sl. no. of species
	Otus bakkamoena Hodgson, 1836	Collared Scops Owl	Nimpokh	40
	Bubo bubo (Franklin, 1831)	Indian Great-horned Owl	Hutum pencha	41
	Ninox scutulata Raffles, 1822	Brown Hawk Owl	Khoira hikrepencha	42
	Glaucidium radiatum (Tickell, 1833)	Jungle Owlet	Chhoto Kalipencha	43
10.Columbiformes 17) Columbidae (Doves & Pigeons)	*Streptopelia chinensis* (Scopoli, 1786)	Spotted Dove	Teela Ghughu, Teelima Ghughu	44
	Streptopelia tranquebarica (Hermann, 1804)	Red turtle Dove	Lal Ghughu, Jongla Ghughu	45
	Columba livia Gmelin, 1789	Rock Pigeon	Jalali Kabutor	46
11. Gruiformes 18) Rallidae (Crakes, Waterhen)	*Amaurornis phoenicurus* (Pennant, 1769)	White-breasted Waterhen	Dahuk	47
	Amaurornis akool (Sykes, 1832)	Brown Crake	Badami Jhilli	48
	Gallinula chloropus (Linnaeus, 1758)	Common Moorhen	Jal Morog	49
	Metopidius indicus Latham, 1790	Bronze-winged Jacana	Dol Pipi	50
12. Ciconiiformes 19) Scolopacidae	*Tringa glareola* Linnaeus, 1758	Wood Sandpiper	Tila Cha Pakhi	51
	Tringa ochropus Linnaeus, 1758	Green Sandpiper	Sabujavo Cha Pakhi	52
	Tringa stagnatilis (Bechstein, 1803)	Marsh Sandpiper	Bil Batan	53
	Actitis hypoleucos Linnaeus, 1758	Common Sandpiper	Cha Pakhi	54
	Calidris ferruginea Pontoppidan, 1763	Curlew Sandpiper	Gulinda Batan	55
	Gallinago gallinago Brisson, 1760	Common Snipe	Kadakhocha	56
20) Accipitridae	*Ichthyophaga ichthyaetus* (Horsfield, 1821)	Grey-headed Fish Eagle	Chhoto Mach-mural	57
	Haliastur Indus Boddaert 1783	Brahminy Kite	Shonkho Cheel, Lalchil	58
	Accipiter badius Gmelin, 1788	Shikra	Turkibaj	59
	Milvus migrans (Boddaert, 1783)	Black Kite	Bhubanchil	60
	Falco tinnunculus Linnaeus, 1758	Common Kestrel	Chhoto Shikari Baj	61
	Falco peregrinus Tunstall,1771	Peregrine Falcon	Peregrine Shahin	62
	Falco chicquera Daudin, 1800	Red-necked Falcon	Toormati	63
	Elanus caeruleus (Desfontaines, 1789)	Black-shouldered Kite	Sada Chil	64
21) Podicipedae	*Tachybaptus ruficollis* (Pallas, 1764)	Little Grebe	Chhoto Dubalu	65
22) Phalacrocoracidae (Cormorants, Garters)	*Phalacrocorax carbo* (Linnaeus, 1766)	Great Cormorant	Boro Pankauri	66
	Phalacrocorax niger Vieillot, 1817	Little Cormorant	Chhoto Pankauri	67
	Phalacrocorax fuscicollis Stephens, 1826	Indian Cormorant, Shag	Majhari Pankauri	68
	Anhinga melanogaster (Pennant, 1769)	Darter or Snake bird	Sap Pakhi, Goyar	69

Order/Family	Scientific Name	English Name	Bengali Name	Sl. no. of species
23) Ardeidae Egrets, Bitterns, Herons)	*Egretta intermedia* (Wagler, 1827)	Median Egret	Maijhla Bok	70
	Egretta garzetta (Linnaeus, 1766)	Little egret	Choto Sada bok	71
	Casmerodius albus (Linnaeus, 1758)	Great Egret	Baro Sada bok	72
	Ixobrychus cinnamomeus (Gmelin, 1789)	Cinnamon Bittern	Lal Bok, Nol Ghonga	73
	Botaurus stellaris (Linnaeus, 1758)	Great Bittern	Boro Nol Ghonga	74
	Ardea cinerea Linnaeus, 1758	Grey Heron	Dhushor Bok	75
	Ardea purpuria (Linnaeus, 1766)	Purple Heron	Beguni Bok	76
	-	Heron (unidentified)	-	77
	Butorides striatus (Linnaeus, 1758)	Little Green Heron	Sabuj Bok	78
	Bubulcus ibis (Linnaeus, 1758)	Cattle heron	Go-Bok	79
	Ardeola grayii (Sykes, 1832)	Indian pond Heron	Kani Bok	80
	Nycticorax nycticorax (Linnaeus, 1758)	Night Heron	Nishi Bok	81
24) Ciconiidae	*Anastomus oscitans* (Boddaert, 1783)	Open-bill Stork	Shamuk Khol	82
13. Passeriformes 25) Lanidae	*Lanius cristatus* Linnaeus, 1758	Brown Shrike	Badami Koshai Pakhi	83
	Lanius cristatus cristatus Linnaeus, 1758	Brown Shrike	Badami Koshai Pakhi	84
	Lanius vittatus Valenciennes, 1826	Baybacked Shrike	Anjon	85
	Lanius schach Linnaeus, 1758	Rufous backed Shrike	Lenja Latora	86
26) Corvidae (Crows, Minivets, Orioles, and others)	*Dendrocitta vagabunda* (Latham 1790)	Rufous Treepie	Harichacha	87
	Corvus splendens Vieillot, 1818	House Crow	Pati Kak	88
	Corvus macrorhynchos (Wagler, 1827)	Jungle Crow	Danr Kak	89
	Corvus corax Linnaeus, 1758	Common Raven	-	90
	Oriolus chinensis Linnaeus, 1766	Black-napped Oriole	Kajolchokh Benebou	91
	Oriolus oriolus Linnaeus, 1758	Golden Oriole	Sonabou	92
	Oriolus xanthornus (Linnaeus, 1758)	Black-Headed Oriole	Kalomatha Benebou	93
	Terpsiphone paradise (Latham, 1758)	Paradise flycatcher	Asio Shabubuli, Dudhraj	94
	Pericrocotus divaricatus (Raffles, 1822)	Ashy Minivet	Dhushor Sat Soheli	95
	Pericrocotus roseus Vieillot, 1818	Rosy Minivet	Golapi Sat Soheli	96
	Pericrocotus cinnamomeus Linnaeus, 1766	Small Minivet	Chhoto Sat Soheli	97
	Pericrocotus flammeus Forster, 1781	Scarlet Minivet	Sindure Soheli, Altapari	98
	Hemipus picatus (Sykes, 1832)	Pied Flycatcher Shrike	Sada-kalo Latora	99
	Tephrodornis pondicerianus (Gmelin, 1789)	Common Woodshrike	Kabashi	100
	Tephrodornis gularis (Raffles, 1822)	Large Woodshrike	Boro Kabashi	101
	Rhipidura albicollis (Vieillot, 1818)	White-throated Fantail	Lej nachuni	102
	Coracina melanoptera (Rupell, 1839)	Black-headed Cuckoo-shrike	Kalomatha Kabashi	103
	Artamus fuscus Vieillot, 1817	Ashy Woodswallow	Mete latora	104

Order/Family	Scientific Name	English Name	Bengali Name	Sl. no. of species
	Aegithina tiphia (Linnaeus, 1758)	Common Iora	Fatikjal, Chatok	105
27) Dicruridae (Drongos)	*Dicrurus macrocercus* (Vieillot, 1817)	Black Drongo	Baro Fingey	106
	Dicrurus aeneus Vieillot, 1817	Bronzed Drongo	Bronze Fingey/ Chhoto Fingey	107
	Dicrurus annectans (Hodgson, 1836)	Crow-billed Drongo	Kak-chonchu Fingey	108
	Dicrurus ludwigii A. Smith, 1834	Squared-tailed rongo	-	109
	Dicrurus leucophaeus Vieillot, 1817	Ashy Drongo	Dhusharavo Fingey	110
	Dicrurus caerulencens Linnaeus, 1766	White-bellied Drongo	Sada-pet Fingey	111
28) Muscicapidae	*Zoothera citrine* (Latham, 1790)	Orange-headed Thrush	Komla Bou/ Komla Dama	112
	Zoothera dauma (Latham, 1790)	Scaly Thrush	Ashtey Dama	113
	Muscicapa dauurica Pallas, 1811	Asian-brown flycatcher	Badami Chatok	114
	Muscicapa muttui (Layard, 1854)	Brown-breasted flycatcher	Lalbuk Chatok	115
	Eumyias thalassina (Swainson, 1838)	Verditer Flycatcher	Firoza Chatok	116
	Cyornis pallipes (Jerdon, 1840)	White-bellied Blue-Flycatcher	Dholapet Neelchotok	117
	Culicicapa ceylonensis (Swainson, 1820)	Grey-headed Canary Flycatcher	Zard Futki	118
	Copsychus saularis (Linnaeus, 1758)	Magpie Robin	Doel	119
	Copsychus malabaricus (Scopoli, 1788)	Shama	Shama	120
	Saxicola leucura (Blyth, 1847)	White-tailed Stone Chat	Sada Lej Fidda	121
	Luscinia svecica (Linnaeus, 1758)	Bluethroat	Neelgola Fidda	122
	Phoenicurus orchruros Gmelin, 1774	Black Redstart	Kalo Girdi	123
29) Sturnidae (Starlings)	*Sturnus contra* (Linnaeus, 1758)	Indian Pied Starling	Gubre Shalik, Pakra Shalik	124
	Sturnus malabaricus (Gmelin, 1789)	Chestnut-tailed Starling	Kath Shalik	125
	Sturnus pagodarum (Gmelin, 1789)	Brahminy Starling	Bamon Shalik	126
	Sturnus vulgaris, Linnaeus, 1758	Common Starling	Chitra Shalik	127
	Acridotheres fuscus (Wagler, 1827)	White-vented Myna/ Jungle Myna	Jhunti Shalik	128
	Acridotheres tristis (Linnaeus, 1766)	Common Myna	Bhat Shalik	129
	Acridotheres ginginianus (Latham, 1790)	Bank Myna	Gang Shalik	130
30) Paridae	*Parus major* Linnaeus, 1758	Great Tit, Grey Tit	Ramgangra	131
31) Picnonotidae	*Pycnonotus jocosus* (Linnaeus, 1758)	Red Whiskered Bulbul	Sipahi Bulbul	132

Order/Family	Scientific Name	English Name	Bengali Name	Sl. no. of species
(Bulbuls)	*Pycnonotus cafer* Sub sp. *bengalensis* (Linnaeus, 1766)	Red Vented Bulbul	Bangla Bulbul, Kalo Bulbul	133
	Pycnonotus xantholaemus (Jerdon, 1845)	Yellow-throated Bulbul	Halud-gala Bulbul	134
32) Cisticolidae	*Cisticola juncidis* (Rafinesque, 1810)	Zitting Warbler	Bhomra Soton	135
	Prinia hodgsonii Blyth, 1844	Grey-breasted Prinia	Metebook Prina	136
	Prinia socialis Sykes, 1832	Ashy Prinia	Dhushor Prina	137
	Prinia inornata Sykes, 1832	Plain Prinia	Sadharon Prina	138
33) Sylviidae	*Orthotomus sutorius* (Pennant, 1769)	Common Tailor bird	Pati Tuntuni	139
	Turdoides striatus (Dumont 1823)	Jungle Babbler, Seven Brothers	Chatarey, Satbhaiya	140
	Turdoides earlei (Blyth, 1844)	Striated Babbler	Chhit Chhatarey	141
	Acrocephalus dumetorum Blyth, 1849	Reed Warbler	Tikra	142
34) Alaudidae	*Alauda gulgula* Franklin, 1831	Oriental Skylark	Udoi Bhorot	143
	Mirafra assamica Horsfield, 1840	Bengal Bush Lark	Bangla Jharbhorot	144
35) Nectariniidae (Flower peckers)	*Leptocoma zeylonica* (Linnaeus, 1766)	Purplerumped Sunbird	Begunikomor Moutushi	145
	Cinnyris asiaticus (Latham, 1790)	Purple Sunbird	Beguni Moutushi	146
	Nectarina lotenia (Linnaeus, 1766)	Loten's Sunbird	Moutushi	147
	Dicaeum erythrorhynchos (Latham, 1970)	Tickell's Flowerpecker	Mete Thot Fuljhuri	148
	Arachnothera longirostra (Latham, 1970)	Little Spiderhunter	Chhoto Makormar	149
36) Passeridae	*Passer domesticus* (Linnaeus, 1758)	House Sparrow	Chorui	150
	Motacilla citreola Pallas, 1776	Citrine Wagtail	Holdematha Khonjon	151
	Motacilla alba alba Linnaeus, 1758	White Wagtail	Sada Khonjon	152
	M. a. leucopsis (Linnaeus, 1758)	White Wagtail	Sada Khonjon	153
	M. a. personata (Linnaeus, 1758)	White Wagtail	Sada Khonjon	154
	Motacilla cinerea Tunstall, 1771	Grey Wagtail	Dhusor Khonjon	155
	Motacilla maderaspatensis Gmelin, 1789	Large Pied Wagtail	Pakra Khonjon	156
	Anthus rufulus (Gmelin, 1789)	Paddy field Pipit	Dhani Tulika	157
	Anthus hodgsoni Richmond, 1907	Tree Pipit	Indian Tree Pipit	158
	Lonchura malacca (Linnaeus, 1766)	Black-headed Munia	Kalomatha Munia	159

Highest number of families (N=14) belonged to the order Passeriformes. The order includes 44 genera (43.04%) and 76 species (66.86%). Maximum number of passerine species was found in the family Corvidae, and the families like Artamidae and Paridae included only one species. Among the non-passerines the order Upupiformes included only one genus and species (0.63%). Among the non-passerines the maximum number of genus was found under the order Ardeidae (12, 8.28%).

Abundance of the observed birds is presented in Table 2. Among these species 30 were found very common (VC), 30 were common (C), 34 were fairly common (FC), 32 were seen few in number (F) and not every time during the study period, and 34 species were noticed rarely (R) (Table 2).

The RU campus had been divided into seven different habitats. Number of the bird species living or foraging

at these habitats, are shown in Table 3. Some of the species share more than one habitat, but their number was few. Birds of all other habitats were seen near the human habitations, but the exceptions were the grassland birds. The assessed status of the birds recorded from the RU campus, are shown in Table 4.

Among these bird species 121 were residents of Bangladesh and 38 were migratory. The number of winter visitors was 29, summer visitor was 2, and 7 species were passage migrants.

According to Haque (1976) the number of bird species of RU campus was only 76. At that time there were more wild habitats in the campus and the number of buildings and people was less. In 2012 the number of species increased to 159 when a large area of the campus has lost under

the bricks and concrete. The reasons behind such a difference of bird species availability are: i) Haque in 1976 did not covered a wide area of the campus; ii) the tenure of study was only one year, where as the present study was carried for 52 months; iii) only binocular was used to see the birds, in the present study powerful camera lenses was used along with video camera, which provided a better chance to record the image of the birds sitting at far; iv) availability of books and internet with birds' photographs made easier to identify the uncommon birds which was not available at that time.

Table 2. Abundance of bird species under different families at RU Campus (by number).

Sl. no of family	Family	VC	C	FC	F	R	Total Species
1	Anatidae	-	-	-	-	5	5
2	Turnicidae	-	-	-	-	1	1
3	Picidae	2	1	2	-	-	5
4	Megalaimidae	-	2	-	1	1	4
5	Upupidae	-	-	-	-	1	1
6	Coraciidae	-	1	-	-	-	1
7	Alcenidae	2	-	-	-	-	2
8	Dacelonidae	-	-	1	-	-	1
9	Cerylidae	-	1	-	-	-	1
10	Meropidae	1	1	-	1	1	4
11	Cuculidae	2	1	2	1	1	7
12	Centropodidae	-	-	-	2	-	2
13	Psittacidae	-	1	1	-	2	4
14	Apodidae	2	-	-	-	-	2
15	Tytonidae	1	-	-	-	-	1
16	Strigidae	1	1	3	-	-	5
17	Columbidae	1	1	-	1	-	3
18	Rallidae	-	-	1	1	1	3
19	Scolopacidae	-	-	-	3	3	6
20	Accipitridae	1	1	1	3	2	8
21	Podicipedae	-	-	-	-	1	1
22	Phalacrocoracidae	-	1	1	2	-	4
23	Ardeidae	3	4	2	2	2	13
24	Lanidae	-	-	3	1	-	4
25	Corvidae	3	4	5	3	2	17
26	Dicruridae	1	2	-	2	1	6
27	Muscicapidae	1	1	4	2	4	12
28	Sturnidae	3	3	-	-	1	7
29	Paridae	1	-	-	-	-	1
30	Aegithinidae	-	-	1	-	-	1
31	Pycnonotidae	2	-	-	-	1	3
32	Cisticolidae	-	-	2	2	-	4
33	Sylviidae	1	1	-	1	1	4
34	Alaudidae	-	-	-	2	-	2
35	Nectariniidae	-	-	2	1	2	5
36	Passeridae	2	3	3	1	1	9
Total		30	30	34	32	34	159

Table 3. Species diversity of birds at different habitats of RU campus

Habitats	G	CL	LF	OW	GL	WL	HH
G	20	1	6	11	2	-	5
CL	-	20	1	1	1	11	1
LF	-	-	24	35	-	2	1
OW	-	-	-	49	5	15	8
GL	-	-	-	-	10	5	-
WL	-	-	-	-	-	30	2
HH	-	-	-	-	-	-	5

Table 4. Assessed status of the recorded bird species of RU campus

Status	Non-Passerine		Passerine		Total	
	No.	%	No.	%	No.	%
Very Common	16	10.12	14	8.86	30	18.98
Common	16	10.12	14	8.86	30	18.98
Fairly Common	14	8.86	20	12.66	34	21.52
Few	16	10.12	15	8.86	30	18.98
Rare	21	13.29	13	8.23	34	21.52
Total	83		76		159	

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Embryonic development of the estuarine crab *Neosarmatium indicum* (Crustacea: Brachyura: Sesarmidae) from the mangroves of the Okinawa Island, Japan

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Abstract : The complete embryonic development of the mangrove sesarmid crab *Neosarmatium indicum* (A. Milne Edwards, 1868) was described based on internal and external morphological changes in live fertilized eggs reared in the laboratory. Several pairs of *N. indicum* were collected from the Nuha River mangrove swamp of the southern Okinawa Island, Japan, which is consisted mainly with the mangrove *Kandelia candel*, and densely populated by the genus *Perisesarma* and *Neosarmatium indicum* . The fertilized eggs were macrolecithal, centrolecithal and spherical in shape, filled with uniform dark olive colour, without evidence of any development. The diameter of fertilized egg was 0.36 mm, which increased to 0.47 mm before hatching. Embryo development from fertilized eggs to hatching (first zoea stage) lasted average of 16 days at 25^0C and salinity at 80‰. Sixteen stages of embryonic development were categorized by following continuous observation using an optical DIC microscope equipped with digital camera, video camera and printer. After 24 hours of incubation, fertilized eggs became 32-celled stage of development. Before hatching, many chromatophores (mostly black) were evident in the abdominal segments and the telson of embryos. At the end of 16 days incubation, the zoea larvae were successfully hatched out, which were reared in the laboratory conditions for further development.

Key words: *Neosarmatium indicum,* zoea, development, fertilization, mangrove.

