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With the increase in population, third world countries today are facing many problems, supply of sufficient food being one of them. In animal sciences we have to understand and preserve the vast diversity of species on our planet. Losing them would be a huge shame and almost a crime of humanity. We have caused a continuous trouble that leads to species extinction. Just because we are the “dominant” species on Earth, it doesn’t mean that we can do whatever we want without suffering consequences. We do not have to protect endangered species only, but we also have to protect species essential for the continuation of Earth’s life. Believe it or not, without animals, humans would die out pretty quickly. First of all, there would be no more meat. But we can’t all become vegetarians either if there are no insects to pollinate the plants. From animals, we can also learn about our anatomy and can understand the function of our bodies in a better way, which help us combat human diseases. In termination, animal’s science is an important field that applies to many real-world situations.

University of Sindh Journal of Animal Sciences (USJAS) will promote and involve the study of various disciplines in Zoological Sciences i-e Entomology, Endocrinology, Molecular biology, Parasitology, Wildlife management and Conservation, animal’s diversity and systematic etc. This journal will be ideal platform for anyone working in Animals Sciences. In addition, the published data to provide additional opportunity for access to advanced standing in existing tertiary level education programs. Researcher will be exposed to the main aspect of animal science including, safety management planning strategies, food and fiber, systematic of individual and making a means to preserve a rapidly declining global ecosystem.



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University of Sindh Journal of Animal Sciences (USJAS) will introduce a viable model of research linkages together with scholarly experience sharing and transferring of inspired evidence. It also promotes the latest emerging trends in zoological sciences along with provision of an awareness of the ethical aspects of animals. Besides its fundamental importance in maintaining the sustainable agricultural, protection and conservation of animals in country it will also educate the public about the need to protect and preserve the environment as a long-range goal for the welfare of future generations. The core focus of (USJAS) is concentrated on promoting and propagating novel and innovative research amongst the readers of this journal.

AIM OF JOURNAL

The primary aim of University of Sindh Journal of Animal Sciences (USJAS) is to gain hands on experience in order to acquire the knowledge necessary for the critical analysis of the results and make appropriate recommendations in all fields of Animal sciences. The aim of this journal is to encourage researchers, investigators and scientists to publish their research findings allowing wider dissemination of their intellectual knowledge, with the aim of applying those for the benefit of the society. The newly launched journal would cover full spectrum of the specialties in Animal's sciences. It would include original research articles, review articles, case reports, short commendation, and scientific findings from within specified domain areas of Zoology. The journal strictly follows the guidelines proposed by Higher Education Commission (HEC) Pakistan. The most important criterion for acceptance/rejection is originality of the material presented in the manuscript.



What is need for publishing this Journal?

The University of Sindh Journal of Animal Sciences (USJAS) with modernized and cost effectiveness will light the tools for numerous directions and problems related to improve identification of pest species, conservation of wild animals, diversity of animals including animal breeding, environmental impact of animal, agriculture, diseases, nutrition and animal products. When animals grow well and stay healthy, farmers can produce more meat, milk or eggs for our consumption. They check meat quality or screen milk for pathogens. Advances in food safety keep humans healthy and increase the world's supply of nutritious food. Beside this, articles regarding entomological science contribute to the betterment of humanity by detecting the role of insects in the spread of disease and discovering ways of protecting food and fiber crops, and livestock from being damaged. Journal provides the way how beneficial insects contribute to the well-being of humans, animals, and plants. This journal will also defend and assess the application of well proven research activities in natural science particularly, Zoology, Physiology, Fresh Water Biology & Fisheries, Biochemistry and Biotechnology of host universities; neighboring and sister universities which are performing research activities on any area of animal's sciences. They have necessity of proper platform for their research exposure around the country as well as in world.



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- ✓ After final Acceptance, the Author/Corresponding author will be notified and paper will be forwarded to Publication Manager/Editorial Assistant for copy-editing, layout editing and proof reading.