Introduction

Information or documentation dealing with physiology, reproduction and embryology of brachyuran crabs are almost entirely lacking (Garcia-Guerrero & Hendrickx 2004), where the other groups of decapod crustaceans including the caridean shrimp, marine lobster and river crayfish, have received most of the attention and their embryology is better documented (Clarke *et al.,* 1990, Helluy & Beltz 1991, Sandeman & Sandeman 1991, Nazari *et al.*, 2000, Müller *et al.,* 2003, Garcia-Guerrero *et al.*, 2003).

In the brachyuran crabs, females incubate their eggs in the body cavity, which remain attached to the pleopods of abdomen, from spawning to hatching (Guinot 1979, Garcia-Guerrero *et al.,* 2003), and show a great diversity of embryonic development with a significant variation in incubation period and egg diameter (Anderson 1982, Hines 1982, Zimmerman & Felder 1991, Nagao *et al.,* 1999, Garcia-Guerrero *et al.* 2003, Garcia-Guerrero & Hendrickx 2004). The embryology of brachyuran crabs are recent and few species are included from different habitats as hair crab *Erimacrus isenbeckii* (Atelecyclidae) by Nagao *et al.,* (1999), estuarine or mud crabs *Chasmagnathus granulata* and *Cytograpsus angulatus* (Varunidae) by Bas & Spivak (2000), fiddler crab or mangrove ghost crab *Uca lacteal* (Ocypodidae) by Yamaguchi (2001), mangrove crabs *Ucides cordatus* (Ocypodidae) by Pinheiro & Hattori (2003), *Goniopsis pulchra* (Grapsidae) and *Aratus pisonii* (Sesarmidae) by Garcia-Guerrero & Hendrickx (2004), green shore crab *Carcinus maenas* (Portunidae)

by Chung and Webster (2004) and Hartnoll & Paul (1982).

However, there is no information available on the embryology of *Neosarmatium indicum* (A. Milne Edwards, 1868), the most common crab typically associated with mangroves in tropical and subtropical estuaries and coastal lagoons in the Indo-West Pacific, being known from the Bay of Bengal to the Andaman Islands, Sri Lanka, India, Malay Archipelago, the Philippines, Hong Kong, Taiwan, Korea, and Tokyo Bay to Kyushu and the Ryukyu Islands of Japan. This species live in burrow constructed in the edges or within the mangroves or in the reed marsh higher than ordinary high water mark, and among the roots, trunk, and lower branches of mangrove trees. This crab commonly occurred in the mangroves of the Ryukyu Islands, Japan (Islam and Shokita 2000, 2002, Islam *et al.*, 2000). The complete larval development of *Perisesarma bidens* has been described in detail by Islam and Shokita (2000), although they did not investigate the embryonic development of this species. The objectives of the present study are to provide the detail descriptions of all embryonic stages, and to compare them with those of other species of brachyuran crab.

Materials and Methods

Several pairs of *Neosarmatium indicum* were collected by hand during spawning season from the Nuha River mangrove swamp, southern Okinawa Island ($26^005'N{\sim}26^052'N$, $127^040'E{\sim}128^016'E$), Japan on 4 June 2005 to June 2006. This swamp is a unique mangrove forest densely populated by *Kandelia candel*

and the experimental species. Collected crabs were brought to the Laboratory of Developmental Biology, Faculty of Science, University of the Ryukyus, Okinawa, and maintained in plastic containers. Among them, two pairs were captured during active in courtship behavior, brought to the laboratory separately and reared as paired in plastic containers (30 cm X 20 cm X 20 cm), filled with filtered seawater, maintained with constant gently aeration, and provided with some rough beach stones as shelter. Photoperiod was kept at 12h dark: 12h light condition. Water was maintained at 25℃ and salinity at 28‰. The filtered seawater was changed daily until hatching. Freshly fallen brawn leaves of *K. candel* were offered as food to the female daily.

After copulation, fertilization occurred within the female body cavity and the fertilized eggs were extruded within 48 hours, which attached to the pleopods of the female abdomen through the stalk or funiculus and the investment coat. Just after oviposition, a sample of at least 10 eggs from each female was removed every hour up to cleavage and then every 24 hours up to hatching to examine the embryonic development. Development duration is expressed as number of hours/ days after oviposition or spawning of the female. Embryos were described as staging method, adapted for crustacean eggs by Sandeman and Sandeman (1991).

Examination of freshly removed (living) eggs was done using an optical DIC microscope (20x magnification) equipped with digital camera, video camera and a printer (Nikon M-B, 291). Fertilized eggs were placed in 80% filtered seawater using excavated slides to observe the cleavage, embryo morphology, growth and yolk-tissues proportion in at least 10 embryos in lateral and frontal views. During examination, shapes, color changes, heartbeat signal and eye formations were also annotated. Diameter of fertilized eggs/ embryos was measured at least 10 embryos to the nearest 0.01 mm in long axis at 20x magnification with a micrometer objective attached to the microscope. Unfertilized eggs were collected directly from the ovary by sacrificing the female to observe their maturation and to compare with fertilized eggs only. After hatching, the larvae were reared under laboratory conditions up to first crab stage, which datum was not included in this manuscript. Specimens of the female *N. indicum* and the embryos have been deposited in the Laboratory of Developmental Biology, University of the Ryukyus, Okinawa, Japan.

Results

The copulation period of *N. indicum* was 5-7 hours. After copulation, fertilization occurred within the female body cavity and the fertilized eggs were extruded within 48 hours, which attached to the pleopods of the female abdomen through the stalk or funiculus and the investment coat, encased. Copulated females were observed successfully from spawning to hatching. Developmental stages, average diameter (long axis)

and incubation periods of fertilized eggs/ embryos are presented in Table 1. Embryonic development of *N. indicum* was completed within 16 days of incubation. A chronology of embryological events was took place at constant room temperature of 25°C.

Table 1. Developmental stages, average diameter and incubation period of the fertilized egg/embryos of *Neosarmatium indicum* (A. Milne Edwards) from spawning to hatching at 25℃ and 28‰ salinity.

Development stages	Size in diameter (µm) (Mean ± SD, N=10)	Durations/ incubation period
Fertilized egg		
Stage-1 (Fig. 1A-B)	368.76 ± 8.48	2-10 h
Cleavage		
Stage-2 (Fig. 1C-G)	370.21 ± 8.12	12-17 h
Blastula		
Stage-3 (Fig. 1H)	375.37 ± 9.30	18-40 h
Gastrula		
Stage-4 (Fig. 1I)	377.71 ± 9.32	41-60 h
Developing embryos		
Stage-5 (Fig. 1J-K)	384.25 ± 8.51	3-4 d
Stage-6 (Fig. 1L)	388.18 ± 8.31	5 d
Stage-7 (Fig. 2A)	391.21 ± 6.97	6 d
Stage-8 (Fig. 2B-C)	395.07 ± 6.88	7 d
Stage-9 (Fig. 2D-E)	401.17 ± 5.39	8-9 d
Stage-10 (Fig. 2F)	405.43 ± 6.79	10 d
Stage-11 (Fig. 2G)	420.33 ± 7.12	11 d
Stage-12 (Fig. 2H)	427.72 ± 8.01	12-13 d
Stage-13 (Fig. 2I)	444.31 ± 8.22	14 d
Stage-14 (Fig. 2J)	459.25 ± 7.42	15 d
Before hatching		
Stage-15 (Fig. 2K)	471.29 ± 8.19	16 d
Hatching		
Stage- Zoea (Fig. 2N-O)	1021.91 ± 7.17	17 d

Descriptions

Fertilized egg

Stage-1 (2-10 hours of incubation, Fig. 1A-B): Before cleavage, the fertilized eggs were macrolecithal, centrolecithal and spherical in shape, and filled with a uniform dark olive colour, without evidence of any development (Fig.1A). No polar bodies were observed in eggs examined immediately after they have been laid/ spawned (Fig.1B). Three distinct egg membranes were observed immediate after egg shifted in to the pleopods. The first polar body like structure was extruded out from the zygote after one hour of incubation. At that time the yolk components were yellowish in colour (Fig.1B).

Cleavage

Stage-2 (12-17 hours of incubation, Fig.1C-G): The first cleavage/ division occurred within 11-12 hours of incubation. The cleavage was holoblastic and characterized by early occurrence of cleavage cavity. At the 2-celled stage, a wide cavity or cleavage furrow was

formed between the blastomeres (Fig. 1C). The first division was typical, passed through the animal vegetal pole, although it might be inclined due to absorbed of first polar body, which ultimately gave rise to a longer blastomere. The 2-celled embryos under went the second equal division of cleavage within the next hour and the embryos reached at 4-celled stage producing four large blastomeres (Fig. 1D). At the end of 14 hours of incubation, the 4-celled embryos underwent to the third cleavage and reached at 8-celled stage (Fig.1E). By the fourth cleavage, developing 8-celled embryos reached at 16-celled stage after the end of fifteen hours of incubation (Fig. 1F). The fourth cleaved embryos usually underwent fifth cleavage within 16-17 hours of incubation (Fig. 1G). Due to the density of blastomeres, the later stage of cleavage could not be detected clearly.
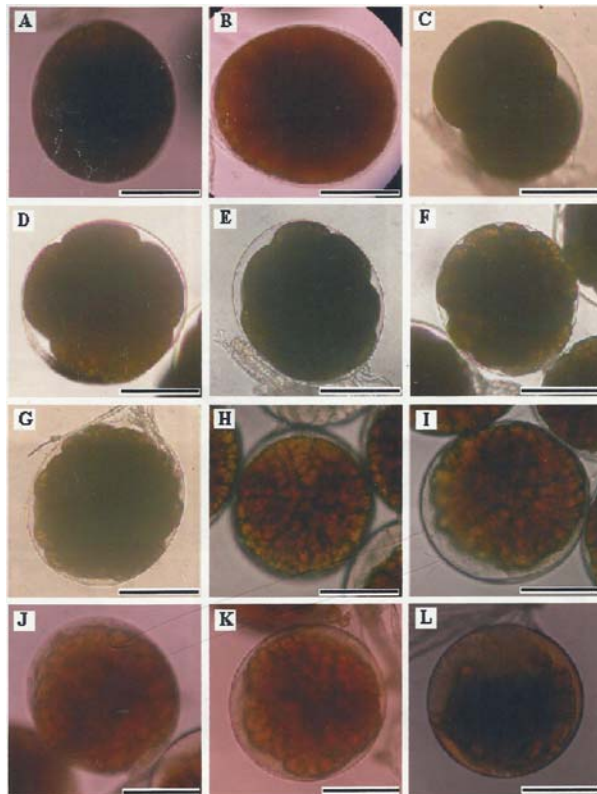
Fig.1. Stages of development in embryos of *Neosarmatium indicum* (A. Milne Edwards). A, unfertilized egg; B, Stage-1 (fertilized eggs before cleavage); C, Stage-2 (cleavage: C, 2-cell; D, 4-cell; E, 8-cell; F, 16-cell; G, 32-cell stage); H, Stage-3 (Blastula); I, Stage-4 (Gastrula); J-K, Stage-5 (developing embryos); L, Stage-6. Scale bars = 100 μm.

Blastula
Stage-3 (18-40 hours of incubation, Fig. 1H): The blastula stage and advanced blastula stage of development is impossible to distinguish individual cells after 18-40 hours of incubation. In these stages, the outer appearances of

developing embryos are very similar to that of undivided or un-cleaved fertilized eggs.

Gastrula
Stage-4 (41-48 hours of incubation, Fig. I: After 41-48 hours of incubation period, the developing embryos reached at the gastrula stage (Fig.1 I).

Developing embryos
Stage-5 (3-4 days of incubation, Fig. 1J-K): Yolk droplets are somewhat larger and with a lighter yellowish color at the beginning of three days of incubation period (Fig1J). Yolk free portion was first observed at the end of four days of incubation. Evidence of tissues was observed in yolk free portion (Fig. 1K).

Stage-6 (5 days of incubation, Fig.1L): Yolk droplets were larger and more distinct with yellowish color at the five days of incubation period. Yolk free portion was increased in size. Any special organ was not observed.

Stage-7 (6 days of incubation, Fig. 2A): At the six days of incubation period, further increase of yolk free portion was observed. Evidence of tissue was more distinct and clear of the yolk free portion.
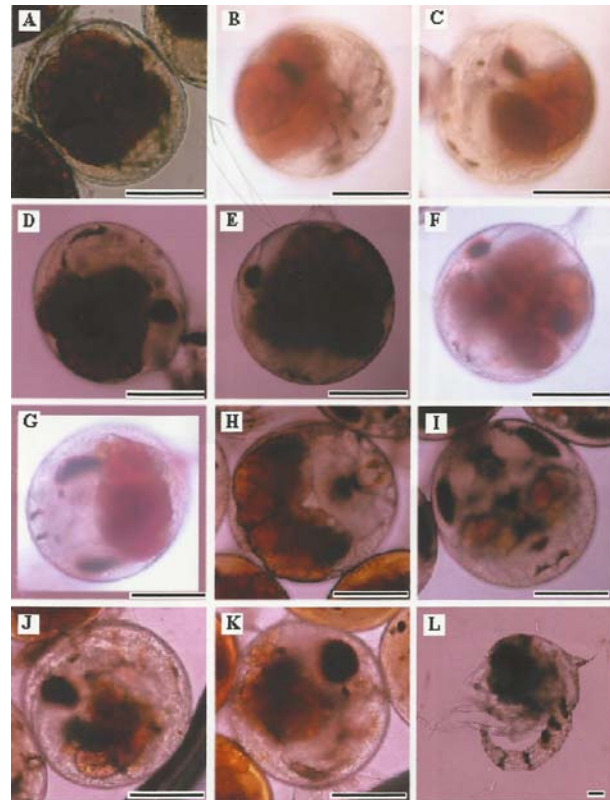

Fig. 2. Stages of development in embryos of *Neosarmatium indicum* (A. Milne Edwards). A, Stage-7; B-C, Stage-8; C, Stage-8; D, Stage-9; F, Stage-10; G, Stage-11; H, Stage-12; I, Stage-13; J, Stage-14; K, Stage-15; M, (embryo ready to hatch); L, zoeal stage. Scale bars = 100 μm.

Stage-8 (7 days of incubation, Fig. 2B-C): First evidence of organs was visible in ventro- lateral position of the yolk free portion at the 7 days of incubation period. Ocular, thoracic-abdominal, and cephalic portion of papilla were observed.

Stage-9 (8-9 days of incubation, Fig. 2D-E): At the eight days of incubation, eyes like organ was first visible (Fig. 2D), and at the end of nine days developing eyes was increased in size (Fig. 2E). No evidence of heart and it beat was observed in this stage.

Stage-10 (10 days of incubation, Fig. 2F): At the ten days of incubation, heart like structure with a little beat jerking was observed first. Eyes were going to more or less complete in size.

Stage-11 (11 days of incubation, Fig. 2G): At this stage, yolk components arranged in four lobes. Developing heart and it beating was observed. Eyes development were incomplete.

Stage-12 (12-13 days of incubation, Fig. 2H): The appearance of eyes was more visible (Fig. 2H). Further reduction of yolk granule, looks like two lobes. Heart beating was faster than previous stage.

Stage-13 (14 days of incubation, Fig. 2I): Embryos occupied half volume with ventral position. Heart was formed in complete shape and beating fast. Eyes were larger in size, and the cornea was forming and eye pigmentation like retina was more intense. Antennae and mandible was comparatively more developed.

Stage-14 (15 days of incubation, Fig. 2J): About 3/4 of yolk granule was consumed, and the remaining yolk was divided into three patches. Embryos were grown considerably and occupied the entire egg volume except reserve yolk. The heart was dorsal position and the beats were regular. Ocular lobes were final position, and eyes were larger and more complex. All appendages were larger, incompletely segmented, and developing setae. First evidence of rostral spine was traced.

Before hatching

Stage-15 (16 days of incubation, Fig. 2K): This stage shows an embryo just before hatching. The embryo occupied almost all the available egg volume. Yolk was almost completely depleted, with traces remaining in the cephalothorax cavity. Carapace, maxillipeds and telson were well differentiated. There were many chromatophores, mostly black ones, in the embryo. The egg membrane was transparent, so the pigment cells were easily distinguished under the stereomicroscope. As hatching approached, the embryo frequently moved its abdomen and appendages in the egg membrane. The heartbeat was faster than the previous stage

Hatching

Stage-First zoea (17 days of incubation period, Fig. 2L): The internal movements of the larvae caused it to rotate 180°, leaving it with the telson and rostral spine facing towards the funiculus. During the hours prior to hatching, the egg dilets as a result of water up take, reached its maximum size.

Discussion

The embryology of the brachyuran crabs was done haphazardly throughout the world by few researcher. Some of the authors ignored the cleavage, blastula and gastrula stage during development, some started from cleavage or developing embryos. So, meaningful comparison is difficult in this field. Staging of embryos is not standard in the brachyuran crabs. Most of the authors recognized as few as five or less stages (Warner, 1967; Henmi, 1989) and as many as sixteen embryonic stages before hatching (Fukui, 1988, Yamaguchi, 2001). In the present study, seventeen stages were recognized, which is defined by the continuous progress. Undivided (before cleavage) eggs were identified as stage 1, which is similar to the findings of Kobayashi & Matsuura (1996), but they recognized 2-celled and 4-celled embryos each as separate stage while 2-celled to 32-celled embryos identified as a single stage in the present study. Yamaguchi (2001) recognized undivided egg to 32-celled embryos as a single stage, which is quit different from the present study. Undivided (just spawned or fertilized) eggs are characteristically different from the cleaved one, must be separated as stage 1, which is more appropriate in the embryology of the brachyuran crabs. Garcia-Guerrero & Hendrickx (2004) applied the term period instead of stage based on the morphological differences but they ignored the cleavage, blastula and gastrula stage, which is not appropriate in the embryology of brachyuran crabs.

There is a relationship between the incubation period and the temperature (Wear, 1974). Incubation period depends on species and temperature (Yamaguchi, 2001). Under low temperature, the incubation period much extended and vice-versa in higher temperature (Yamaguchi, 2001). Daily or continuous progress of development is very useful where the length of the incubation period is assumed to be proportional to that of each stage (Kobayashi & Matsuura, 1996). It is possible to estimate the days needed to develop the stages shown in the present study when the total length of the incubation period is known.

In the present species, the largest diameter of the undivided (just fertilized not cleaved) egg of *N. indicum* is 0.36 mm, which increased to 0.47 mm before hatching. Yamaguchi (2001) reported that the largest diameter of the undivided egg of *Uca lectea* is 0.24 mm and it increased to 0.32 mm in stages 14 and 15. Garcia-Guerrero & Hendrickx (2004) investigated the diameter of egg is 0.57-0.62 mm in *Aratus pisonii* and 0.60-0.63 mm in *Goniopsis pulchra*, which is more larger than those of Yamaguchi's investigation and the present study. Comparison of embryonic stages, egg diameters, and incubation periods and temperatures of four species of mangrove brachyuran crabs are shown in Table 2. The eggs (fertilized to embryos) of *N. indicum* was always spherical, which is the most common shape in brachyuran eggs (Hines, 1982; Nagao *et al.*, 1999; Yamaguchi 2001; Pinheiro & Hattori, 2003; Garcia-Guerrero & Hendrickx, 2004).

Table 2. Comparison of embryonic development of four species of mangrove crabs including *N. indicum* (A. Milne Edwards). References: 1, Garcia-Guerrero and Hendrickx (2004); 2, Present study; 3, Yamaguchi (2001).

Family & Species	Embryonic stages	Incubation period (days	Diameter of embryos (mm)	Incubation temp. (℃)	References
Grapsidae, *Goniopsis pulchra*	9 periods	15	0.60-0.64	26-28	1
Sesarmidae, *Aratus pisonii*	8 periods	14	0.57-0.62	26-28	1
N. indicum	15	16	0.36-0.47	25	2
Ocypodidae, *Uca lactea*	15	15.4	0.24-0.32	28	3

The embryonic development of *N. indicum* is similar and matches the general embryonic pattern observed in many brachyuran crabs, including in much larger tropical and subtropical species (Garcia-Guerrero & Hendrickx, 2004; Pinheiro & Hattori, 2003) and in cold water species that experience a very slow embryonic development (Nagao *et al.*, 1999). Total incubation period of embryos was 17 days in the present species where it was 14 days in *Aratus pisonii* and 15 days in *Goniopsis pulchra* observed by Garcia-Guerrero & Hendrickx (2004). The development duration of *P. bidens* is little longer that that of *A. pisonii* and *G. pulchra*, although the incubating temperature was similar as in *A. pisonii* and *G. pulchra*. Just before hatching or the final days of the embryonic development, all features that characterized the first zoeal stage of *N. indicum* was present, when compared with the illustrations of first zoea reported by Islam & Shokita (2000).

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Physico-chemical variables and their effect on the growth performance of some major carps in some ponds of a matsaya gram (Fish village) in Natore

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Abstract: An experiment was carried out to asses the physico-chemical characteristics, water quality and fish growth suffered by pollution hazards created by unethical use of agrochemical in few ponds in a matsaya gram (fish fillage) in northern part of Bangladesh. The range of free CO_2, DO, HCO_3, CO_3 and p^H were 2-30mg/l, 3.14-11.86 mg/l, 86.31-143.84mg/l, 0 -50mg/l and 8.5-9.5 respectively. The BOD of the studied ponds found quite less than optimum level. The study of relationship between fish growth and the soil characters showed negative impact. It revealed that due to minimum rainfall the ponds were not washed out that could decrease the rate of pollution created from the nearest house and residual chemicals, those used in upland crop field beside it. But the ponds are still suitable for fish culture and should keep them at least in present position for good crop.

Key words: Impact, pollutants, pond fisheries, matsaya gram.

Introduction

It is well recognized that the water quality plays an important role on fish production. A pond with good water quality will produce more healthy fish than a pond with poor water quality. So, the production of fish in any water body depends largely on the availability of food organisms of the water body. The role of water pollutants has a direct affect on fish health. Several environmental factors, which determine the character of water, have great importance upon the growth, maturity, reproduction and development of fishes. The relationship between fishes and their biotic and abiotic factors is not an isolated phenomenon, but change in one may reflect on the other effectively. Fishes are more dependent on water temperature, pH, dissolved oxygen, free CO_2, alkalinity and some other salts for growth and development (Nikolsky, 1963).

Since the last few years due to natural and man made causes biodiversity especially species diversity of fishes and other aquatic organisms in open water have been decreasing drastically. This situation is creating a lot of problem for human and as well as organisms of aquatic environment.

The importance of the assessment of limnological factors is of great importance. Soil quality and water quality study are also important. The study helps to formulate the guideline for management of fish culture practices. The limnological factors on hydrology deal with many interested phenomena, such as, hydrophytes phytoplankton, zooplankton to benthic fauna, fish diversity, physical and chemical nature of water body (Banerjea, 1967). Fishes are more dependent on water temperature, pH, DO, free carbon dioxide, alkalinity, hardness and some salts for growth and development (Nikolsky, 1963). Any change of these parameters may affect the growth, development and maturity of fishes. (Nikolsky, 1963). In short, water quality is directly related to productivity of fish and determines the quality of their lives.

Considering the importance of pond fishery to the economy and to human health, this study was conducted on few selected ponds in a matshya village. The study has been conducted to asses the water quality of the ponds and their impacts on pond fishes.

Materials and Methods

The physico-chemical study was started from October 2008 to March 2010. The water sample, for test was collected from 10 ponds in matsaya villages of Baraigram upazilla, Natore. Sampling and physical test was done weekly on all the 10 ponds. The methods used to test different parameters are described below:

Turbidity: Turbidity was measured by Seechi disc. The disc was slowly lowered in the water and the depth at which the disc becomes invisible was noted from the scale reading. The turbidity was then calculated from turbidity chart. Turbidity of water is expressed in mg/l.

***p*H** (Potenz Hydrogen) ∶ *p*H refers to the amount of hydrogen ions in a solution. P^H of pond water was measured by a digital *p*H meter (HANNA model HI 96107) made in Italy. The *p*H was measured directly by immerging the meter knod into the water.

Temperature: The temperature of water was measured at the time of sample collection by an alcoholic centigrade thermometer with the range 0ºC to 110ºC. The thermometer was immerged directly into the water and the reading was noted.

Dissolved oxygen: The dissolved (DO) oxygen concentrations of the collected samples were determined immediately by titration method following the Winkler's method (APHA 1976). DO was measured weekly.

Free carbon-dioxide: Free carbon-dioxide (CO_2) in the water was determined by the method of titration of sample with N/44 NaOH solution using phenolphthalein as an indicator (Welch, 1948). The test was done immediately after collecting the water sample. Samples were collected from the pond four times in a month.

Carbonate: Carbonate (CO_3) alkalinity was determined by titration of 100 ml of water sample with N/50 sulfuric acid using phenolphthalein as indicator (Welch, 1948). This test was completed within thirty minute of sample collected.

Bicarbonate alkalinity: Bicarbonate (HCO_3) alkalinity was determined by titration of 100 ml. of water sample with N/50 sulfuric acid using methyl orange as an indicator (Welch, 1948).

Biological oxygen demand : For the measurement of biological oxygen demand *(BOD)* Two bottles each of 250 ml capacity were taken and then the bottles were filled with water from the pond as sample. The dissolved oxygen in one bottle was determined immediately by using the DO measurement method.   But the other bottle was incubated at 20ºC for 5 days. After 5 days, the DO was measured by the same method as described above. The value of BOD was determined by subtracting final dissolved oxygen (DO) from initial dissolved oxygen.

Fish collection: The weight of fishes were taken after every three months. During harvest rui, mrigel, catla, silver carp, and mirror carp fishes were taken as sample and weight was taken near the pond site.

Results and Discussion

Quarterly average turbidity, *p*H and water temperature of the studied ponds are presented in Table 1 and dissolved O_2, CO_2, CO_3 and HCO_3 concentration in the water is presented in Table 2. The water remained more turbid in the 1st quarter of 2010. The DO and bi-carbonate also remained the higher during that quarter. The *p*H amount did not differ much. But the concentration of CO_2 and CO_3 observed higher in the 3rd quarter of 2009. The measured BOD (mg/ l) in the water of those ponds is presented in Table 2.

Table 3. Biological oxygen demand (BOD) of ten ponds tested quarterly.

Pond no.	Biological oxygen demand (BOD mg/ l)					
	4th quarter 2008	1st quarter 2009	2nd quarter 2009	3rd quarter 2009	4th quarter 2009	1st quarter 2010
1	6.28	4.19	3.49	2.64	6.28	5.24
2	7.14	2.40	2.79	1.70	5.59	1.40
3	6.25	2.79	2.09	1.31	5.59	2.66
4	5.32	2.80	3.49	2.17	0.69	3.84
5	2.12	3.49	2.55	2.07	1.40	4.40
6	1.32	3.49	3.32	2.44	1.47	4.19
7	5.42	2.09	3.50	2.78	6.29	3.15
8	5.70	2.09	2.97	2.29	4.19	0.70
9	4.12	2.10	3.02	2.10	2.09	3.01
10	2.62	2.79	2.56	3.01	2.79	4.19
Mean	4.63	2.82	2.98	2.25	3.64	3.28

The higher mean of biological oxygen demand (BOD) was recorded in the 4th quarter of 2008 (Table 3). The values were analyzed by analysis of variance.

Table 4: Quarterly growth and total weight (in g) of different fishes in 10 ponds.

Pond	Weight of fishes in g									
	Silver carp		Rui		Catla		Mrigel		Mirror carp	
	quarterly	Total	quarterly	Total	quarterly	Total	quarterly	Total.	quarterly	Total.
1	320	1800	285.00	1710	288.33	1730	193.33	1160	301.66	1810
2	400	2400	316.66	1900	345.83	2075	226.66	1360	534.16	3225
3	583	3500	346.66	2150	510.00	2060	400.00	2400	783.33	4700
4	235	1415	163.33	980	160.00	960	122.50	735	500.00	3000
5	312	1870	308.33	1850	475.00	2850	163.33	980	600.00	3600
6	397	2380	283.33	1700	245.83	1475	303.33	1820	428.33	2570
7	374	2245	300.00	1800	272.50	1635	256.66	1540	419.66	2950
8	610	3660	328.33	1970	260.00	1560	341.66	2250	795.83	4775
9	513	3075	225.00	1350	204.16	1225	275.00	1650	808.33	4850
10	478	2870	327.50	965	230.50	1385	230.83	1535	876.66	5260
Mean	**422.2**	**2521.5**	**288.41**	**1637.5**	**299.22**	**1695.5**	**251.33**	**1543**	**604.80**	**3674**

Quarterly (during 3 months) growth and total weight of different fishes (after 18 months) in 10 ponds are presented in Table 4. The growth rate of catla and mrigel are comparatively lower than other fishes observed in every pond. Mirror carp grow very rapidly and the average weight become 3600g after 18 months.

The relationship between the fish growth and different physical and chemical factor of waters of different ponds were studied and (coefficient correlation) r values were calculated. Turbidity showed direct relation with the growth of silver carp, rui and catla. BOD of water has inverse relation with fish growth.

During the present study different physicochemical variable in 10 ponds and growth of fishes upto 18 months were studied. The growth of some carps (biota) was also taken into consideration. The productivity of an aquatic ecosystem is governed by the physical, chemical and biological characteristics of the ecosystem. The overall behaviour and nature of an environment is largely governed by the interaction of the meteorological, physical, chemical and biological parameters, but a factor is not always independent. There remains a close relationship among the factors. A little variation in one of the factors may influence the other. Similar type of interaction was observed in the present study.

Temperature is one of the most outstanding and biologically significant phenomenona of aquatic environment. All metabolic and physiological activities and life processes such as feeding, reproduction, movement and distribution of aquatic organisms are greatly influenced by water temperature. Bhuiyan and Nessa (1996) observed limited degree of positive relationship between water temperature and water turbidity. They observed positive relationship betweemn pH and water temperature (r=0.09) and inverse relationship with free CO_2 (r=-0.66) and alkalinity (r=-0.87). Temperature tolerance limits for Indian major carps and common carps is reported to be 18.3 $^\circ$C and 37.8 $^\circ$C. The temperature recorded from the studied ponds was within the acceptable limit for the species cultured.

The concentration of free carbon-dioxide was found to be directly related to the amount and nature of biological activity in the water. In the present study the high free CO_2 content during spring months was possibly due to low temperature and low rainfall. Which caused low decomposition of organic matter and addition of free CO_2 high photosynthesis which consumed high precipitation of free CO_2 agreeing with Michael (1968) in India and Patra and Azadi (1987) in Bangladesh.

The low temperature and low rainfall results the lower decomposition of organic matter causing lower production of free CO_2 and lower consumption of dissolved oxygen Patra and Azadi, 1987).

Michael (1968). found that the bicarbonate alkalinity of pond water ranging from 86.31 to 143.84 mg/l is suitable for fish cultivation. The alkalinity above 40 mg/l is considered to be hard water characteristic, which help to maintain the pH value in alkaline condition. Bicarbonate constitutes the chief source of alkalinity at a pH range of 7.00 to 9.00. It may also be assumed that in pond the main source of alkalinity may be due to a high content of bicarbonate. Yaron (1964) observed that the alkalinity of an aquatic habitat partly depends on the amount of water in it.

In most water bodies bicarbonate and carbonate are the predominant bases that contribute to the alkalinity. The alkalinity of water is considered one of the most important aspects of the chemistry of water to be used in aquaculture ponds (Boyd 2000). During the study period its higher values were observed in the winter season whereas the lower values were observed in the others season. The observed pH value of ponds water showed to be alkaline in nature with small variation.

The BOD is the quantity of oxygen in mg/l used in oxidatoin in water. BOD represent the concentration of organic matter remaining in the water at any time and DO shows the ability of the water body to purity itself through biochemical. According to Khanna (1992), BOD may be measured at a rate of removal of oxygen from natural water by microorganisms in aerobic degradation of the dissolved or even particulate organic matter in winter. Palharya et al. (1993) noticed that seasonal variation in the value of BOD appears to be a function of changes in the degree of dilution, quantity of organic matters and activities of microorganisms carrying out decomposition of carbonaeous and nitrogenous matter. The highest value of BOD indicated very high degree of organic pollution. In the present study the recorded highest value 4.29 mg/l is alarming. As BOD is a good measure of the contamination level of a water and it is used primarily for waters that receive pollution from sewage and industrial waters it should be within range for better aquaculture. The result

obtained from this study suggest the alarming situation of the ponds. The water characters are somewhat beyond the range and these conditions are caused due to over household use that contaminating the water particularly in the winter season when water reserve / containing become very low. It is also observed from this study that the heavy rainfall normalize the all conditions and washed away the pollutants developing the water quality in many cases.

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Screening of *Derris indica* Bennet. for cytotoxicity against *Artemia salina* and phytotoxicity on mustard seeds

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Abstract: Chloroform extracts of the fruit shell, leaves, root bark, root wood, seeds, stem bark and stem wood of *Derris indica* Bennet. were tested against the brine shrimp, A*rtemia salina* nauplii. All the test extracts of *D. indica* were found to be effective. The LC_{50} values of the extracts were 15312.37, 92.074 and 29.661 ppm for the fruit shell; 60922.83, 61.522 and 23.777 ppm for the leaf; 15312.37, 51.477 and 19.169 ppm for the root bark; 2598.584, 30.480 and 8.260 ppm for the root wood; 545.025,26.730 and 7.719 ppm for the seed; 60922.83, 114.549 and 29.572 ppm for the stem bark and 7734.618, 58.501 and 23.694 ppm for the stem wood at 30 minute, 24 hours and 48 hours post exposures respectively at doses 200, 100, 50, 25, 12.5, 6.25, 3.125 and 1.563 ppm against *A. salina* . The toxicity of the extracts could be arranged in the order: seed > root wood > root bark> stem wood > leaf > fruit shell > stem bark extract. However, the extracts did not significantly inhibit the germination of mustard oil seeds, and thus its application to crops or to the crop field may not cause any harm to crop plants.

Key words: *Derris indica*, cytotoxicity, A*rtemia salina* nauplii, phytotoxicity, mustard seeds.
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Introduction

Natural products derived from plants, as an alternative to conventional insecticides for insect control, is now-a-days very popular among the IPM practitioners. Plant-derived pesticides are more readily biodegradable; therefore they are less likely to contaminate the environment. Moreover, the botanical pesticides break down readily in soil and are not stored in plant or animal tissues.

Being a medicinal plant, *Derris indica* Bennet. might contain some antipathogenic properties. Powdered seeds of this plant are valued as febrifuge and tonic, and are used in bronchitis and whooping cough. The seeds are also reported to be used as a fish poison (Kirtikar and Basu, 1935), and the seed oil is used as a soap liniment to treat scabies, herpes and rheumatism (Burkill, 1966). The leaf extract is active against *Micrococcus pyogenes* var. *aureus* (Anon, 1969). The leaf juice is prescribed in flatulence dyspepsia, diarrhoea and cough and also considered as a remedy for leprosy and gonorrhoea. The root juice is used for cleansing foul ulcers and sores, and to treat gonorrhoea. Roots are used as fish-poison by the aborigins of Australia (Kirtikar and Basu, 1935). The dried flowers are used as decoction to quench thirst in diabetes. The fresh bark juice is given internally in bleeding piles (haemorrhoids) and a decoction of bark is used against beri-beri (Anon, 1969). Cheema *et al.* (2003) have advocated the commercial utilization of sorghum water extracts for weed management in wheat. Ethanol extracts of *D. trifoliata* showed different mortality rates of brine shrimp which increased proportionally with the increasing concentrations of the extracts (Saifullah and Azam, 2011). Daruliza et al. (2012) traced the anti-Candida activity and brine shrimp toxicity assay of *Ganoderma boninense.* The insecticidal activity of this plant against *Callosobruchus maculatus* has been determined (Mondal and Islam, 2008), and antibacterial and larvicidal potentials have also been worked out (Mondal *et al.,* 2010). Germination characteristics of Maize seeds under different ageing treatment have been done by Siadat *et al.*, (2012). Another seed germination test was conducted by Geetha *et al.*, (2011). Seed priming is known as technique of seed enhancement that improves germination or seedling growth in many crops such as Dry bean (*Phaseolus vulgaris* L.), cordia (*Cordia millennii*) (Adebisi, 2011), coffee (*Coffea arabica* L.) (Gebreselassie *et al.*, 2010), capsicum (*Capsicum annum*) and *Agropyron elongatum* (Tavili *et al.*, 2010)

Various workers investigated *D. indica* giving emphasis mostly on the chemical constituents and their medicinal profile but very few works have been done on its pesticidal importance. In this investigation, cytotoxic and phytotoxic activity tests of *D. indica* were carried out on the brine shrimp, *Artemia salina* nauplii and the musatard seeds respectively to evaluate the efficacy of the plant parts as a possible source of potential secondary metabolites to be used as environment friendly pest control agents.

Materials and Methods

Preparation of plant materials for extraction: The fresh leaves, fruit shell, root bark, root wood, seeds, stem bark, and stem wood of *D. indica* were collected from the campus of the University of Rajshahi, Bangladesh. After drying under shade the plant materials were powdered in a grinder machine.

Chemical extraction of the collected materials: Chloroform was selected as a solvent to extract seven different parts of *D. indica* separately. The ground dried materials, *viz.* leaves, fruit shell, root bark, root wood, seeds, stem bark, and stem-wood were extracted with sufficient amounts of chloroform (500g × 1500ml × 3 times) for each of the items. Separate extracts were collected by the cool method after 72 hours of plunging for each of the plant parts. Extracts were subjected to

filtration and evaporation of the solvent. The residues were kept in a refrigerator after proper labeling.

Since the lethality test involves the culture of brine shrimp nauplii, i.e., the nauplii should be grown in water with salinity similar to that of sea water, while the seawater contains 3.8% sodium chloride. Accordingly, a 3.8% sodium chloride solution was prepared by dissolving 38 gm sodium chloride in 1000 ml distilled water. The P^H of the brine water thus prepared was maintained between 8 and 9 using NaHCO$_3$.

Brine water was taken in a small tank and shrimp eggs (1.5 gm/L) were added to one side of the perforated tank with a constant oxygen supply. A constant temperature (37ºC) and sufficient light were maintained. After 48 hours, shrimp nauplii were collected and used for the experiment. For the seed germination test, fresh and healthy mustard seeds were collected from the market.

Cytotoxicity test:
Preparation and application of doses on *A. salina*
Chloroform extracts of the *D. indica* samples were applied against the brine shrimp nauplii. For the fruit shell, leaves, root bark, root wood, stem bark and stem wood samples 4mg were initially dissolved in 200µl of pure dimethylsulfoxide (DMSO) to make them hydrophilic before adding 19.98 ml of water to get a concentration of 200 ppm for each of the samples separately which were used as stock solutions for all the extracts and from these concentrations other successive doses were prepared separately for each of the extracts through the serial dilution method. A series of concentrations, e.g. 200, 100, 50, 25, 12.5 and 6.25 ppm were prepared for the extracts separately. However, for the seed extract 2 mg was initially dissolved in 100 µl of DMSO to make it hydrophilic before adding 19.98ml of water to get a concentration of 100 ppm which was used as the stock solution for the seed extract. The following concentrations were made from the stock solution: 100, 50, 25, 12.5, 6.25, 3.125 and 1.563 ppm.