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DIETARY EFFECTS OF IRON AND IRON OXIDE NANOPARTICLES ON GROWTH, MUSCLE COMPOSITION AND HEMATOLOGICAL ASPECTS OF *LABEO ROHITA*

FARKHANDA ASAD, SAMINA QAMER*, ZUNAIRA SHAHEEN, RAFIA JAMAL, SHEHAR BANO, AIMAN NADEEM, NAVAIRA BATOOL, NOSHABA ANWAR

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FA contributed to experimental design, SQ supervised the research, provided guidance in experimental design and data interpretation, ZS, RJ, SB, AN, NB, and NA conducted laboratory experiments, collected and analyzed data, and contributed to manuscript preparation.

Key words:

Labeo rohita, Hematology, Iron oxide, Iron, Growth and Muscle composition.

ABSTRACT

Two hundred *Labeo rohita* fingerlings of uniform size (2-3g), irrespective of sex, were used to investigate the effects of dietary Fe and Fe₂O₃-NPs on growth, muscle composition and hematological aspects of fish. Fish maintained on 32% crude protein, conventional basal feed was supplemented with Fe & Fe₂O₃ nanoparticles (each at 0.6 & 0.8 mg/kg supplementation levels) to replicate groups of fish for 90 days. Morphometric characteristics i.e. wet body weight (g) and body length (cm) was recorded fortnightly. It was found that the weight gain, body length, SGR and PER were significantly improved in the fish fed on nano iron oxide supplemented diet (T₂). Protein and lipid nutrients retention in the body meat was improved in group of fish fed with nano iron supplemented diet (T₃). A significant increase in hemoglobin (Hb) content, red blood cell (RBC) count, white blood cell count (WBC) and hematocrit (Ht) value was noticed throughout the study period when compared to control group. From the results, it was concluded that Fe-NPs@0.6mg/Kg level is more suitable for *Labeo rohita* with great growth potential and improved muscle composition and hematology as compared to other levels of Fe and Fe₂O₃ NPs.

1. INTRODUCTION

Nano technology can be defined as the accommodating and regulation of substances at the nano-scale, at magnitudes roughly between 1 and 100 nm, where characteristic phenomenon permit novel applications. When adapted into nano-size, the materials indicate exclusive chemical and physical properties (Khan *et al.*, 2019). Nanoparticles are considered to be reasonably well-accepted in the body of human and have major uses in magnetic detection, magnetic resonance imaging (MRI), and hyperthermia and drug delivery. They have used in magnetic resonance imaging as distinctive agents and can afford larger imaging capability owing to their greater half-life (Han *et al.*, 2019). Nanotechnology have capability for both nutrition and medication, because at the nm dimensions materials show innovative properties contrasted to the secluded atoms and unpackaged materials in size, shape, surface area to volume ratio and conductivity.

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Among the metal-oxide NPs, Iron oxide nanoparticles are broadly used in bioengineering, biomedical and clinical applications because to their greater catalytic properties and exclusive magnetic abilities (Vallabani *et al.*, 2018). Iron is the major essential micronutrient because of its influence on the effectiveness of immune system and also defense against several infections. It is vital element for the effectiveness of the tissues androgens' of higher class animals including fish because of its vigorous role in the biological processes including lipid oxidation reactions, cellular respiration and oxygen transport (Uzo *et al.*, 2019).

Fishes are the main protein source among aquatic life, providing 7% of entire protein supplies with 80% of the animal protein consumption. Per capita yearly fish consumption in Pakistan is around 1.9 kilogram which is lowermost contrasted to the advanced countries i.e. about 20kg (FAO, 2017). Pakistan's native carps like *Labeo rohita*, *Cirrhinus mrigala* and *Catla catla* are cultured in private and government sectors, also topmost the natural

aquatic systems (Hussain et al., 2011). For the period of former twenty years cultivation of fish has been renowned as the endorsing world food industry (Yildirim et al., 2014).

The chief objectives of fisheries industry are, primarily to produce better quality fish and secondly, to augment growth. The feed that used for fish cultivation should be inexpensive, enhance immunity, growth and rigorous health of fish, to attain extreme benefits from the fish culture (Ringo et al., 2016). Fisheries industry can be progressed by using nanoparticles in the fish feed.