Brine shrimp eggs were hatched in simulated seawater to get nauplii. Test samples were prepared by the addition of the requisite amounts of DMSO for obtaining desired concentrations of the test sample. The nauplii were counted by visual inspection and were taken in vials containing 5ml of brine water. Then samples of different concentrations were added to the pre-marked vials with the help of a micropipette. The vials were left for 24 hours and then the nauplii were counted again to find out the cytotoxicity of the test agents and compared to the results with positive control.

Preparation and application of doses on mustard seeds:
In this experiment 4 doses from the fruit shell, leaves, root bark, root wood, seed, stem bark and stem wood extracts of *D. indica* were made as 1 mg, 0.75 mg, 0.50 mg and 0.25 mg/ml freshwater. Because of insolubility of the extract in water it was needed to add 100µl DMSO with the weighed extract before mixing with water.

For application of doses a number of petridishes 60mm diam. were used. Filter papers were placed inside the petridishes and doses were applied separately. Five mustard seeds were put in every petridish and three replications were set for each concentration and a control with three replications was also maintained. All the petridishes were kept covered to avoid drying. The humid condition inside the petridishes helped the seeds to germinate. Then the petridishes were placed in a safe place with plenty of light and air. Germination (%) was carefully recorded at various concentrations of different extracts of *D. indica*.

Collection and analysis of data for cytotoxicity
The test tubes containing the nauplii with the treated brine water were kept on a rack near the window in the laboratory. The recorded mortality was corrected by the Abbott's (1925) formula:

$$P_r = \frac{P_0 - P_c}{100 - P_c} \times 100$$

Where,
P_r = Corrected mortality (%),
P_o = Observed mortality (%), and
P_c = Control mortality (%).

Mortality data were subjected to statistical analysis according to Finney (1947) and Busvine (1971) by using software developed at the Department of Agricultural and Environmental Science, University of Newcastle-upon -Tyne, U.K. The dose-mortality relationship was expressed as median lethal concentrations (LC$_{50}$).

Results and discussions
The results of dose-mortality assays of *D. indica* extracts against *A. salina* nauplii are presented in table 1 and illustrated in Fig. 1. Most of the test extracts showed remarkable dose-mortality effects against the 1 day old nauplii of *A. salina.* The degrees of activity of the extracts against the brine shrimp nauplii could be arranged in the order: seed > root wood > root bark> stem wood > leaf > fruit shell > stem bark extract.
In general, the application of the chloroform extracts of different parts of *D. indica* on the germination of mustard seeds produced significant effects (Table 2).

The ethanolic extracts of *D. scandens* (Roxb.) Benth, along with other test extracts showed cytotoxicity (LC$_{50}$<30 µg/ml) against lung and prostate cancer cell lines (Acharya and Thomas, 2007). LC$_{50}$ values of petroleum ether, chloroform and methanol extracts on *A. salina* Leach were recorded as 1.14, 1.1, and 54.9mg/l respectively. Chemical analysis revealed the presence of fatty acids, steroids, triterpenoids, alkaloids, phenols, and phenyl propanoids, tannin, and mucilage in the extracts (Uyub *et al.,* 2010).

The present results are more or less similar to those of Mondal and Islam (2008). However, the fruit shell and the leaf extracts did not offer any mortality of the test insect (*Callosobruchus maculatus*) and the intensity of activity could be arranged in a descending order as seed > root wood > root bark >stem wood > stem bark.
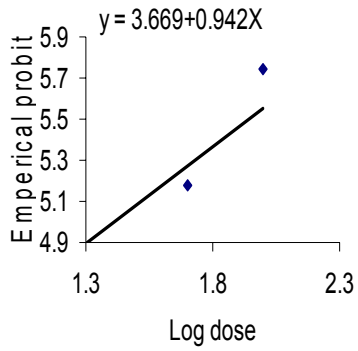
Table1: Cytotoxicity of *D. indica* extracts against *A. salina* nauplii.

Test extract	Time exposed	LC$_{50}$ value (ppm)	95% Confidence limits		Regression equation	χ^2 Value(df)
			Lower limit	Upper limit		
Fruit shell	30 min	15312.7	34.995	6700098	Y=2.791+0.528X	0.546 (4)
	24 h	92.074	50.777	166.959	Y=3.182+0.925X	0.319 (4)
	48 h	29.661	20.117	43.734	Y=3.284 + 1.166X	0.994 (4)
Leaf	30 min	60922.83	5.791	6.409e+08	Y=2.721+0.476X	0.550 (4)
	24 h	61.522	37.700	100.397	Y=3.259+0.973X	0.278 (4)
	48 h	23.777	16.546	34.168	Y=3.194+1.312X	0.911 (4)
Root bark	30 min	15312.37	34.995	6700098	Y=2.791+0.528X	0.546 (4)
	24 h	51.477	27.505	96.342	Y=3.766+0.721X	0.279 (4)
	48 h	19.169	11.440	32.122	Y=3.788+0.945X	1.225 (4)
Root wood	30 min	2598.584	110.001	61387.03	Y=2.737+0.663X	0.204 (4)
	24 h	30.480	16.123	57.620	Y=3.989+0.682X	5.686e-02(4)
	48 h	8.260	4.036	16.905	Y=4.125+0.954X	2.352 (4)
Stem bark	30 min	60922.83	5.791	6.409e+08	Y=2.721+0.476 X	0.550 (4)
	24 h	114.549	47.100	278.582	Y=3.612 + 0.674 X	0.374 (4)
	48 h	29.572	17.862	48.960	Y =3.712 + 0.876 X	8.718e-02(4)
Stem wood	30 min	7734.618	68.723	870519.9	Y =2.530 + 0.635 X	0.389 (4)
	24 h	58.501	28.500	120.083	Y =3.859 + 0.646 X	0.356 (4)
	48 h	23.694	13.599	41.286	Y = 3.867+0.824 X	0.597 (4)
Seed	30 min	545.025	81.267	3655.26	Y= 2.452 + 0.931X	0.799 (4)
	24 h	26.730	16.271	43.912	Y 3.678 + 0.926 X	1.335 (4)
	48 h	7.719	4.673	12.750	Y =4.063 + 1.056 X	1.568 (4)

Table2. Germination (%) of Mustard seeds by extracts of different parts of *D. indica*

Treatment with following extratives	Germination %age
	Mustard seed
Fruit shell	90
Leaf	92
Root bark	85
Root wood	90
Stem bark	80
Stem wood	75
Seed	85
Control	95

It is important to evaluate the newly found bioactive agents to try against some test crop plants to see whether or not they cause any detrimental effects on target crop(s). Thus, phytotoxicity tests are necessary. Ndakidemi and Dakora (2003) employed legume seed flavonoids and nitrogenous metabolites for an improvement in their understanding of seed chemistry whether they would permit manipulation of these molecules for effective control of pathogens, insect pests, *Striga* and destructive weeds, as well as for enhanced acquisition of N and P via symbioses with soil rhizobia and AM fungi.
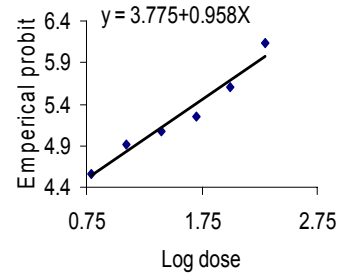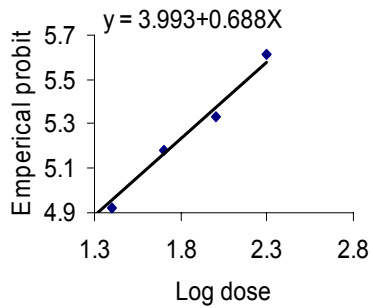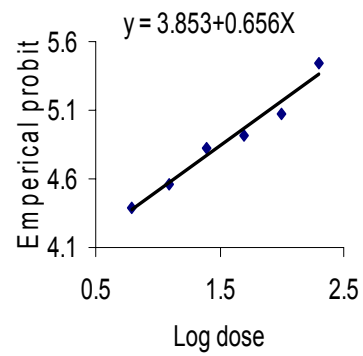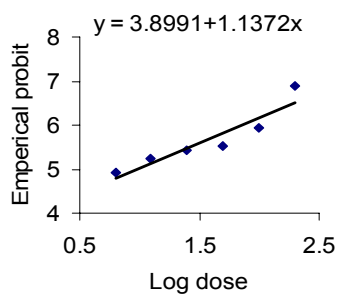
A

y = 3.669+0.942X

E

y = 3.784+0.711X

B

y = 3.9852+1.1381x

F

y = 3.775+0.958X

C

y = 3.993+0.688X

G

y = 3.853+0.656X

D

y = 3.8991+1.1372x

H

y = 3.837+0.853X
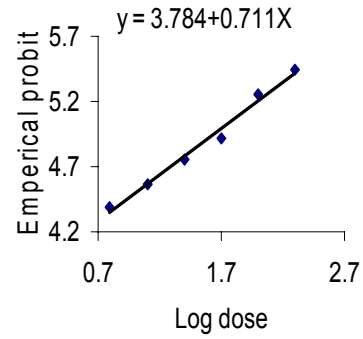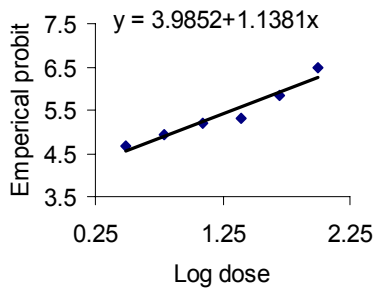
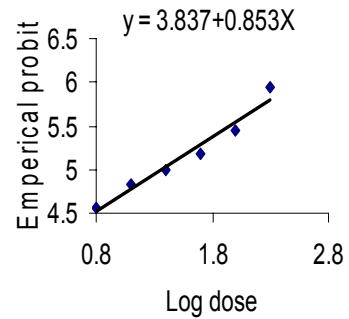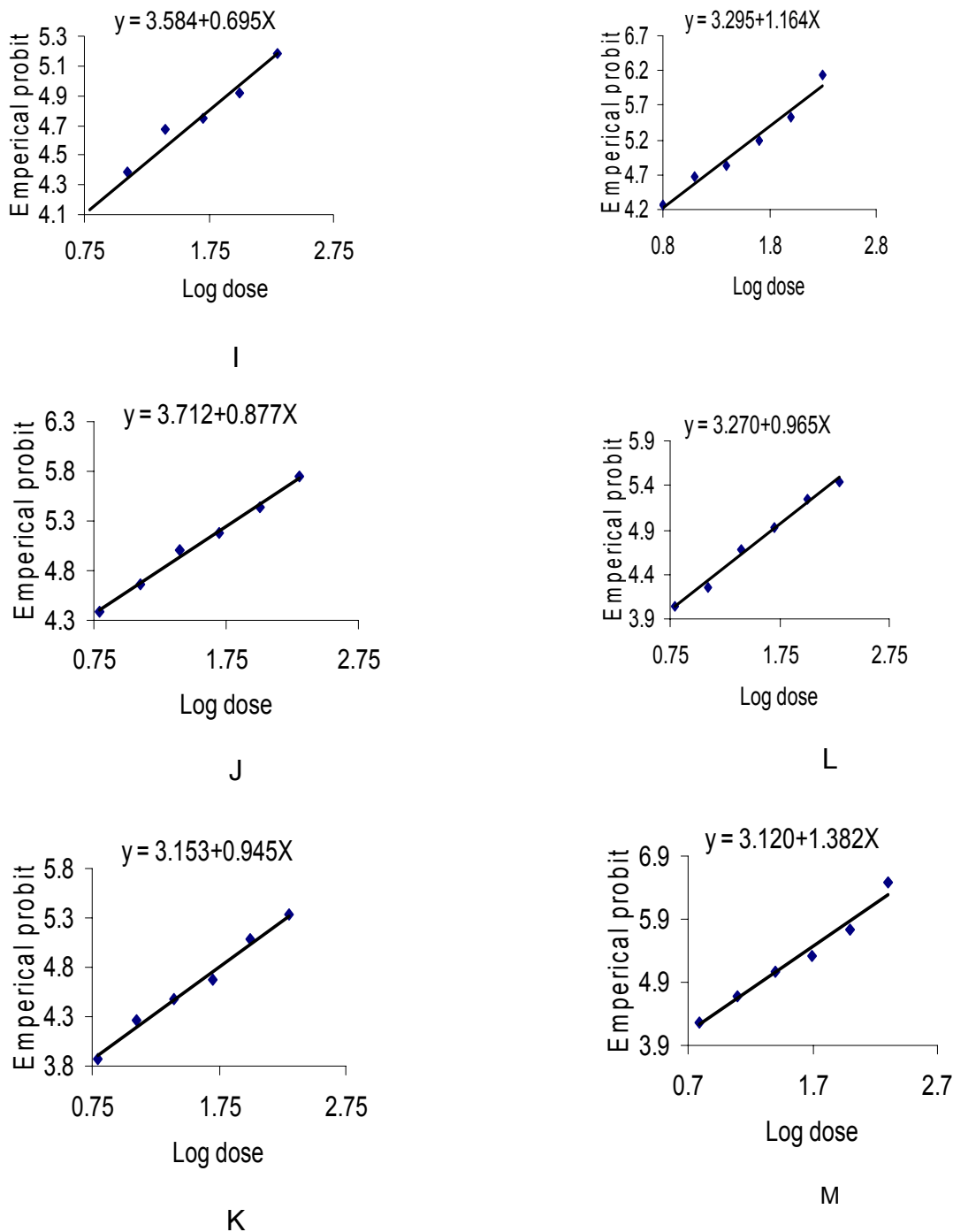**Fig. 1.** Probit mortality regression lines of the chloroform extracts of *D. indica*: A- Seed/ 24 h; B- Seed/ 48 h; C- root wood/ 24 h; D- root wood/ 48 h ; E- root bark/ 24 h and F- root bark/ 48h;.G- stem wood/ 24 h; H- stem wood/ 48 h; I- Stem bark/ 24 h; J- Stem bark / 48 h; K- fruit shell/ 24 h and L- fruit shell / 48 h; M- leaf/ 24and N- leaf / 48 h of exposure against *A. salina*

However, it is also important to see whether or not the plant secondary metabolites having insecticidal or biological activity cause any barrier to the sprouting and growth of seedlings. Phlomina and Srivasuki (1996) reported that leaf leachates of 5 multipurpose tree species (*Eucalyptus camaldulensis, Acacia nilotica, Derris indica, Cassia siamea* and *Sesbania grandiflora)*

had varying degrees of inhibitory and stimulatory effects on germination percentage. Velu *et al.*(1996) reported that *Acacia* sp. retard the plant growth and development. Thakur and Bhardwaj (1992) reported that when wheat seeds were exposed to leachates from leaf extracts of *Eucalyptus globulus, Populus ciliata, Juglans regia* and *Robinia pseudoacacia* germination was not affected.

A perusal of the data shows that *D. indica* extracts produced significant mortalities against *A salina* nauplii. But the extracts had, in general, no significant phytotoxicity against mustard seeds. However, more comprehensive studies are needed in this line.

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Non-protein nitrogen compound poisoning in cattle

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Abstract : The study was carried out in Chittagong Veterinary and Animal Sciences University (CVASU), Chittagong to find out the causes of sudden death of cattle in the Military Dairy Farm, Chittagong. To explore the cause of death, clinical history, clinical signs were recorded. Finally, postmortem was done and samples were collected for laboratory diagnosis. The ruminal pH was 9.0 and the clinical history along with signs suggests possible non-protein nitrogenous compound poisoning. The laboratory diagnosis coincides with nitrate poisoning which focuses possible relationship between non-protein nitrogenous compound and nitrate poisoning. The study recommends maintaining proper cautions to use urea in concentrate feed.

Key words : Non-protein, urea, ammonia, nitrate, poison

Introduction

Urea poisoning is one of the common toxicities found in ruminants especially cattle. It is used as an alternative nonprotein nitrogen (NPN) source in ruminant diet and its nitrogen is liberated as ammonia in rumen and then the released ammonia is used by rumen microbes to synthesize their own protein. But in urea, nitrogen can be replaced by up to 16% of the dietary nitrogen along with 2 or more feeds for dairy cows (Froslie, 1977). Dietary urea has been used for decades as an effective and inexpensive source of nitrogen for ruminal microbial utilization. It is rapidly hydrolyzed upon entry into the rumen resulting in peak rumen ammonia concentrations within the first hour of consumption. However, ammonia that is not utilized for microbial synthesis is absorbed across the gastrointestinal tract, with increasing ruminal ammonia concentrations resulting in increased rate of absorption (Huntington, 1986). Increased blood ammonia concentrations alter hepatic metabolism by increasing ureagenesis may also affect glucose metabolism in the liver and peripheral tissues (Huntington *et al.*, 2006). Poisoning may occur periodically when ruminants gain access to large quantities or are fed large amounts of urea; when they are not adapted to it or when feeds are improperly mixed or high urea concentration is present in low energy, low protein, and high roughage diets (Ortolani *et al.*, 2000). In Bangladesh, urea is also used in urea molasses straw preparation and/or sometimes only urea is supplemented with cattle feed more frequently in beef fattening programme or in dairy production. The present study was, therefore, focused on the NPN poisoning in Military Dairy Farm, Chittagong to find out the cause behind death of some cattle in their shed.

Materials and Methods

The authority of Military Dairy Farm, Chittagong reported that sudden death of some cattle occurred after concentrate feeding. Based on the complain, the Toxicology Laboratory of Chittagong Veterinary and Animal Sciences University (CVASU) collected feed, water and molasses samples from that mentioned farm. The whole study was conducted based on direct interview of the farm's staff, postmortem examination of the dead animals and laboratory diagnosis.

Description of the farm*:* The Military Dairy Farm is located in a hilly area of the Chittagong Cantonment, Baluchara, Chittagong. There is a total of 8 sheds for cattle with available cultivable land for fodder. All cattles were crossbreed between 1.5-2 years of age.

Case history: The cattle were regularly fed with fodder along with concentrated feeds. On the day of case fatality, the caretaker of the farm reported that cattle in the affected shed out 8 sheds, cattle were offered concentrated feed mixed with a new pack of molasses at 3 pm. The new pack of molasses was carried in the same vehicle as for urea and one sack of urea fertilizer was found opened. After almost 40 minutes of feeding, the cattle were found with labored breathing, mydriasis, salivation, convulsion, bloat and increased body temperature between 104°-105°F. Among 24 cattle of affected shed, 19 (male=2, female=17) cattle showed clinical sign and within 2-3 hours of onset of clinical sign 17 cattle were dead.

Treatment given: After the onset of clinical signs the animals of the case shed were treated with 1% methylene blue@ 20 mg/kg body weight introvenously, vinegar @ 2L orally, 5% Dextrose saline @1000ml, atropine sulphate @ 0.25mg/body weight intramuscularly. Trocarizations of some animals were done by trocar and canula and carminative mixture (No Bloat® suspension-Square Pharmaceuticals Bangladesh) was given. Later, Streptomycin-Penicillin combination (SP-vet®-Acme Pharmaceuticals Bangladesh) was administered intramuscularly.

Post-mortem inspection: Postmortem examinations of dead cattle were performed and various organ lesions were noted.

Laboratory tests: The various organs from dead animals and feed samples were collected by the personnel of the Military Dairy Farm. Samples were packed properly within sterile zipper clip bag and submitted to the Toxicology Laboratory, CVASU. Two blood samples were also given to the Physiology Laboratory for hemoglobin level analysis. After taking the *p*H of rumen content by Hanna pH meter (Hanna instruments, USA) toxicological testing was done according to joint reaction (Sandhu, 1999).