Fish can absorb soluble iron from the water across the gill membrane but feed is considered to be the main source of iron for fish because of the limited concentration of soluble iron in water. Fish accept and take feed comprising NMs. At the nano-size dietary minerals can pass into the cells more freely than their larger complements. This hastens their process of integration into the fish and also augments the capability of fish to quickly absorb drugs such as vaccines, hormones and nutrients (Terova et al., 2018). Deficiency of iron causes growth reduction, immune damage, changes in the hematological parameters, poor food conversion, microcytic anemia and vulnerability to diseases in common carp (Akbari et al., 2019).

However, very little research has been done to incorporate nano forms of iron into fish feed based on hematology and muscle composition. Little information is present for the mineral desires of *Labeo rohita*, Indian major carps, but these have greatest significance in feed preparations. Under applied farming conditions, deficiencies of iron occur primarily because of low bio-available iron in marketable fish feeds. Therefore, in this study we assessed the applications of different nano forms of iron (nano-Fe & Fe₂O₃) as feed additives in feed for *Labeo rohita*, rohu which is commercially important and broadly cultured fish particularly in Indian subcontinent and generally in Asia (Iqbal et al., 2014).

The necessary tasks of this study were to produce high quality fish with high muscle protein, and to produce fish with better immune functions by improving its hematology and to determine the optimum level of iron and iron oxide nanoparticles for fish feed.

2. MATERIALS AND METHODS

Experimental fish

For the execution of this feeding trial, a total of 200 *Labeo rohita* fingerlings were bought from fish seed hatchery, Satyana road, Faisalabad. Before the commencement of the experiment fingerlings were acclimatized to laboratory conditions for about 15 days, weaned on the control diet (32% crude protein) once daily on 4% live wet body weight (Allan and Rowland, 1992).

Feed ingredients and diet preparation protocol

The basic diet ingredients were sunflower meal, fish meal, gluten, wheat bran, rice bran, vitamin premixes and canola oil. These ingredients were purchased from local market and in laboratory they were grinded to acquire fine powder and were weighed out. To prepare the four experimental diets, both iron and iron oxide nanoparticles (>50nm) (0.6 and 0.8 mg/kg) were mixed in 1 liter of de-chlorinated water per kg and used this water to prepare the dough (Lovell, 1989), whereas the control diet was maintained without nanoparticles supplementation. The dough was pelletized in a laboratory pelletizer fixed with 2 mm diameter and the pellets were collected in trays and dried at room temperature. Diet was formulated by linear formulation method using Win feed 2.6 (Win Feed (UK) Ltd., Cambridge, UK) (Table 1).

Experimental design

After acclimatization, fingerlings of uniform size (2-3g), irrespective of sex, were randomly selected and evenly distributed in 10 glass aquaria (90L×30W×45H with 29L water capacity) at 20 fingerlings per aquarium with two replicates. The experiment was conducted as a Complete Randomized Design (CRD) with one control and four treatments viz. T₁: Fe₂O₃-NPs 0.6; T₂: Fe₂O₃-NPs 0.8mg/kg; T₃: Fe-NPs 0.6 and T₄: Fe-NPs 0.8mg/kg of fish feed. The fingerlings were fed to apparent satiation once a day at 4% of live wet body weight. The water quality parameters i.e. pH, dissolved oxygen and temperature through YSI pro-series multi parameter professional plus meter were monitored. Dissolved oxygen (5ppm) was maintained by air pumps through capillary system (APHA, 1998).

Growth Studies

During the trial, on the basis of fortnight from control and treated groups morphometric characteristics of sampled fish (10) were recorded which include live wet body

weight and body length. Fish fingerlings growth was calculated according to the method followed by (Brown, 1957).

Increase in wet body weight (g)

Average body weight of 1st observed fortnight - Average body weight of next observed fortnight

Increase in net total body length (cm)

Average body length of 1st observed fortnight - Average body length of next observed fortnight

$$\text{Condition factor (K)} = W \times 10^5 \div L^3$$

$$\text{SGR} = \frac{\ln(\text{Final wet body weight}) - \ln(\text{Initial wet body weight}) \times 100}{\text{Time duration (Days)} \times 100}$$

$$\text{Weight gain} = \text{Final weight} - \text{initial weight}$$

$$\text{Weight gain\%} = \frac{\text{Final weight} - \text{Initial weight}}{\text{Initial weight}} \times 100$$

$$\text{PER} = \frac{\text{Final body weight} - \text{Initial body weight}}{\text{Amount of protein fed}}$$

Analytical methods/ Calculations

A representative sample of feed and body meat were homogenized utilizing a motor and pestle and examined chemically by AOAC (1990) procedures: dry matter (DM) by oven drying at 105 °C; crude protein (CP) by microkjeldahl investigation, gross energy by using formula and electric furnace (Eyela-TMF 3100) for ash. Lipid was determined by chloroform methanol extraction technique (Bligh and Dyer, 1959) through 10454 soxtec system HTz.

Dry matter (DM)

$$\text{Moisture\%} = \frac{W_1 - W_2}{\text{Wt. of sample}} \times 100$$

$$\text{Dry matter} = 100 - \text{moisture\%}$$

Crude protein analysis:

$$\text{N}_2\% = \frac{\text{Volume of 0.1N (H}_2\text{SO}_4\text{) used} \times 0.001 \times 250}{\text{Weight of sample} \times 10}$$

$$\text{Crude protein percentage} = \text{N}_2\% \times 6.25$$

$$\text{Percentage of crude fat} = \frac{W_2 - W_1 \times 100}{\text{Wt. of sample}}$$

$$\text{NFE \%} = 100 - (\text{crude protein} + \text{fat} + \text{moisture})$$

$$\text{GE (Kcal/g)} = (5.64 \times \text{protein \%}) + (9.44 \times \text{lipids \%}) + (4.11 \times \text{NFE\%}) \text{ (Henken et al., 1986)}$$

$$\text{Ash} = \frac{\text{Weight of ash} \times 100}{\text{Wt. of sample}}$$

Study of hematological parameters

After providing the different concentrations of iron and iron oxide nanoparticles for 90 days, fish from control and treated group were sacrificed and blood was drawn from caudal vein using plastic disposable syringe. The collected blood sample was transferred into small vials, which were previously rinsed with heparin. The whole blood sample was used for the estimation of hematological aspects like hemoglobin, RBCs and WBCs count. HGB was determined with Drabkin's (Drabkin, 1945) cyanmet hemoglobin method. Microhematocrit method was used in measuring the hematocrit described by Svobodova et al. (1991) and RBC and WBC was determined with a Burkner hemocytometer using Natt and Herrick (1952) stain.

Red blood cell indices were calculated in accordance with Greer et al. (2014).

$$\text{MCV} = \frac{\text{Hematocrit}}{\text{RBC}}$$

$$\text{MCH} = \frac{\text{HGB}}{\text{RBC}}$$

$$\text{MCHC} = \frac{\text{HGB}}{\text{Hematocrit}}$$

Statistical Analysis

After finding possible results, information of growth, muscle composition and hematology were subjected to statistical analysis ANOVA and \pm SE values was evaluated by using SPSS (Steel et al. 1996) and difference between means ($P < 0.05$) were evaluated by the Tukey's HSD Test (Snedecor & Cochran, 1991).

3. RESULTS AND DISCUSSION

Growth performance

Results revealed that Iron NPs and Iron oxide NPs had pronounced effect on the growth performance and muscle composition of *Labeo rohita* fingerlings. After the period of 90 days, body weight, body length and SGR were recorded highest in Fe-NPs and FeO₃ NPs treated groups. Moreover, highest body weight gain, body length and SGR was recorded in group T₂ treated with FeO₃ NPs 0.8 mg/Kg (Table 2).

Body composition

Body composition parameters are the key indicator of the physiological status of fish as well as feed composition

and feeding rates. The results revealed various levels of Fe-NPs and Fe₂O₃ NPs in diet showed pronounced effect on the muscle composition protein, lipid, ash and dry matter of experimental fish *Labeo rohita*. Crude lipid and crude proteins content were found highest in group T3 treated with Fe-NPs 0.6mg/Kg whereas ash contents and gross energy were recorded highest in group T4 treated with Fe-NPs 0.8mg/Kg. Moisture contents were recorded highest in group T1 treated with Fe₂O₃ NPs 0.6mg/Kg and dry matter recorded lowest as well (Table 3).