Results
The young are less affected (13%) whereas the adult are more affected (67%) as shown in Table-1. Besides that, Male were less prone to be affected (8%) with less case fatality (5%) in comparison to highly affected female (72%) with high case fatality (84%).

On postmortem examination congested liver and kidney, frothy bloat in rumen, gastroenteritis with hemorrhagic intestine, edema of lung were observed. Toxicological testing was done on the supplied samples according to Sandhu (1999) and Nitrate and Nitrite (Ammonia) positive reactions were found in some samples.

Table-1: Attack rate and case fatality according to animals

Factor	Category	Total(N=24)	Affected animals (N=19)	Death (N=17)	Attack rate		Case fatality	
					Overall	According to factor	Overall	According to factor
Age (Years)	1.5	3	3	1	3/24=0.13	3/3=1	1/19=0.05	1/3=0.33
	2.0	21	16	16	16/24=0.67	16/21=0.76	16/19=0.84	16/16=1
Sex	Male	2	2	1	2/24=0.08	2/2=1	1/19=0.05	1/2=0.5
	Female	22	17	16	17/24=0.72	17/22=0.77	16/19=0.84	16/17=0.94

Table-2: Test results of supplied samples

Type of samples (Total number)	Ammonia test
Rumen content (2)	+
Liver (2)	-
Kidney (2)	-
Molasses (1)	+
Concentrate feed with molasses (1)	+
Concentrate feed without molasses (1)	-
Supplied water (1)	-

Blood samples were analyzed at Physiology Laboratory, CVASU and average hemoglobin level was found 5.35±0.2 gm/dl. The pH of rumen fluid was determined and was found 9.0.

Discussion
Non-feed commodities were commonly found in the farm that can create nitrate toxicity problems. Ammonium nitrate and urea fertilizers, have been implicated in poisoning cases. As cattle graze pastures, or forage around buildings, they will locate fertilizer spills and quickly consume the material. When urea fertilizer is consumed, the urea molecule is broken down into two ammonia units. Rumen and blood ammonia levels increase dramatically within 20-30 minutes of consumption (Mathew, 1989). Blood ammonia concentrations generally cause the toxicity problems with symptoms of ammonia poisoning in animals are sunken eyes and loss of elasticity of the skin due to dehydration, high temperature, laboured respiration, muscle tremors or tetany and a fluid

filled rumen of pH of 8 – 9 (Bartley *et al.*, 1976) which coincides with our findings. Mydriasis occurs in parasympatholytic status or a sympathetic stimulation (Mathew, 1989). The presence of signs such as mydriasis and tachycardia suggests that ammonia poisoning could elicit a sympathetic or a parasympatholytic status. This hypothesis contradicts with Haliburton and Morgan (1989) statement that during ammonia poisoning the parasympathomimetic status predominates and could be responsible for signs such as bradycardia and profuse salivation. The presence of congestion of liver with some damage matched with findings Horner (1982) who reported liver congestion and pericarditis. It is generally agreed that urea toxicity is equivalent to ammonia poisoning (Shirley, 1986). Ammonia toxicity prevents the release of carbon dioxide from the red blood cells while nitrites prevent the red blood cells from carrying oxygen to body tissue. Some cattle survived after treating with vinegar. The antidote for a mature cow suffering from ammonia toxicity is the oral administration of 4L vinegar and this may need to be repeated every 20-30 minutes until the symptoms disappear (Horner, 1982). Toxicity problems are not usually associated with the ingestion of protein but rather with ingestion of excess levels of urea. The utilization of ammonia depends upon the growth rate of ruminal microbes and is usually limited by the availability of readily fermentable carbohydrates (i.e. grains). After an animal consumes feed that contains nitrate, rumen ammonia levels may increase significantly and it is unusual to have blood ammonia levels increased (Kemp, 1977). Healthy animals have normal methemoglobin levels that are relatively constant at 2-3% of total hemoglobin. When high nitrate feeds are consumed, moderate nitrate poisoning symptoms appear and 20-40% of the hemoglobin is converted to methemoglobin (Johnson, 1983; Pfister, 1988) and severe symptoms or death can occur when blood methemoglobin levels rise up to 67-90% (Asbury and Rhode, 1964). Veterinarians have various drugs and antidotes that can be given to animals to relieve from acute poisoning symptoms. Chronic cases are not cured by the administration of these products (Page, 1987). Methylene Blue is able to convert methemoglobin

back to hemoglobin. The dosage must be within a specified narrow range otherwise it can intensify the problem. Intravenous injection of methylene blue in saline solution (4%) for horses must be in the 1-2 mg/kg range while cattle and sheep require 20 mg/kg to obtain satisfactory results (Blood *et al.*, 1989).

Conclusion

Nonprotein nitrogenous sources such as urea has benn used as feed additive for a long time in cattle feeding. The conventional and proper dose maintaining in mixing of urea with feed can be a handy and economic source of protein from non-protein nitrogenous substances for animals. The deliberate use of urea in cattle feed can be fatal and cause severe farm animal loss. So, farmers should have proper knowledge about the dose and method of urea supplementation in cattle feed and should be cautious enough in this situation.

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Constraints and prospects of carp production in Rajshahi and Natore districts, Bangladesh

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Abstract : This study was conducted in Rajshahi and Natore districts of northern area of Bangladesh for a period of three years (2007 to 2009). One hundred carp ponds were selected randomly and fish farmers were interviewed to reveal the existing constraints and prospects. Ten percent farmers reported multiple ownership as a problem and 16% farmers stated that lease value of pond is too high which reducing their profit level. Lack of quality fish seeds was reported by 33% farmers which results in low growth and high mortality of fishes. Nine percent carp farmers reported that inorganic fertilizers were not giving expected performances. Thirty three percent carp farmers failed to apply required inputs in due time due to lack of sufficient credit. Plankton bloom was recorded in 18% ponds. Three percent fish farmers were found to be affected by flood and drought. Eight percent farmers reported non-severe attack of disease. Fish poaching and poisoning were revealed as major constraints**.** Thirty six percent farmers mentioned lack of technical supports. Due to found constraints, the farmers failed to use inputs and experiences properly resulted in unsatisfactory fish production (3598.72±785.83 kg/ha/yr) to the carp farmers. Further research efforts are recommended to assess the specific impacts of recorded constraints on fish production.

Introduction

Fisheries is one of the major components of agricultural activities in Bangladesh and plays a vital role in nutrition, employment, income generation and foreign exchange earnings (Bhuiyan *et al.*, 2011). Carp polyculture is the most popular form of aquaculture practice in Rajshahi and Natore districts. Though there are at least 265 freshwater fish species in the country (Rahman, 2005) but only 4 native and 12 exotic carp species are cultured in Bangladesh (DoF, 2012). Some non-carp species are also brought under culture lately in the country. The total amount of cultured fishes in the country is produced in ponds and ditches (86%) and other water bodies (14%) (FRSS, 2009). Among the fishes produced in ponds and ditches, 88.18% are carp species and 11.82% are non-carp species (FRSS, 2009). Average fish production (2839 kg/ha) in aquaculture ponds and ditches of Bangladesh is still much lower than many other carp producing countries like China (4474 kg/ha) (FRSS, 2009; Dey *et al.*, 2005). Though the aquaculture production rate has been increased much in recent time in Bangladesh, but still not satisfactory in comparison to most other Asian countries due to existence of some constraints and problems in culture system. To increase the production, we need to identify the existing constraints and problems first. But in Bangladesh, research on this issue is not satisfactory. The present study was conducted with a view to identifying the existing problems and constraints of carp culture.

Materials and Methods

Study sites and duration: Field survey was carried out in Rajshahi and Natore districts, situated in the northern part of Bangladesh, for a period of three years from 2007 to 2009.

Sampling framework: A total of 100 carp polyculture ponds were selected randomly in the study areas. Pond operators of those ponds *i.e.* carp farmers were interviewed with a selected questionnaire to reveal the existing problems and constraints of fish production. The questionnaire was prepared, pre-tested in field situation and then necessary modifications were made prior to final data collection.

Data analyses: All the collected data were tabulated, and subjected to common analyses by MS Excel.

Results and Discussion
Existing problems and constraints

Various constraints and problems of carp culture recorded in the study areas are mentioned here under different headings. All the recorded constraints are displayed in figure 1.
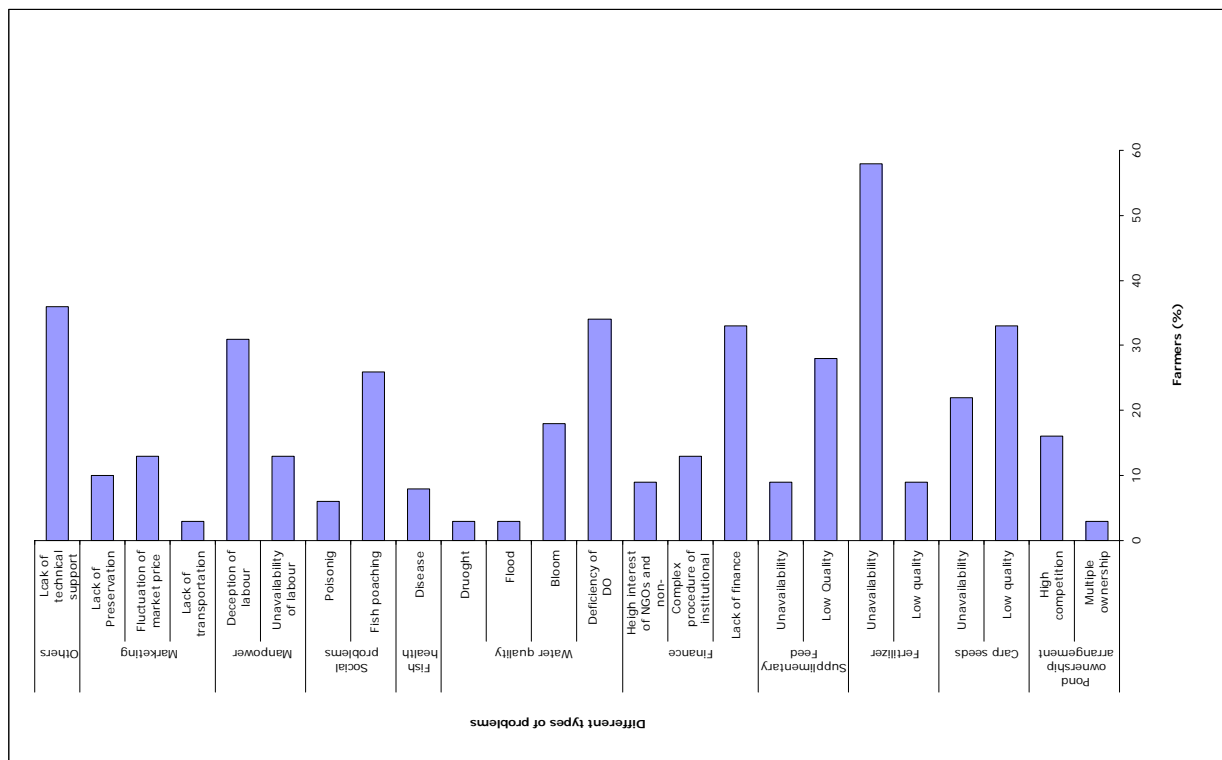
Fig. 1: Constraints of carp culture in the study area

Ownership problems: A few farmers (10%) reported multiple ownership of the culture pond as a problem in the study areas. Respondents have mentioned that multiple-ownership of pond was a vital problem in study areas in past, and they were trying to avoid this problem by leasing out the pond. However 16% farmers stated that lease value of pond was too high which reduced their level of profit. However, multiple-ownership of water bodies is a well documented constraint for aquaculture in Bangladesh (Mollah *et al.*, 1990; Bhuiyan, 1999; Alam, 2005).

Input problems: Lack of quality fish seeds was reported by 33% farmers which resulted in low growth and high mortality of fishes. Almost all the farmers preferred wild seeds than that of hatchery seeds to avoid these problems, but unavailability of wild seeds in due time was also mentioned by 22% farmers. Hossain *et al.* (2008) stated that healthy fish seeds are the prerequisite for fish production. 10% farmers stocked both wild and hatchery produced carp seeds into their ponds. Similar findings were also reported by Mohsin and Haque (2009), Alam (2005), Awal *et al.* (2001), Khaleque *et al.* (1998), Hossain *et al.* (1991) and Khan *et al.* (1991).

Nine percent carp farmers reported that inorganic fertilizers were not giving expected outputs. They have suspected that these fertilizers were impure. Moreover supply of fertilizers, especially inorganic fertilizers, was not regular in the study areas. 58% farmers reported unavailability of desired fertilizers when needed which greatly affected their production. Similar findings were also reported by Ahmed *et al.* (1995).

All the farmers mentioned high price of commercial carp supplementary feeds. To minimize the feeding cost local farmers were interested to use locally available low cost fish feed ingredients like rice bran, wheat bran, oil cakes etc. But mixture of other particles to these feed ingredients was reported by 28% farmers, which resulted in low performances of ingredients. Moreover, unavailability of these ingredients when needed was found in case of 9% farmers. Mohsin and Haque (2009) mentioned similar findings in his research work.

Credit problem: Thirty three percent carp farmers failed to apply required inputs, fertilizers and feeds, in due time due to lack of sufficient credit. In this case, loan from the bank or other organizations may be an alternative. But, difficulties in getting loan from government banks

were mentioned by 13% farmers. However loan from non-government organizations was easy to get but interest rate was high. Present findings are similar to the findings of Alam (2005). Sayeed *et al.* (2007) mentioned that our farmers do not have enough money to invest in aquaculture.

Water quality problems: Thirty four percent farmers reported that their stock grasped at the water column due to lack of sufficient dissolved oxygen (DO) level in pond water. All the farmers did not use any water quality test kit. While facing this problem, the farmers followed traditional methods to increase DO level, like swimming in pond to agitate water.

Plankton bloom was recorded in 18% fish ponds. Over fertilization of pond, especially with organic fertilizers, was responsible for this and this problem was common in winter season. This result was similar to Sayeed *et al.* (2007) who mentioned that fish farmers use fertilizers at different doses, sometimes three to four times more than that of standard dose.

Carp farmers of the study areas were also affected by natural disasters like flood and drought. 3% fish farmers were found to be affected by flood and drought. During flooding, farmers tried to create barricade with 'bana' and nets to restrict fish from escaping. Effects of drought were common in small sized ponds and they farmers placed water hyacinth as a shelter in the deeper part of pond to shelter the fish stock. Islam (1986) mentioned that 13% farmers were suffering from sufficient water in dry season.

Fish disease: Eight percent fish farmers reported that disease has been affected their stock, but the attack was not severe. High stocking density during winter season was found as the prime cause of disease which was similar to the result of Hossain *et al.* (2008). According to FFP (2004), 30% fish farmers treated fish disease as a major problem. Whereas Mohsin and Haque (2009) described disease as minor problem in their study.

Social problems: Fish poaching was a severe problem in the study areas and 26% farmers have been victimized by this problem. To overcome this problem, farmers placed bamboo or tree branches into the pond water. Security guards have also been employed in some cases too. 6% farmers were the victim of fish poisoning. This was an important risk of aquaculture in the study areas. Similar findings were also reported by Alam (2005) and Islam (1986).

Manpower problems: Thirty one percent farmers mentioned deception of labour as a problem. They have also mentioned high rate of daily labour. 13% farmers reported unavailability of labour timely.

Marketing problems: Problem of slow transportation to fish market was reported by 3% farmers. For which the farmers have to sell their fishes in the nearest fish markets and was not getting expected price. Fluctuation of market price was treated as problem by 13% farmers. Lack of proper preservation facilities was also mentioned by 10% farmers.

Others: Thirty six percent fish farmers stated that they were not getting technical supports from relevant government or non-government organizations and they had to discuss with neighbor farmers to solve their problems. Positive relationship between level of knowledge and fish production has been reported by Rahman *et al.* (1989) and Hossain *et al.* (1991). According to Islam (1986) some fish farmers have basic information regarding pond aquaculture but they have no scientific knowledge about technologies. Pekar *et al.* (2002) mentioned that better knowledge of functioning of aquaculture ecosystems would help to improve the management of culture sustain high pond productivity.

Prospects of carp polyculture

The carp polyculture in ponds of Rajshahi and Natore districts was a common aquaculture practice and the most important means of animal protein production for local people. The minimum, maximum and mean (±SD) fish production was found 2017.65, 5458.88 and 3598.72±785.83 kg/ha/yr respectively. According to Sayeed *et al.* (2007), fish production can be increased up to 5000 kg/ha by feeding and fertilization which is much higher than that of the mean production. This indicates that there is enough scope of increasing the existing production. Due to found constraints, the farmers failed to use inputs and experiences in culturing fish properly. Farmers said that due to mentioned constraints overall fish production rate was not satisfactory and they did not get expected profits. They also believed that these constraints would badly affect the aquaculture production in future.

Emphasis should be given on expansion of hatchery facilities to supply high quality fish seeds required to support high aquaculture production in the study area. Similar comment also made by Bhuiyan *et al.* (2011). Several problems (e.g.

plankton bloom) can easily be avoided by maintaining standard doses of fertilizers. This not only helps to control the bloom, but also reduce the input cost resulting in more profit.

The production of aquaculture in a unit area is poor comparing to other neighboring countries (Sayeed *et al.*, 2007). This may be due to presence of so many problems. Further research efforts are necessary to understand the specific impacts of recorded constraints on fish production.

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The efficacy of diatomaceous earth in mixed formulation with other dusts and an insecticide against the pulse beetles, *Callosobruchus chinensis* L. and *Callosobruchus maculatus* (F.)

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Abstract: Effectiveness of diatomaceous earth (DE) and other inert dusts (kaolin powder, paddy husk ash, coal ash, alluvial soil, china clay) and a dust formulation insecticide carbaryl have been tested on the pulse beetles *Callosobruchus chinensis* L. and *C. maculatus* (F.). The bioassay of the dusts was done on adult beetles by mixing them with normal food (lentil and black gram seeds). The LD_{50} of the combined doses of mixtures for *C. chinensis* have been calculated as 12703.57 and 859.36 ppm for DE+ kaolin powder; 2432.78 and 274.00 ppm for DE+ paddy hush ash; 3430.036 and 426.16 ppm for DE+ coal ash; 12563.47 and 652.29 ppm for DE+ alluvial soil; 2242.81 and 325.76 ppm for DE+ china clay; and 21.33 and 14.45 ppm for DE+ carbaryl after 24 and 48 h after treatment respectively. The LD_{50} of combined doses of different mixtures for *C. maculatus* have been calculated as 3640.65 and 503.74 ppm for DE+ kaolin powder; 54946.68 and 987.2394 ppm for DE + paddy hush ash; 61029.04 and 3229.436 ppm for DE+ coal ash; 61029 and 4265.599 ppm for DE+ alluvial soil; 4648.786 and 642.278 ppm for DE+ china clay; and 24.12017 and 15.47023 ppm for DE+ carbaryl after 24 and 48 h after treatment respectively. The co-toxicity coefficient has been calculated and all ratios showed synergistic action. The highest co-toxicity coefficient was recorded as 88885.15 and 92107.22 in DE+ carbaryl at 24 and 48 h after treatment for *C. chinensis* and 78615.55 and 86004.88 in DE+ carbaryl at 24 and 48 h after treatment for *C. maculatus*.

Introduction

Inert dusts, especially DE dusts and silica gels, are suitable for disinfesting empty storage facilities and for grain treatment (Aldryhim, 1990). Their use is more appealing in view of the widespread development of resistance in stored-product insects to conventional pesticides. Fields (2000) studied that the minimum effective rate of dust needed for suppression of progeny production of bruchid beetles 50% less in mixed form with other dusts than that needed for complete mortality used alone.

Admixture of dust formulations of insecticides with grain is a widely used method of protection against stored product pests and has particular advantages when used to treat small batches for local storage (LaHue, 1978). In some cases insecticides are supplied by manufacturers as dust concentrates for dilution with locally available mineral bases; in other cases formulation is carried out in the user country using local mineral carriers and imported technical grade insecticide. The addition of suitable stabilizing agents is usually necessary to prevent rapid decomposition of the insecticide on the mineral surface, and a small proportion of amorphous silica is sometimes added to dust concentrates as an anti-caking agent.

The integration of non-chemical control methods can mitigate problems related to residues in food and pest resistance by overuse of these products (Beckel *et al.*, 2004). The manipulation of grain temperature and the use of inert dusts such as diatomaceous earth are examples of promising non-chemical methods for the integrated management of insect pests of stored products (Flinn, 1998; Flinn & Hagstrum, 2002; da Conceição *et al.*, 2012). Diatomaceous earth is derived from sediment diatomaceous alga shell, and when in contact with the insects causes the removal of the wax layer of the cuticle, causing its death by desiccation (Korunic, 1998).

Diatomaceous earth mixed with grain via dusting or spraying, controls most of the pests effectively. This product works on larvae and adult insects, clinging to their bodies as they move on the surface or within the treated grain mass (Alves *et al.*, 2006). Moreover, it presents some advantages such as low toxicity to mammals and environment; it does not leave harmful residues in the treated product; it is effective against insect species resistant to insecticides, and it is persistent and stable at high and low temperatures (Collins, 2006).

However, studies are needed on the toxicity of diatomaceous earth in combination for populations of insect pests of stored products with

different standard of susceptibility to the insecticides currently in use. These studies are important because populations of the same species with different genotypes may show different responses to the same treatment (McKenzie, 1996; Li *et al.*, 2007).

Inert dusts, especially DE dusts and silica gels are suitable for disinfesting empty storage facilities and for grain treatment. Their use is more appealing in view of the widespread development of resistance in stored-product insects to conventional pesticides. On grain, different treatment techniques (treating partial layers) should be explored. Locally available different inert dusts are easily available and initiatives should be given to develop insecticides with these materials. Keeping these in mind the present study was undertaken to find out the toxicity of DE and some other inert dusts on the adult pulse beetles, *C. chinensis* and *C. maculatus.*

Materials and Methods
Test Insects
The pulse beetles *C. chinensis* and *C. maculatus* were collected from Shaheb Bazar, Rajshahi. Mass cultures were maintained in earthen pots and sub-cultures in glass jars (500 ml) or beakers (500 ml) with the food medium in the Crop Protection and Toxicology Laboratory, Department of Zoology, University of Rajshahi, Bangladesh. All the equipments were kept in an oven for sterilization, about six hours at 60°C. Lentil (*Lens culinaris* Medic.) and black gram (*Vigna mungo* (L.)) seeds were used as food medium for *C. chinensis* and *C. maculatus* respectively through the experiment. A large number of beetles were thus reared for continuous supply of the newly emerged adults.

Diatomaceous earth and others dusts
SilicoSecs was obtained from Agrinova GmbH (Germany). SilicoSec is a relatively new DE formulation of freshwater origin, and contains approx. 92% SiO_2, 3% Al_2O_3, 1% Fe_2O_3, and 1% Na_2O (McLaughlin, 1994). A dry lump of kaolin was purchased from the market. The lumps were crushed in boiling distilled water and then homogenized. The preparation was left to cool at 29°C. It was then filtered through a 53 μm mesh sieve, a piece of 45 μm fine steel gauze and a piece of cotton fabric (25 μm of fine-knit). The resulting suspension, called "kaolin milk", was left undisturbed for 3 days and protected from dust

with a fine steel gauze. The particulates were recovered by draining of the water and they were placed in the sun to dry. The dried material was crushed in a porcelain mortar and the powder was sifted (53 μm mesh) and stored in a container away from moisture.

Paddy husk and coal ashes were collected from rice mills and brickfields respectively. The ashes were sieved with a fine net (mesh 600) and placed in an incubator for an hour at 60ºC to dry up excessive moisture. Alluvial soil was collected from the riverbed of the Padma, Rajshahi. The china clay was procured from the local market. Both soil and clay were powdered in a mortar and pestle and finally meshed and dried. For comparison the insecticidal activity of the inert dusts, a commercial insecticide dust formulation "Sevin 85 SP" of Bayer CropScience was used.

Experiments
For combined treatment DE was mixed in different ratios with kaolin powder, paddy hush, coal ash, alluvial soil, china clay and carbaryl. The ratios were 1:1, 1:5, 1:10, 1:20, 1:50 and 1:100 for kaolin powder, ashes and clays and for carbaryl it was 1:1, 1:2, 1:5 and 1:10. In all cases the unit of DE was 50 ppm excepting for carbaryl which was 5 ppm. The mixed formulations were used for the bioassay on the newly emerged adults of *C. chinensis* and *C. maculatus* with respective food and in each case a control batch was maintained on untreated food. The mortality of the beetles was recorded 24 and 48 h after treatment.

Data analysis
The mortality percentage was corrected using Abbott's formula (Abbott, 1925). Probit analysis was done according to Finney (1947) and Busvine (1971) using a software developed in the Department of Agricultural and Environmental Science, University of Newcastle upon Tyne, UK. Co-toxicity coefficients were calculated following Sun and Johnson (1960) as:

$$\text{Co} - \text{toxicity coefficient} = \frac{\text{LD50 of the toxicant alone}}{\text{LD50 of the toxicant in the mixture}} \times 100$$

Results and Discussion
The results of LD_{50}, 95% confidence limits, regression equations (Y) and χ^2 of diatomaceous earth in mass ratio mixtures with tested dusts on *C. chinensis* and *C. maculatus* are presented in Table 1.

The LD_{50} and the co-toxicity coefficient for DE

and dusts has been separated as ratios and are presented in Table 2. The result shows that the LD$_{50}$ values of diatomaceous earth in mixture were decreased in different ratios as the different dusts were mixed. The synergism was in the order of DE: carbaryl> DE: china clay> DE: paddy hush ash> DE: coal ash> DE: alluvial soil> DE: kaolin powder at 24 h and DE: carbaryl> DE: paddy hush ash> DE: china clay> DE: coal ash> DE: alluvial soil> DE: kaolin powder at 48 h for *C. chinensis* and the synergism was in the order of DE: carbaryl> DE: kaolin powder> DE: china clay> DE: paddy hush ash> DE: coal ash> DE: alluvial soil at 24 and 48 h for *C. maculatus*.

Table 1. LD$_{50,}$ 95% confidence limits and regression equations of diatomaceous earth in mass ratio mixtures with tested dusts on adult pulse beetles.

Pulse beetles	Diatomaceous earth: Dust	Combined LD50 (ppm)	95% confidence limits		Regression equation	χ^2 (4 df)
			Lower (ppm)	Upper (ppm)		
	24 hours					
	Kaolin powder	12703.57	2380.47	67793.47	Y= 1.960363+ 0.7406656 X	0.3381
	Paddy hush ash	2432.78	1556.73	3801.82	Y= 0.1815667+ 1.423003 X	5.9025
	Coal ash	3430.03	1486.49	7914.71	Y= 1.966581+ 0.8580376 X	0.3197
	Alluvial soil	12563.47	911.16	173229.7	Y= 3.228549+ 0.4321551 X	0.0747
	China clay	2242.81	1218.96	4126.62	Y= 1.664071+ 0.9955641 X	0.4389
	Carbaryl	21.33	17.70	25.710	Y= 0.1733399+ 3.631431 X	2.4141
C. chinensis	**48 hours**					
	Kaolin powder	859.36	449.24	1643.86	Y= 2.835927+ 0.7375404 X	1.1209
	Paddy hush ash	274.00	151.06	496.99	Y= 2.507756 + 1.022351 X	3.0830
	Coal ash	426.16	253.33	716.87	Y= 2.298666 + 1.02729 X	1.7954
	Alluvial soil	652.29	296.75	1433.82	Y= 3.301301+ 0.6035645 X	0.5115
	China clay	325.76	208.27	509.54	Y= 1.698464 + 1.313831 X	0.8225
	Carbaryl	14.451	11.95	17.47	Y= 0.5489369 +3.837429 X	4.1731
	24 hours					
	Kaolin powder	3643.651	1299.44	10216.85	Y= 2.480977+0.7072854 X	1.2359
	Paddy hush ash	54946.68	2051.36	1471774	Y= 2.156692+0.5998614 X	0.2658
	Coal ash	61029.04	686.56	5424904	Y= 3.077287+0.4017759 X	0.4222
	Alluvial soil	61029.04	686.56	5424904	Y= 3.077287+0.4017759 X	0.0422
	China clay	4648.78	2040.53	10590.94	Y= 1.317287+1.004192 X	0.1762
	Carbaryl	24.12	19.95	29.14	Y= 0.0678453 +3.567871 X	2.4422
C. maculatus	**48 hours**					
	Kaolin powder	503.74	301.46	841.763	Y= 2.31615+0.9932041 X	1.2084
	Paddy hush ash	987.23	694.52	1403.312	Y= 0.5533071 + 1.484992 X	5.3390
	Coal ash	3229.43	686.94	15182.06	Y= 3.439218+0.444778 X	0.2714
	Alluvial soil	4265.59	978.81	18589.1	Y= 3.09993+0.5234382 X	0.4148
	China clay	642.27	410.31	1005.385	Y= 1.840817+1.125176 X	1.6445
	Carbaryl	15.47	12.85	18.614	Y= 0.4231234+3.847742 X	4.6092

Table 2. Co-toxicity coefficient of mixtures of Diatomaceous earth in mass ratio mixtures with tested dusts on adult *C. chinensis* and *C. maculatus*

Species	DE : Dust	Combined LD50 (ppm)	LD50 of DE in mixture (ppm)	Co-toxicity coefficient
	24 hours			
	Kaolin Powder	12703.57	396.986	149.117
	Paddy Hush Ash	2432.782	76.024	778.668
	Coal Ash	3430.036	107.188	552.277
	Alluvial Soil	12563.47	392.608	150.780
	China Clay	2242.814	70.087	844.628
C. chinensis	Carbaryl	21.33705	0.666	88885.150
	48 hours			
	Kaolin Powder	859.3629	26.855	1546.838
	Paddy Hush Ash	274.0051	8.562	4851.721
	Coal Ash	426.1612	13.317	3119.348
	Alluvial Soil	652.2975	20.384	2037.890
	China Clay	325.7676	10.180	4080.585
	Carbaryl	14.45133	0.451	92107.228
	24 hours			
	Kaolin Powder	3643.651	113.864	519.896
	Paddy Hush Ash	54946.68	1717.083	34.475
	Coal Ash	61029.04	1907.157	31.039
	Alluvial Soil	61029.04	1907.157	31.039
	China Clay	4648.786	145.274	407.488
C. maculatus	Carbaryl	24.12017	0.753	78615.551
	48 hours			
	Kaolin Powder	503.7491	15.742	2638.823
	Paddy Hush Ash	987.2394	30.851	1346.483
	Coal Ash	3229.436	100.919	411.620
	Alluvial Soil	4265.599	133.299	311.632
	China Clay	642.278	20.071	2069.670
	Carbaryl	15.47023	0.483	86004.886

In the present investigation the possibility of using DE as an extender or carrier for some other inert dusts or insecticidal dust formulations used to protect stored grain from insect attack. Formulations of this type would have the advantages of reducing levels of toxic residues and of having greater activity against insect populations with low levels of resistance to the chemical component of the formulation. Le Patourel and Singh (1984) showed that formulations of permethrin, cypermethrin and deltamethrin on absorptive silica had the additional advantage of a greater than additive action between the insecticidal components when tested against *T. custaneum* in grain of low moisture content.

The trials in the study indicate that SilicoSec can be used successfully as a protectant against adults of the bruchid beetles *C. chinensis* and *C. maculatus*. Regardless of the synergism of other factors, the effect of DE is dose-dependent (Fields & Korunic, 2000; Athanassiou *et al.*, 2003; Stathers *et al.*, 2004; Mahdi & Khalequzzaman, 2006), as in the case of residual insecticides used as grain protectants. However, the dose rate is more important in the case of inert dusts, given that the presence of dust in grain highly affects the physical properties of grain (Korunic *et al.*, 1996). In addition, dust formulations that are effective at high application rates are usually not acceptable, for health and environmental reasons (Subramanyam & Roesli, 2000). Nevertheless, higher application rates are recommended in cases of increased humidity, or as a surface treatment in bulked grain (Nickson *et al.*, 1994; Bridgeman, 2000; Subramanyam & Roesli, 2000; Cook & Armitage, 2002).

In the present study, the rates of 1600 ppm produced high mortality levels, though 100% was not achieved in most of the cases examined. However, given that pulse beetles can survive at application rates that are effective against other stored-grain beetle species (Aldryhim, 1990; Arthur, 2000a, b; Fields & Korunic, 2000), higher dose rates or longer exposure intervals are needed to achieve 100% mortality for adults of this species. The efficacy of SilicoSec is determined by the type of the product the dust is applied to. Athanassiou *et al.* (2003), using SilicoSec in dose-response tests against *S. oryzae* adults in peeled rice, paddy rice, barley and maize, found that mortality notably varied in different types of grain.

The efficacy of diatomaceous earth on the mortality of insect pests of stored products is usually affected by several factors among which stands out the temperature (Chanbang *et al.*, 2007). Generally, the increase in temperature favors the increase in the effectiveness of this product by stimulating the movement of insects within the grain mass, providing an increased contact of them, with the diatomaceous earth (Chanbang *et al.*, 2007; Vayias *et al.*, 2009). In addition, the insects have higher respiration rates at higher temperatures (Cotton, 1932), and consequently the greater water loss via spiracles promoting desiccation (Zachariassen, 1991). However, it was shown in some studies that the insect mortality can vary between species (Arthur, 2000a; Vayias & Athanassiou, 2004; Athanassiou *et al.*, 2005; Vayias *et al.*, 2009).

Increased exposure time is highly important for DE efficacy, since surviving individuals may disperse from the treated substrate and colonize untreated parts of the product mass (Subramanyam & Roesli, 2000). This fact must be seriously taken into account in cases of partially treated grain masses with DE, such as the surface treatment in grain bulks, when DE in the surface is used alone, as a barrier to infestation (Korunic & Mackay, 2000).

Conclusion

Diatomaceous earth is a potential alternative to be used in the development of strategies for management of resistance in insect pests of stored products, since a uniform response was observed among populations of *C. chinensis* and *C. maculatus*. In the present study, the rates of 1600 ppm produced high mortality levels, though 100% was not achieved in most of the cases examined. However, given that pulse beetles can survive at application rates that are effective against other stored-grain beetle species. Other dusts and clays used were inactive to *C. chinensis* and *C. maculatus* but in combination with DE they also provide some sort of synergistic effects.

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Prevalence and determination of occupational diseases of leather tannery workers

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The tannery industry in Bangladesh is concentrated in the Hazaribagh area. It is the largest leather-processing belt of Bangladesh. The total waste discharge area is about 25 hectares, where 20,000 people are presently living in a slum area, under extremely densely populated and unhygienic conditions. Health impact from the tannery disposal wastes is either death or increased probabilities of death and sufferings by illness including skin diseases, fevers, headaches etc. In Hazaribagh and surrounding areas, many vegetables farms are irrigated with waste water from polluted Buriganga rivers. Studies by FAO/WHO (1993) have found that metal concentrations are high and increased consumption of these vegetables, health problems for consumers in future are inevitable. Severe effects include reduced growth and development, cancer, organ damage, nervous system damage, and in extreme cases, death.

There are 206 tannery industries in Hazaribagh area. Thirty-five tannery industries were selected and visited and discussed with workers about their health problems, socio-economic conditions, knowledge about the diseases, their attitude and practice towards the prevention and control of the diseases. More than four thousands people are working in the 35 industries. Most of the workers are suffering from different diseases. About 35% of the tannery workers are suffering from gastrointestinal disease, 25% from dermatological disease, 10% from Headache, Hypertension and Lethargy (Fig. 1 and Plate 1).

In an open question to workers on predominant health problems, the respondents mentioned that skin diseases, gastric ulcers, gastroenteritis, respiratory illnesses (asthma), anemia, dysentery, headache, hypertension and lethargy were the most common health problems amongst the population in the area.

The distribution of the most commonly cited health problems are shown in Fig. 1. Gastrointestinal and skin diseases were the health problems ranked as most frequently occurring in the workers of the industry. Headache, lethargy and hypertension were mild health problems experienced by the workers of tannery industry.

The interviews were conducted to local doctors practicing in the area and provided similar results in terms of the five most prevalent health hazards in the area. The doctors identified ten health problems that were prevalent in the area. Of these five most frequently diseases were gastric ulcers, skin disease, asthma, diarrhoea and hypertensions. According to doctor's perception, skin disease, gastrointestinal problems, respiratory disorders (asthma), diarrhea and fever were the main health problems in the area throughout the year.

Environmental pollution is a major threat facing humanity in view of increasing industrialization, urbanization and population growth (Fitzgerted, 1993). Cities are becoming more and more polluted every day due to increasing discharge of untreated wastewater effluents into water reservoirs and the rivers. The polluted water poses serious health hazards to residents. As will become readily apparent to any visitor, the noxious odor of rotting flesh has created the worst case of pungent atmospheric pollution in Dhaka.

Hazaribagh area has a high incidence of a number of health problems. In this area the peoples are frequently suffering from abdominal skin ulcer, scabies, discomfort/gastritis, peptic ulcer, lung diseases, respiratory diseases, dermatitis, nasal ulcer/loss of smelling capacity, red eye/other eye illness, running nose, erosion and discolouration of teeth, asthma, puffines of face and oedema, diarrhoeal disease, high fever, conjunctivitis, urinary tract infection, jaundice, hypertension etc. A high number of mentally retarded children have been found, most of them were born in this area (IULTCS, 2004).

According to the report of the Bangladesh Society for Environment and Human Development, about half a million residents of Hazaribagh, Bangladesh, are at risks of serious illness due to chemical pollution from tanneries near their homes. The report says, large numbers of the 8000-12000 tannery workers aged 30-35 years suffer from gastrointestinal disease (58%), dermatological disease (31%), hypertension (10%), and jaundice (10%) that could be related to the pollution. Ninety

percent of these workers die before the age of 50 *Vs* less than 60% for the country as a whole. About a quarter of these workers are under 11 years of age (Maurice, 2001).

Chromium is one of the most harmful chemicals found in the tannery waste because of its carcinogenic potential. It may cause cancer. Chromium wounds skin, liquor chrome enters the body through hair pores and comes into direct contact with the skin (O'Flaherty *et al.,* 1956-65). Acidic effluents can cause severe respiratory problems. Gaseous emissions from the tanneries contain sulphur dioxide that is converted into sulfuric acid on contact with moisture and can damage lungs (SEHD, 1998).

The most of the workers performed their duties in acid solution at pickling stage without wearing musk, gloves, boots and apron. The sulfuric acid is strongly corrosive, which may cause permanent damage to skin if any worker worked bare hand in acid solution. Harmful chemicals like sodium sulphate and sodium bisulphate when mixed with blood for a long time it may cause cancer (Salam *et al.*, 2002).