Hematology

All the hematological parameters were lower significantly ($P < 0.05$) in fish fed on control diet compared to those fed on experimental diets. Higher hemoglobin (Hb) contents were observed in fish fed with Fe NPs supplemented diet compared to those fed with Fe₂O₃ NPs which was 7.42 g/dl and red blood cell (RBC) count was significantly ($P < 0.05$) lower ($1.54 \times 10^3 / \mu\text{L}$) in fish fed the Fe₂O₃ NPs supplemented diet than in those fed with Fe NPs supplemented diet while all of the red blood cell (RBC) indices MCV, MCH and MCHC were higher in fish fed with Fe₂O₃ NPs supplemented diet than in those fed with Fe NPs supplemented diet which were 160.98 fl, 58.8pg and 48.58 g/dl, respectively. In comparison to fish fed with Fe NPs supplemented diet, white blood cell (WBC) count was significantly ($P < 0.05$) lower ($9.55 \times 10^3 / \mu\text{L}$) in fish fed with the Fe₂O₃ NPs supplemented diet. In case of hematocrit (PVC%), it was maximum (23.97%) in fish fed with Fe NPs supplemented diet compared to those fed with Fe₂O₃ NPs supplemented diet (Table 4).

Discussion

Growth in fish is considered in terms of increase in volume and the volume is represented by weight. Results indicated that body weight, body length and SGR were observed highest in group T2 treated with Fe₂O₃ NPs 0.8mg/Kg. These results are supported by Behera et al., (2014) who found the significant ($P < 0.05$) changes in growth parameters FW, WG, %WG, SGR and CF with the inclusion of Fe₂O₃-NPs. Small size of nano iron have facilitated the passage of nanoparticles across tissues and cell membrane leading to improved gastrointestinal absorption because due to much smaller size nano Fe can penetrate the cells easily and is more efficient in action thus leading to increase of body cells of the experimental fish and its growth increased. Protein content deposition in *Labeo rohita* body meat was recorded highest in group T3 treated with Fe-NPs 0.6mg/kg as compared to control

group-T₀ and Fe₂O₃ NPs treated groups after the period of 90 days. These results are in agreement with the findings of El-Shenawy et al., (2019) who found significantly high protein retention in the muscle of Nile Tilapia (*Oreochromis niloticus*) by administering Fe-NPs at various levels in diet.

The reason is the adsorption of proteins occurs on the surface of nanoparticles. Nano-protein coronas can interfere with protein folding and can enhance protein cross linking and fibrillation thus lead to high protein retention in the body meat of experimental fish. Crude lipid deposition in *Labeo rohita* body meat was recorded highest in group T3 treated with Fe-NPs@0.6mg/kg as compared to control group-T₀ and Fe₂O₃ NPs treated groups which is in agreement with Akter et al., (2018) who found significantly high lipid retention in the muscles of Bagridae catfish (*Clarias batrachus*) by administering Fe-NPs at various levels in fish feed. The reason is Iron NPs bind lipoprotein in the blood formed complex resulted in high lipid metabolism high retention of lipid in the muscles of *Labeo rohita*. Highest ash contents were recorded in group T4 treated with Fe-NPs@0.8mg/Kg which is in agreement with the findings of two researchers El-Shenawy et al., (2019) and Akter et al., (2018) who stated that ash contents increased in Nile Tilapia (*Oreochromis niloticus*) and Bagridae catfish (*Clarias batrachus*) with the increasing level of Fe-NPs and high ash value observed in group treated with high concentration of Fe-NPs as compared to control group-T₀ and Fe₂O₃ NPs treated groups. Highest ash contents in fish treated with Iron-NPs are may be due to more bioavailability and more intake of iron NPs. Gross energy also recorded highest in group T4 treated with Fe-NPs@0.8mg/Kg because ash contents were highest in this group. Moisture contents recorded highest in group T1 treated with Fe₂O₃ NPs@0.6mg/Kg which may be due to higher surface area to volume ratio of NPs and dry matter is vice versa (Behera et al., 2014).