Skin problems, allergic conditions, itching and other skin lesions are contact type disease. Usha (1989) reported that the water of khal, beel and parts of the Buriganga river are generally alkaline in nature, which is likely to be attributed to the extensive use of the alkalis soda ash, caustic soda, heavy metal salts in the tannery and dyeing industry. This alkalinity is likely to be a key factor in the skin diseases and irritations reported by local communities as they reported that the symptoms manifest themselves when their skin has come into physical contact with khal water or sediments. The majority of the respondents reported that children and tannery workers are suffered from skin diseases. Most of workers expressed that they have experienced skin problems because of their frequent contact with chemicals, and some of them were currently suffering from skin problems. They willingly showed the skin lesions on their bodies, particularly on hands, fingers and legs. The symptoms of the skin conditions include a rash, boils and irritation. While talking to the local pharmacy, they reported that the drugs for skin problems were the highest selling drugs in the tannery locality.

The tannery workers and local people believe that there are two main causes of skin problem. The first is that physical contact especially among children who are living in unhealthy environments. The second and more frequently reported cause is contact with the chemicals used in the tannery industry.

Skin disease has increased in tannery area. Besides the workers, farmers, children and fisherman are mainly affected as they work in the polluted water. The pollutants from tannery industries are responsible for it. Pollutants from industries enter in the Buriganga river through the drain, khal and beel and end up here.

Stomach problems; the majority of the respondents also blamed the lack of proper sanitation system, tanning wastes (liquid and solids) and lack of knowledge about hygiene for diarrhoea and dysentery, which are frequently among children, slum dwellers and tannery workers. Gastrointestinal diseases have been identified as a common health problem for workers in the area, including tannery workers. The local doctors interviewed opined that this was due to irregular eating habits and the length of time between meals. Many studies confirm the occupational health problems associated with working in the tannery industry, and textile dyeing industry. Usha (1989) notes the high incidences not only skin problems but also asthma, chronic bronchitis, tuberculosis, bladder cancer and irritation of the eyes among the workers of tannery and dyeing industries.

After tanning comes the process of finishing leather, a huge amount of dyes, pigments and chemicals are used again. During making the finished products, leather particles mixed with the air and also causes respiratory problems of the workers. Most of the workers said, some of the illnesses automatically disappear when they take a leave or stop working temporarily.
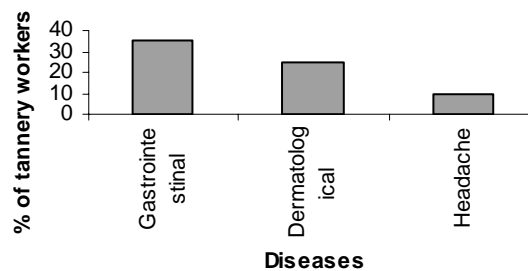

Fig. 1. Percentage of tannery workers suffering from different diseases.

Plate1. Dermatological Diseases of Tannery Workers

From the findings, the present study concludes that the Hazaribagh area has a high incidence of different number of health problems. In this area the peoples are frequently suffering from a variety of health problems that could be a direct or indirect relating to the activities of local industries. These problems include skin disease, gastrointestinal disease, respiratory illnesses, jaundice etc. The workers believe that these problems are a result of increases in the number of industrial units in the area. They expressed that tanning effluents entering the surface water bodies in the area including drain, canal, khal, is reducing the quality of water and as a result they are unable to use it for the purpose for which it was used in the past, such as bathing and washing cattle. When they do use it, they suffer from health impacts like skin rashes and sores. Gastrointestinal problems, such as gastric ulcer or other similar gastric problems may be related to diet and the impacts of the pollution on crops, vegetables and fishes consumed by people living around the Hazaribagh

area. It is also possible that ground water is being polluted by infiltration of tannery effluent but similarly there has been no empirical research into this. The problems of diarrhoea and dysentery are unlikely to be caused directly by the tanning effluents, as they are usually the result microbial contamination. None of these findings have been confirmed with rigorous epidemiological studies. Epidemiological studies, are necessary to better determine the impact these industries having on the environment and the people who interact with it. Such evidence is crucial if policy makers and industry owners are going to be influenced to control and mitigate for environmental pollution. In order to improve the situation interventions both at the national and local levels are required. The implementation of legislation on safety precautions, banning toxic chemicals and pollutant concentrations in industrial discharges into water sources are all required. In 2003, the then government took up a Tk. 175 crore project to shift the tanneries to a 'leather estate' in Savar. The project was inaugurated in 2005. The deadline for relocating the tanneries to Savar is 2010. Sources said complexities in setting up the Common Effluent Treatment Plant (CETP) are hindering the process of shifting the tanneries. Tannery owners are also reluctant to shift their business until the government provides them with compensations and other facilities. However, education and communication campaign would be beneficial to aware the community about risks and possible ways to minimize them, and to inform the Bangladesh public about the problems. Finally, it can be concluded that government should take necessary measured to control this problems.

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Anatomy of digestive and respiratory system of Indian grey mongoose (*Herpestes edwardsii*)

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Mongooses (Herpestidae) are small, widespread carnivores occupying various habitats from Africa to Southeast Asia (Thulin *et al.,* 2006). The genus *Herpestes* contains 10 species (Nowak, 1999) and is considered the oldest genus within the order Carnivora, dating back approximately 30 million years (Hinton and Dunn, 1967). The Indian gray mongoose or common grey mongoose (*Herpestes edwardsii*) is a species of mongoose mainly found in southern Asia mainly India, Pakistan, Nepal, Sri Lanka and some other parts of Asia (Choudhury *et al.,* 2011). According to IUCN Red list 2012 (IUCN, 2012) status they are listed as Least Concern. They are terrestrial, diurnal and commonly found in open forests, scrublands and cultivated fields, often close to human habitation (Prater, 1980). This omnivorous scavenger preys on rodents, snakes, birds' eggs and hatchlings, lizards and variety of invertebrates (Choudhury *et al.,* 2011). The Indian gray mongoose is one of the few animals that can survive a cobra attack, which makes it one of the deadly snake's few predators. As there is limited information about the anatomy of digestive and respiratory system of mongoose, this will be a platform for better understanding of physiology, pathology of diseases and subsequent application in clinical examination and surgical intervention if needed.

An adult male Indian grey mongoose (*Herpestes edwardsii*) was brought in Sahedul Alam Quadery teaching veterinary hospital, Chittagong for surgical treatment but it was found clinically dead. Before post mortem examination species name, body weight, body length, tail length, external features were recorded in hospital case record sheet. Keeping in dorsal recumbancy, a longitudinal incision was made at the ventral midline from pharynx to the pelvic inlet. Thus organs were examined sequentially without any damage or distortion. After thorough post mortem examination it was subjected to anatomical measurement in Anatomy laboratory of the same University. Different views were examined to describe the organs. Length of coiled portion of the respective organ was measured by thread placing on along the long axis of the tubular organ. Circumference was taken with full of content of the organ. Diameter was measured by using slide caliper (0-150 digital caliper, Shinko Denshi Co. Ltd, Japan). Tracheal length was measured from last laryngeal cartilage to the tracheal bifurcation. Each lobe of the lung was studied well by removing the loose attachment within them. For nomenclature Nomina Anatomica Veterinaria, 1994, 4th edition was used.

Digestive system

The digestive tract of Mongoose consist of oesophagus, stomach, duodenum, Jejunum, ileum, caecum, colon and rectum. Accessory digestive organs includes teeth, tongue, salivary glands, liver, pancreas. Among them liver and pancreas were studied well. Oesophagus was straight tube with a length of 12.3 cm and diameter at cranial and caudal portion was 6.2 mm and 4.7 mm respectively. The stomach was the carnivore type. It was 'J' shaped with a total length of 9.5 cm and circumference at cardiac, fundic, pyloric region was 6 cm, 6 cm and 4 cm respectively (Fig.1). Duodenum starts from the pyloric sphincter and continues about 10 cm with a maximum diameter of 5.0 mm. Pancreas is located along the border of the duodenum. The jejunum was the longest portion among the three parts of the small intestine which had a total length of 92 cm with a maximum diameter of 4.92 mm. Unlike other carnivore they have their centrifugal and centripetal coiled portion having length of 16 cm with cranial and caudal continuation of 'U' shaped loops (Fig.2).
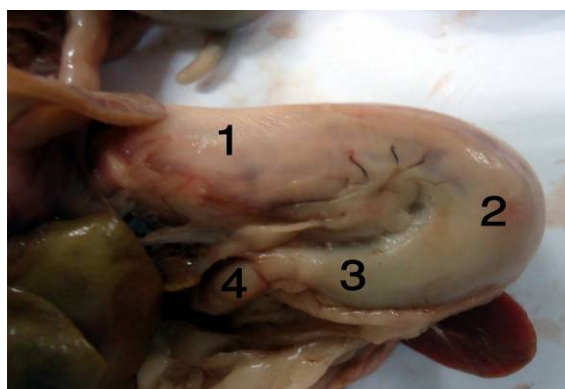

Fig. 1. Simple stomach of Indian Grey Mongoose. 1.Cardic region, 2. Fundic region, 3. Pyloric region 4. Pyloric sphincter
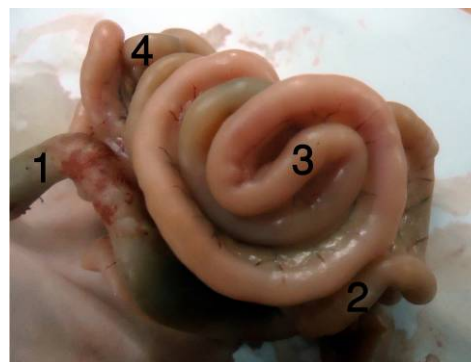

Fig. 2. Jejunum of Indian Grey Mongoose. 1.Duodenum, 2.Cranial part of Jejunum , 3.Centripetal and centrifugal coiling of Jejunum, 4.Caudal part of Jejunum.

Coiled portion starts 14 cm away from the starting point of the jejunum and rest of the part holds a length of 62 cm with uniform diameter. In dog and cat there is no such concentric coil (Getty, 1975). Ileum, the straight portion of the small intestine has its length and diameter, 9 cm and 4.2 mm respectively. Caecum was elongated comma shaped structure situated at the junction of the ileum and colon with a length of 3.8 cm (Fig.3). Its diameter at base and apex was 4.0 mm and 3.0 mm respectively. Colon was straight tube with length 4.3 cm and diameter 5 mm. Rectum was also the straight tube which terminates at the anus. Its maximum length was 7.1 cm with circumference 2.7 cm. Liver was divided into five chief lobes and two processes by fissures which converge at the portal fissure. During examination in soft condition the lobes were spread out so as it was well visible (Fig.4).
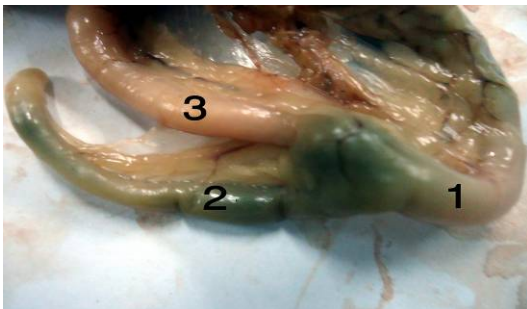

Fig. 3. Elongated comma shape caecum of Indian Grey Mongoose. 1.Ileum, 2.Caecum, 3.Colon.

Fig. 4. Parietal surface of the liver of Indian Grey Mongoose. 1. Right medial lobe, 2.Gall Bladder, 3.Quadrate process, 4.Left medial lob.
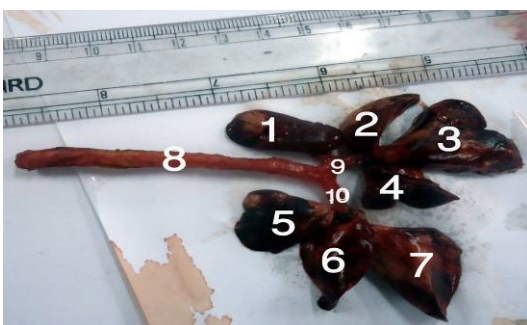

Fig. 5. Right and left lobes of lungs with trachea of mongoose.

The right medial lobe was the largest and rectangular in outline. The left lateral lobe was second in size and was somewhat tongue shaped. But the left medial lobe was third in size and was prismatic. Caudate lobe had deep impression of right kidney. In dog and cat left lateral lobe was largest and was oval in outline. The left medial lobe was smaller and prismatic. The right medial lobe was second in size and was somewhat tongue shaped (Getty, 1975). Usually it does not reach to the ventral border of the liver. It was visible well from the parietal surface of the liver (Fig.4). Pancreas was located along the border of the duodenum with a length of 15 cm.

Respiratory system

Respiratory system of mongoose consists of nasal cavity, larynx, trachea and lungs. Trachea was 8 cm long tube consisted of incomplete 'C' shaped cartilaginous ring in which incomplete part meet face to face at the dorsal aspect. The cranial and caudal part was 5.16 mm and 4.60 mm diameter respectively. Trachea bifurcates into right and left principal bronchi. Diameter of right and left principal bronchi at their origin was 4.42 mm and 3.64 mm respectively. The right apical lobar bronchus originated from 2.30 mm away from the tracheal bifurcation whereas left apical lobar bronchus was originated 4.46 mm away from the bifurcation. Right lung possess four lobes namely apical (cranial), middle (cardiac), diaphragmatic (caudal) and accessory (intermediate) lobes whereas left lung possess 3 lobes namely apical (cranial), middle (cardiac) and diaphragmatic (caudal) lobe (Fig.5). Right lung was larger than the left. Right apical lobe originated more craniad than the left. All the lobes were well separated. In left lung there had no distinct cardiac notch as in dog (Miller *et al.,* 1964). Accessory lobe was conical in shape with concave basal border.

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Egg morphometric analyses in chickens and some selected birds

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Morphometrics in general refers to measurements of the body parts. The knowledge and information on morphometric parameters is therefore essential for understanding an animal and its reproductive biology in particular (Danilov, 2000). Egg morphometric parameters such as egg weight, egg width, albumen and yolk weights are very important in poultry because these factors influence egg quality and grading (Farooq *et al.*, 2001), reproductive fitness of the chickens and embryonic development (Onagbesan *et al.*, 2007). Effects of feed (Shapira, 2010) and housing system (Wang *et al.*, 2009) on egg composition and its quality have been reported. Internal egg quality parameters such as albumen weight and yolk weight are very important from nutritional and cholesterol content for human consumption (Sparks, 2006). Egg characteristics of Fayoumi (Islam, 2005), broiler chickens (Mamun, 2005) and indigenous fowl (Sarker, 2006) have previously been reported. In recent years egg quality traits of various chicken breeds (Islam & Dutta, 2010; Jones *et al.,* 2010; Momoh *et al.,* 2010) revealed results that are important to poultry breeders. Here we report a detailed account of egg morphometrics from six available chicken breeds and five other bird species.

Experimental: Eggs from breeder hens of an indigenous (non-descriptive, *Deshi*), five purebred exotics *viz.*, Cobb-500, RIR, ISA Brown, ISA White and Fayoumi, and a crossbred called *Sonali* (derived from RIR♂ × Fayoumi♀) were collected for this study. Moreover, eggs from five selected pet birds namely goose, duck, pigeon, dove and quail were also collected. A total of 120 fresh eggs (12 birds × 10 replicates each) were collected for estimating egg quality traits *viz.* egg length (EL in cm), egg width (EW in cm), egg volume (EV in cm^3), gross egg weight (GW in g) and shell weight (SW in g). In addition, four internal egg quality traits *viz.* shell index (SI=EW÷EL×100), shell ratio (SR=SW÷EW×100), yolk weight (YW in g) and albumin weight (AW in g) were taken into account. The eggs were numbered first and then weighed on an electronic balance to determine their weights. Subsequently, EV was determined using the formula, EV= π × EL × EW^2/6 (cm3). Each egg was broken on a table and its contents poured into a plate or small pot. Then the yolk was separated from the albumen with the help of a spoon and weighed. Moreover, the phenotypic associations between the relevant external and internal egg quality traits were determined by Karl Pearson's product moment co-efficient of correlation (r). Mean, standard deviation (SD), analysis of variance (ANOVA), least significant differences (LSD) and r values were computed using the SPSS (version 11.0 for Windows). Data on various egg morphometrics and external and internal egg quality traits were subject to these statistical procedures to detect the significant differences between the genetic groups of chicken under study.

Egg morphometric parameters in chickens: It is apparent from the results presented in Table 1 that the parameters like EL, EW, EV, GW, SW and AW were found to be the highest in ISA Brown and the lowest in the indigenous chickens. This trend was altered for YW, SI and SR traits where the highest values were recorded respectively in Cobb 500, Cobb 500 and RIR, whereas the indigenous, ISA White and ISA White showed the lowest values. A descending order of ISA Brown > ISA White > Cobb 500 > Fayoumi >RIR > *Sonali* > indigenous was obvious for EV. Depending on GW, the chicken breeds could be arranged in a descending order of ISA Brown > Cobb 500 > ISA White > Fayoumi >RIR > *Sonali* > indigenous. The AY of the chickens was recorded as follows: ISA Brown > Cobb 500 > ISA White> Fayoumi > RIR > *Sonali* > indigenous while the YW was recorded as Cobb 500 > ISA White> ISA Brown > Fayoumi > *Sonali* > RIR > indigenous. One-way ANOVA demonstrated that all the egg morphometric parameters varied significantly among the chicken breeds (P<0.001) except for EL ($F_{6,\ 63}$= 1.24; P>0.05).

Egg morphometric parameters in other birds: In birds other than chickens, goose had the highest values for EL, EW, EV, GW, SW, AW and YW, whereas quail and pigeon attained the highest values for SI and SR, respectively. On the other hand, quail (EL and AW), dove (EW, EV, GW and SW), pigeon (YW) and goose (SI and SR) showed the lowest values for the parameters in parentheses (Table 1). On the basis of EV and GW, a descending order of goose > duck > pigeon

> quail > dove was recorded for each parameter. On the other hand, the sequences of AW and YW were goose > duck > pigeon > dove > quail, and goose > duck > quail > dove > pigeon, respectively. Unlike chicken breeds, one-way ANOVA revealed highly significant variations among the five bird species under study (P<0.001).

Table 1. Egg morphometric parameters in chickens and some other birds

Breeds	EL	EW	EV	GW	SW	AW	YW	SI	SR
Indigenous	4.59 ±0.47[a]	3.56 ±0.19[a]	30.72 ±5.81[a]	20.20 ±4.76[a]	4.20 ±1.69[a]	8.10 ±2.08[a]	7.90 ±2.03[a]	78.10 ±7.11[a]	20.17 ±4.65[b]
Cobb 500	5.86 ±0.15[a]	4.09 ±0.17[bh]	51.45 ±5.18[b]	56.20 ±1.62[b]	9.20 ±0.92[b]	32.00 ±2.40[b]	15.00 ±2.87[b]	69.79 ±1.95[c]	16.35 ±1.35[c]
ISA Brown	5.93 ±0.54[a]	4.56 ±0.14[c]	62.01 ±4.80[c]	57.50 ±2.72[bc]	10.20 ±1.14[bc]	35.50 ±2.17[c]	11.90 ±1.10[ce]	79.90 ±2.83[a]	17.78 ±1.53[c]
ISA White	5.90 ±0.14[a]	4.25 ±0.10[bd]	55.78 ±2.58[bdh]	53.90 ±2.64[bd]	8.50 ±1.51[bd]	31.70 ±1.4[bd]	13.70 ±2.67[bde]	72.08 ±2.70[c]	15.75 ±2.59[c]
RIR	5.11 ±0.15[a]	3.83 ±0.07[ef]	39.26 ±2.20[ef]	28.80 ±0.80[e]	6.70 ±0.68[e]	13.30 ±1.25[e]	8.80 ±1.40[a]	74.99 ±1.93[b]	23.25 ±2.09[a]
Fayoumi	5.06 ±0.23[a]	3.92 ±0.13[fgh]	40.73 ±3.31[f]	38.00 ±3.30[f]	8.60 ±0.84[bf]	19.40 ±2.01[f]	10.00 ±1.63[ac]	77.64 ±4.80[a]	22.71 ±2.24[a]
Sonali	4.96 ±0.14[a]	3.77 ±0.09f[g]	36.94 ±2.55[efg]	26.60 ±4.81[eg]	5.10 ±2.18[ae]	12.50 ±3.21[eg]	9.00 ±1.33[a]	76.04 ±2.15[b]	18.94 ±6.00[b]
Goose	8.80 ±0.19[a]	5.96 ±0.19[a]	63.91 ±3.57[a]	66.30 ±3.34[a]	20.00 ±1.25a	81.60 ±1.35[a]	64.70 ±1.83[a]	67.57 ±1.16[c]	12.02 ±0.62[c]
Duck	5.87 ±0.17[b]	4.07 ±0.09[b]	51.42 ±2.85[b]	53.50 ±5.76[b]	9.00 ±2.16[b]	28.70 ±3.02[b]	15.80 ±2.53[b]	69.36 ±1.51[c]	16.74 ±3.34[b]
Pigeon	3.82 ±0.19[c]	2.73 ±0.12[c]	14.97 ±1.97[c]	11.70 ±0.82[c]	2.40 ±0.52[c]	6.30 ±0.68[c]	3.00 ±0.82[c]	71.50 ±1.64[b]	20.69 ±5.18[a]
Dove	3.26 ±0.08[d]	2.30 ±0.08[d]	9.05 ±0.84[cd]	9.40 ±0.52[cd]	1.60 ±0.52[cd]	4.30 ±0.48cd	3.30 ±0.48[cd]	70.55 ±1.49[b]	15.78 ±4.96[b]
Quail	3.15 ±0.09[de]	2.41 ±0.09[de]	9.59 ±0.81[ce]	10.90 ±1.73[ce]	2.20 ±0.63[ce]	3.70 ±0.68[def]	5.00 ±1.56[ce]	76.54 ±3.04[a]	19.11 ±6.14[a]

EL= egg length; EW= egg width; EV= egg volume; GW= gross egg weight; SW= shell weight; AW= albumen weight; YW= yolk weight; SI= shell index; SR= shell ratio. Figures (mean ± SD values) followed by different superscripts for each parameter in the same column (chickens and other birds considered separately) differ significantly by LSD (P<0.05).

Associations between egg morphometric parameters: The GW was significantly correlated with EV in indigenous (r= 0.88; P<0.001) and ISA Brown (r= 0.72; P<0.001), with SW in indigenous (r= 0.88; P<0.001) and Cobb 500 (r= 0.72; P<0.05), with AW in indigenous (r= 0.84; P<0.01), ISA Brown (r= 0.77; P<0.01), Fayoumi (r= 0.90; P<0.001)and *Sonali* (r= 0.81; P<0.01), with YW in indigenous (r= 0.75; P<0.05), Cobb 500 (r= 0.69; P<0.05), ISA White (r= 0.74; P<0.05), Fayoumi (r= 0.64; P<0.05) and *Sonali* (r= 0.71; P<0.05), with SI in ISA White (r= -0.78; P<0.01) and Fayoumi (r= -0.65; P<0.05); and with SR in indigenous chickens (r= 0.63; P<0.05) only. In birds other than chickens, significant correlations were found to exist between GW and EV for goose (r= 0.91; P<0.001), duck (r= 0.83; P<0.01) and pigeon (r= 0.66; P<0.05); between GW and SW and GW and AW for goose (r= 0.64, 0.67 and 0.89, respectively), duck (r= 0.66, 0.69 and 0.89, respectively) and dove (r= 0.67, and 0.80, respectively); between GW and YW for goose (r= 0.89; P<0.001), duck (r= 0.89; P<0.001), pigeon (r= 0.66; P<0.05) and quail (r= 0.82; P<0.01); and between GW and SR for dove only (r= 0.73; P<0.05). All other correlations between the egg parameters in chickens and other bird species were statistically insignificants.

Economically important egg morphometric parameters such as weight, size, albumen and yolk contents are quantitative traits that show continuous variability (Chatterjee *et al.*, 2007; Islam & Dutta, 2010). It is also an established fact that the weight of an egg is a direct proportion of shell, albumen and yolk that it contains and this varies significantly between breeds or strains of the bird species (Jones *et al.*, 2010; Momoh *et al.*, 2010). The present results lend support to the findings of Yeasmin & Howlider (1998) and Islam

(2006) for indigenous, Nahar *et al.* (2007) for broiler, Islam & Nahar (2008) for White Leghorn, RIR and indigenous, and Miazi (2008) for Fayoumi and *Sonali* chickens. Internal egg parameters such as AW and YW are very important from nutritional and health viewpoints (Sparks, 2006). In this regard, ISA Brown eggs showing the highest albumen contents (35.50±2.17g) and indigenous eggs showing the lowest yolk content (7.96±2.03g) could be considered preferable. Significant correlations between GW and various external and internal egg parameters of the present study agree with Pohle & Cheng (2009) and Momoh *et al.* (2010). But unfortunately, owing to scarcity of experimental data on egg morphometrics of such birds as goose, duck, dove, pigeon and quail, the present results could not be compared.

Conclusions: Chicken eggs contribute substantially to the human nutrition and so their dietary profile including lipid, cholesterol and antioxidant contents are particularly important. In addition, because yolk weight is related to the amount of cholesterol, choice for the nutritionally potential and healthier eggs is a matter of considerable concern, especially to patients suffering from cardiovascular diseases, oxidative stress, endothelial dysfunction and inflammatory syndromes. Apart from these, information on the egg morphometric parameters is also vital for an understanding of fertility, development of embryo, egg quality and disease of the poultry and other pet birds.

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The time of embryonic axis formation in quail eggs

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The chalazae constitute an axis of rotation around which the egg can rotate, maintaining the germ upward within the inner liquid albumen (Burley & Vadehra, 1989; Rahman *et al.*, 2007, 2009). When the outer part of the egg envelope, the eggshell, rotates in the uterus, the egg yolk remains nearly motionless, and the chalazae become coiled (Clavert, 1962). As a result of this rotation, the blastoderm is forced into an oblique position, and the embryo develops an antero-posterior axis with its head in the direction of rotation (Kochav & Eyal-Giladi, 1971). Vintemberger & Clavert (1960) showed that most eggs rotate in the uterus with their sharp end toward the cloaca, in which case the embryo develops according to Von Baer's rule; its tail develops toward the observer holding the sharp end towards the right of the observer. The present study aimed to find out the time of embryonic axis formation by incubating oviductal eggs of quail.

Japanese quail (*Coturnix japonica*) of the wild-type strain were reared individually in cages in a poultry house with 16 h illumination per day. They were fed a layer diet *ad libitum* and killed by cervical dislocation, which was approved by an Institutional Animal Care and Use Committee of Gifu University (No. 06120). The oviductal eggs were removed from 20-week-old females at an appropriate time after oviposition.

Collected eggs were positioned horizontally with the equatorial side up, marked at the top of the eggshell, and incubated at 39ºC and at 60% humidity until Day 3 of incubation, at which time the embryonic axis appears. After incubation, the eggshell was cut around the mark, inverted, and the orientation of the embryonic head was determined, with the orientation of naturally spawned eggs being 0° according to the rule of Von Baer (Fig.1). If the anterior direction shifted toward the blunt end, the value is expressed with a plus sign, whereas if it shifted toward the sharp end, it is shown with a minus sign.

Ovulated eggs were located in the oviductal uterus during 5-25 h after the previous oviposition in Japanese quail (Iwasawa et al., 2010; Rahman et al., 2009; Woodard and Mather, 1964). Overall, 114 oviductal eggs were examined. Among them, 26 eggs were collected from females during 5-17 h

after the previous oviposition, but incubation of these eggs resulted in arrested development at the blastoderm embryo stage, with no axis formation (data not shown).
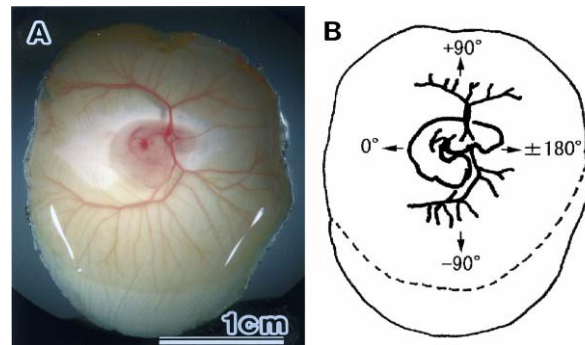

Fig. 1 Micro-photograph of oviductal egg incubated for 3 days (A) and its illustration (B), showing the definition of axis orientation with the anterior direction of embryo being 0°, according to the rule of Von Baer. The blunt end of the egg is at the top. View from inside.

Forty-four eggs collected during 17-25 h developed an embryonic axis (Fig. 2A). Of these, the direction of the antero-posterior axis of the embryo in the eggs collected during 17-21 h deviated widely from the fundamental position determined by the rule of Von Baer (see, Clavert, 1962; Kochav and Eyal-Giladi, 1971), in a random direction. On the other hand, eggs collected during 21-25 h developed an embryonic axis converging into the fundamental position.

In the remaining 44 eggs collected during 17-25 h, development was arrested at the thin blastoderm with occasional blood cells, and the embryonic axis was not formed (Fig. 2B). Such arrested development was mostly observed in eggs collected during 17-21 h. Since the location of these blastoderms was just beneath the mark made on the top of eggshells and since the location of embryonic bodies, if developed, always shifted from the mark, some shift of the blastoderm from the top position may happen in the latter but not in the former. This means that embryonic development does not proceed unless its axial formation occurs. The reason for the developmental arrest observed in eggs collected during 5-17 h is unknown.
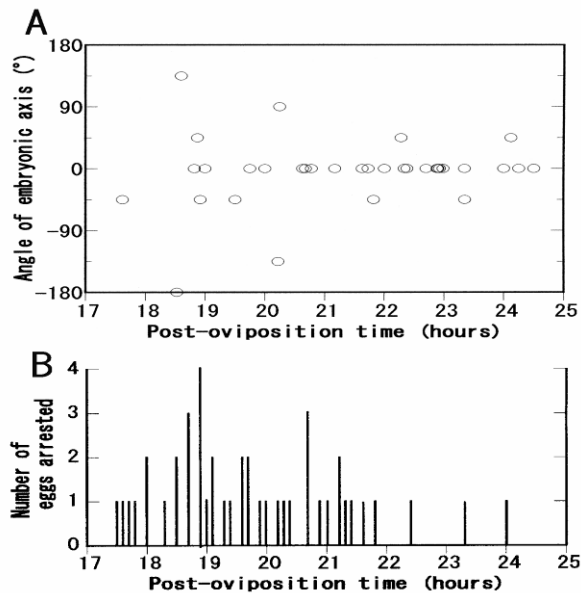
Fig. 2 Angle of the anterior direction of embryonic axis that developed in eggs collected from oviducts at various hours after the previous oviposition (A) and the number of eggs in which development arrested (B).

Eggshells and the cuticle layer are formed in the uterus. The matrix of eggshell is produced by granular cells of mucosal epithelia and calcified between 7 and 20 h after the previous oviposition (Iwasawa *et al.*, 2010). The cuticle materials were produced by ciliated cells of mucosal epithelia between 21-25 h (Rahman *et al.*, 2009). The appearance of 10-µm-wide posts on the surface of the mucosal epithilia during eggshell formation and their disappearance during cuticle formation was interesting (Iwasawa *et al.*, 2010). Retreat of the posts leads to the formation of air canals in the eggshell, but it also means that the egg in the uterus is immobile from 7 h to 21 h and becomes mobile after 21 h (Rahman *et al.*, 2009). This transition may be triggered by progesterone, which stimulates the release of the resident sperm from the sperm storage tubules in the utero-vaginal junction (Ito *et al.*, 2011) with the same time schedule as that of cuticle formation.

It is said that the peristalsis of the uterus wall rotates the shell materials slowly in one direction while the yolk tends to return the egg to a vertical position, keeping the blastoderm tilted obliquely through the commitment of chalazae (Gerhart and Kirschner, 1997). Kochav & Eyal-Giladi (1971) experimentally showed that the embryo head pointed in the direction of rotation. The present study showed that the embryonic anterio-posterior axis is formed during 21-25 h after the previous oviposition with an aid of cuticle material as a lubricant for egg rotation; however, the molecular

mechanism of axis formation is still unknown. Such a study may be possible in the future using eggs collected during 17-21 h, since they are competent for axis formation but this has not been determined yet.

The present findings disclose the determination of the time of antero-posterior axis formation in embryos of the Japanese quail (*Coturnix japonica*). Oviductal eggs from the females during 5-25 h after the previous oviposition incubated for several days in a horizontal position with the equatorial side up. Eggs during 5-17 h after the previous oviposition showed arrested development at the blastoderm embryo stage, with no axis formation. The direction of the embryonic axis in the eggs collected during 17-21 h showed a random orientation or developmental arrest. But the eggs collected during 21-25 h showed the development of the embryonic axis converging into the fundamental position determined by the rule of Von Baer. These results indicate that the embryonic antero-posterior axis is formed during 21-25 h after the previous oviposition in quail.

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Influence of farm conditions on the production of hygienic milk

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Milk is one of the most common food sources in the human diet and is also a product that is directly available for consumption (Grimaud *et al.*, 2009). In Bangladesh the production of milk is very low. Most of the cows are indigenous and produce a little more than one liter milk per day (Kabir & Islam, 2009). Although measures are being taken to improve the production of local cows through artificial insemination, the improvement is not rapid due to the shortage of exotic bulls, frozen semen, trained personnel and technical facilities. At present many private dairy farms have been established but most of them follow no hygienic points for milk production.

Maintaining a high standard of hygiene is one of today's most important milk production objectives. The hygiene level directly influences economic production and dairies are enforcing this by steadily raising their quality requirements for raw milk (Jansen, 2003; Lues *et al.*, 2003; Dovie *et al.*, 2006). More importantly consumers are concerned about the safety of dairy products and the conditions under which these are produced. It is therefore important to ensure high quality raw milk from healthy animals under good hygienic conditions and that control measures are applied to protect human health.

For the study Zihan dairy farm (ZDF), Sherpur, Fatema Multi Project (FMP) (Vita Milk), Mymensigh and Beltali Krishi Khamar (BKK) (Pusti), Mymensingh were selected.

Raw milk samples (100ml) were collected aseptically into sterile bottles from three different points of each farm- one (point-1) from milking bucket of each milk collection unit/point (MCU) immediately after milking from individual cow, another (point-2) from bulk cans of each MCU after mixing of milk of different cows and last (point-3) from polythene packaged milk sold to retail stores. Bottles were kept in a large wide mouthed thermo flasks kept cool in ice and then processed immediately for delivery to the Laboratory of Microbiology, Department of Microbiology and Hygiene, Bangladesh Agricultural University (BAU), Mymensingh. A total of 30 samples (10 samples from each unit) of each farm were bacteriologically examined and microorganism counts were expressed in colony forming units (cfu) per ml of milk. The foregoing prepared diluted samples were subjected

to Total Viable Count (TVC) in the Laboratory as well as to detect and enumerate coliform and staphylococci. TVC was accomplished according to the method described by Quinn *et al.* (1994) who prescribed serial tenfold dilution of the milk samples. The technique followed to determine the TVC by pour plate method on nutrient agar, MacConkey agar and Manitol salt agar.

The bacterial load was not uniform in different points of production in the same farm and within farms. From the results it is observed that in all three points TVC were the lowest (54.5×10^4/ml) in ZDF followed by a higher count (58.8×10^4/ml) of FMP (Vitamilk) and the highest (77.6×10^4/ml) in BKK (Pusti). The TVC increased progressively from the first point to the third point as recorded in sample of all farms but this increase was found to be the lowest in milk samples of ZDF. It may be due to hygienic care and handling of milk, proper cleanliness of utensils and minimum period required during production at different points and rapid cooling of milk. The TVC/ml of milk samples of FMP (Vitamilk) as well as BKK (Pusti) from point-1 towards point-2 increased poorly but from point-2 to point-3 the rate of increase was rapid. It may be attributed to longer time interval of packaging of milk and unhygienic practices.

The results of TVC were more or less similar to Golubeva (1981) who reported that the bacterial counts in unstored and stored milk sample ranged from 100000 to 5.5 million/ml and 400000 to 23.5 million/ml respectively.

The coliform count was the lowest (73.3 cfu/ml) in FMP (point-1) and the highest (154.8 cfu/ml) in BKK (point-3). In ZDF coliform increase rate from point-1 to point-3 was fairly uniform. But in FMP coliform counts from point-1 to point-2 increase rapidly which indicate post milking contamination due to faulty handling and poor hygienic practices. The BKK milk samples from point-1 to point-2 coliforms increased very slowly but from point-2 to point-3 they increased sharply. This may be due to longer holding period and contamination during transportation and measuring. The results are in agreement with the findings of Ahmed & Salam (1991) who examined bacteriologically 100 samples of market raw milk and

Domiati cheese (50 each) with a mean coliform count of 3.8 x 108.

The lowest count of staphylococci was noted in the first point of ZDF and the highest count in the third point of BKK. The average staphylococcal count in all points of individual farms was lower in point-1 and was higher in point-3. The result of the present study similar to the findings of Nader *et al.* (1990) who

examined raw bulk milk collected from a milk processing plant and found S*taphylococcus aureus* having a mean count of 1190/ml.

The milk samples of first point of all the farms were of the best quality because TVC, coliform count and staphylococcal count/ml were the lowest at this point of production at all the farms.

Table 1. Examination of milk samples immediately after milking in selected farms. (Point-1)

Source of sample	Average TVC/ml (TVC/mlx10^4)	Range of TVC/ml (TVC/mlx10^4)		Average coliform/ml (cfu/ml)	Range of coliform/ml (cfu/ml)		Average staphylo-cocci/ml (cfu/ml)	Range of staphylococci /ml (cfu/ml)	
		Max.	Min.		Max.	Min.		Max.	Min.
Zihan dairy farm	54.5	75	40	90.7	115.0	64.0	24.8	42.0	14.0
Fatema Multi Project	58.8	72	41	73.3	102.0	56.0	30.9	42.0	24.0
Beltali Krishi Khamar	77.6	96	50	86.4	106.0	65.0	38.8	62.0	24.0

Table 2. Examination of milk samples from bulk cans (Point-2).

Zihan dairy farm	85.5	120.5	59.5	110.0	142.0	89.0	58.2	80.0	40.0
Fatema Multi Project	76.4	100	54	121.0	182.0	102.0	66.2	88.0	51.0
Beltali Krishi Khamar	87.8	107	66	97.6	124.0	69.0	64.9	80.0	46.0

Table 3. Examination of packaged milk samples of selected dairy farms. (Point-3)

Zihan dairy farm	109.4	120	54	119.4	131.0	108.0	71.0	92.0	63.0
Fatema Multi Project	149.5	182	108	150.4	192.0	112.0	79.1	110.0	50.0
Beltali Krishi Khamar	197.0	260	155	154.8	195.0	100.0	89.0	106.0	66.0

The hygienic production of milk requires stringent measures against contamination at every stage of dairy practices.

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