Hematological parameters are one of the significant indicators, for observing health condition of fish in terms of physiological and pathological approach. A higher red blood cell (RBC) count and RBCs indices (MCV, MCH, MCHC) was observed in fish fed the nanoparticles supplemented diet compared to those fed control diet, which may be due to more iron intake in nano forms because at the nano-size dietary minerals can pass into the cells more freely than their larger complements and iron

is a major co-factor in blood formation in common and a main component of all RBCs in particular. It can be said that fish may experience gill injury because of iron toxicity. This may cause oxygen deficiency, causing more production of RBCs to lessen the effect. Gill injury induced by nanoparticles have reported by [Ates et al. \(2013\)](#) and [Li et al. \(2009\)](#) in gold fish and medaka fish, while similar results were found by [Behera et al. \(2014\)](#) who reported that RBCs count was significantly higher in Fe NPs- treated groups as compared to the control group. Similar results were shown by [Karthikeyan et al. \(2013\)](#) who have reported higher MCV, MCH and lower MCHC values in groups fed with Iron oxide nanoparticle compared to the control group. While [Remya et al. \(2015\)](#) reported in results that MCV, MCH and MCHC were decreased in group of fish fed with Iron oxide nanoparticles compared to the control group.

During the study period, WBCs count of fish blood were found positively correlated with dietary nanoparticles supplementation and expressively higher WBCs were observed for the fish group fed on the feed with 0.6mg/kg Iron nanoparticle. Increased numbers of WBCs might be linked with immunological reaction to produce antibodies to handle with stress induced by the nanoparticles. [Uzo et al. \(2018\)](#) demonstrated in results that fish fed with diet containing Iron oxide nanoparticles showed a significantly ($P < 0.05$) higher white blood cell (WBC) count compared to the control group.

4. CONCLUSION

The results revealed that both iron and iron oxide nanoparticles improved the growth, muscle composition and hematology of *Labeo rohita* as compared to the control diet. Some parameters were improved in the iron nanoparticles supplemented diet compared to the iron oxide nanoparticles supplemented diet and vice versa. Lower doses of the nanoparticles may provide good results so it is suggested that further research in this matter should be in that direction.

Highlights

- Low production of aquaculture is primarily due to improper diet (leads to retard growth).
- Diet supplemented with iron and iron oxide nanoparticle show improved health of *Labeo rohita* in terms of hematology and body composition.

- Iron oxide nanoparticle holds the abilities to enhance growth of *Labeo rohita*.

5. CONFLICT OF INTERESTS

All authors have declared that there is no conflict of interest regarding this publication.

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Iron Nanoparticles' Dietary Effects on Labeo rohita Growth and Hematological Aspects

Table 1. Composition of Control and Experimental Diets

Ingredients (g)	Control diet	T₁ Iron Oxide NPs (0.6mg/kg)	T₂ Iron Oxide NPs (0.8mg/kg)	T₃ Iron NPs (0.6mg/kg)	T₄ Iron NPs (0.8mg/kg)
Fish meal	25	25	25	25	25
Sunflower meal	18	18	18	18	18
Gluten	50	50	50	50	50
Wheat bran	1.7	1.7	1.7	1.7	1.7
Rice bran	1.7	1.7	1.7	1.7	1.7
Vitamin premix	1.3	1.3	1.3	1.3	1.3
Canola oil	2.3	2.3	2.3	2.3	2.3
Total	100	100	100	100	100
Iron oxide NPs (<50nm)	0	0.6	0.8	0	0
Iron NPs(<50nm)	0	0	0	0.6	0.8

Table 2. Comparison of means of Growth performance of *Labeo rohita* cultured under control and different treatments

Parameters	T₀ (Control)	T₁	T₂	T₃	T₄
	NPs (0.0mg/kg)	Iron Oxide NPs (0.6mg/kg)	Iron Oxide NPs (0.8mg/kg)	Iron NPs (0.6mg/kg)	Iron NPs (0.8mg/kg)
Body weight	4.97±0.36B	5.40±0.44B	5.59±0.46A	5.21±0.4A	5.25±0.41A
Body length	7.39±0.28A	7.21±0.26B	7.41±0.29A	7.23±0.2B	7.30±0.29AB
SGR	0.985 ± 0.055A	1.100 ± 0.0A	1.130 ± 0.010A	1.115±0.01A	1.085 ±0.045 A

Table 3. Comparison of means of approximate muscle composition of *Labeo rohita* cultured under control and different treatments

Parameters	T₀ (Control)	T₁	T₂	T₃	T₄
	NPs (0.0mg/kg)	Iron Oxide NPs (0.6 mg/kg)	Iron Oxide NPs (0.8mg/kg)	Iron NPs (0.6mg/kg)	Iron NPs (0.8mg/kg)
Moisture (%)	2.000 ± 0.000B	7.0 ± 0.00 A	2.50± 0.50 B	6.500 ± 0.50A	3.00±0.000B
Dry matter (%)	98.0 ±0.0A	93.0 ± 0.00 B	97.50± 0.50 A	93.500±0.50B	97.000 ±0.0A
Ash (%)	78.0±1.00B	87.0 ± 0.00 A	81.0± 1.0 B	85.500 ±0.5 A	88.50±0.50A
Crude lipid (%)	21.500 ± 1.50B	25.50±0.50AB	29.0± 0.0AB	33.000 ± 0.0A	32.5± 0.50 A
Crude protein (%)	25.0 ± 0.00 B	8.590 ± 0.78B	10.930±0.0 B	28.12 ±0.0A	27.3±0.78AB
GE(Kcal/g)	555.62± 7.99 B	531.28± 3.85B	572.02±2.05A	603.18±2.05A	613.71 ±3.8A

Table 4. Comparison of means of approximate hematological analysis of *Labeo rohita* cultured under control and different treatments

Parameters	T₀ (Control)	T₁	T₂	T₃	T₄
	NPs (0.0mg/kg)	Iron Oxide NPs (0.6mg/kg)	Iron Oxide NPs (0.8mg/kg)	Iron NPs (0.6mg/kg)	Iron NPs (0.8mg/kg)
Hemoglobin (g/dl)	6.67± 0.010 A	7.33± 0.215 B	7.03± 0.115 B	7.42± 0.130 B	7.40± 0.215 B
WBCs (x10 ³ / μL)	7.17± 0.020 B	11.05± 0.050 A	9.55±0.020 A	12.235± 0.565 A	11.295± 1.065 A
RBCs (x10 ⁶ /μL)	1.33± 0.020 A	1.65± 0.050 B	1.54± 0.135 B	8.615± 0.185 C	8.565± 0.135 C
HCT (PVC)%	20± 1.000 A	21.82± 0.410 B	18.3± 0.4 B	23.97± 0.135 B	21.77± 1.065 B
MCV (fL)	107.3± 0.000 D	160.98± 10.72 A	150.5± 8.270 B	122.47± 6.435 C	114.89± 13.165 CD
MCHC (pg)	34.17± 0.000 D	52.47± 2.500 B	58.8± 4.230 A	40.47± 0.035 C	41.80± 0.900 C
MCH (g/dl)	30.73± 0.000 C	48.58± 3.035 A	44.315± 0.850A	41.295± 1.235 B	39.74± 0.885 BC

EFFICACY OF DIFFERENT NEONICOTINOIDS AND BIO PESTICIDES ON OKRA AGAINST *BEMISIA TABACI*

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ABSTRACT

The current study was investigated at the Agricultural Research Institute Tarnab, Peshawar during 2020 in a Randomized Complete Block Design (RCBD) with three replications. The study aimed was determining the efficacy of different neonicotinoids insecticides and bio-pesticides against *Bemisia tabaci* on okra crop. Results revealed that significantly maximum number of whitefly (*Bemisia tabaci*) population reduction was recorded in imidacloprid (7.55plant⁻¹) treated plot followed by acetamiprid (8.88plant⁻¹) and bio-pesticides (11.99 plant⁻¹) while minimum population reduction was recorded in clothianidin (14.66 plant⁻¹) treated plot over checked plot after 1st application while similar observation was recorded after the 2nd application. In terms of CB ratio, highest CB ratio was recorded by imidacloprid treated plot followed by bio pesticide, acetamiprid and clothianidin while lowest CB ratio was recorded by acetamiprid. Similarly, the research work concluded that overall best performed by imidacloprid after 1st and 2nd application while minimum control was observed by clothianidin treated plot over the control treatment.

1. INTRODUCTION

Okra (*Hibiscus esculentus* L) usually called bhindi or lady's finger belongs to family *Malvaceae* (Bayer *et al.*, 2003 and Naveed *et al.*, 2009). Okra is grown in summer season crop of Pakistan, the cultivated area of okra was 232.05 hectares which produced 303.16 tons/year (Kashif *et al.*, 2008). Many biotic and abiotic factors significantly decreased the okra production (Mani *et al.*, 2005). The okra cultivated area of Sindh is about 35.06 thousand hec with an annually productivity of 219.7 thousand tones (Larik, 2002). Losses in yield and fruit quality was effected by sucking insect pest and fruit borers pests from sowing till harvesting. The main sucking insect pests viz., whiteflies, jassids, aphids, thrips and mites. Among all those sucking insect pests, whitefly, *Bemisia tabaci* severally damaged okra crop by sucking cap and in result

produced honey dew which provides medium for sooty mould (Oliveira *et al.*, 2001). It's not only damaged plant through direct feeding in order to physiological disorder but it may carrier of different viruses (Oliveira *et al.*, 2001). The yield was decreased is 94.0% by whiteflies and 54.04% by aphids (Meenambigai *et al.*, 2017). For the management of whitefly different groups of insecticides have been recommended. Though, the synthetic insecticides use during the fruit bearing stage is challenging because the fruit is picked at regular intervals, making the opportunity that toxic residues might carriage a health hazard. Previously researcher has estimated lowest toxic and more environmentally nontoxic insecticides. For example, Mishra and Mishra (2002) described that the botanical insecticide, Neem seed kernel extract and Multi neem (neem oil) controlled infestation of this whiteflies (Acharya *et al.*, 2002).

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Keeping in view the above importance the current study was determining the effectiveness of different neonicotinoids and bio pesticides on okra against *Bemisia tabaci*.

2. MATERIALS AND METHODS

Study area

The experiment was conducted at the Agricultural Research Institute Tarnab, Peshawar during 2020.

Experimental design

Dibbling method was used for sowing the local okra variety with spacing 45 cm x 30 cm with 2 to 3 seeds per hill. For optimum plant density gap filling and thinning was done in order to prevent the competition between the plants. The experiment was performed out in randomized block design and triplicated with 4 different treatments.

Methodology

The number of whiteflies were recorded a day before and after spraying 3, 7 and 14 days. The data were recorded from 3 leaves each from top, middle and bottom leaves from 5 randomly chosen and labelled plants from every plot without troubling the plants to reduce the observational errors. Whiteflies population was recorded from each net plot and the populace was worked out per three leaves.

Cost benefit ratio

To analyzed that among the tested treatments which was good to control of sucking insect pest as well economical one cost, benefit ratio was calculated with using following formula:

$$\text{Cost benefit ratio} = \frac{\text{Value of yield over control}}{\text{Total cost of plant protection}}$$

Statistical analysis

The data were subjected to the analysis of variance in order stated whiteflies population by number based. ANOVA was constructed by using STATISTIX 8.1 software.

3. RESULTS AND DISCUSSION

Efficacy of different insecticides against whiteflies (*Bemisia tabaci*) after 1st application

Results of the study on the effectiveness of targeted insecticides against whiteflies population are presented in Table 1. Before spraying showed significant difference

among all the treatments. After 3 days of spray, maximum number of whitefly was recorded in control plot (33.00plant⁻¹) while minimum number of whitefly was recorded in imidacloprid treated plot (13.66plant⁻¹) followed by acetamiprid treated plot (14.33plant⁻¹), bio pesticides (18.33plant⁻¹) and clothianidin (19.33plant⁻¹) which were significantly different with each other respectively. Similarly, after 7 days of spray, maximum number of whitefly was recorded in control plot (30.66plant⁻¹) while minimum number of whitefly was recorded in imidacloprid treated plot (4.00plant⁻¹) which was statistically significant with acetamiprid treated plot (5.33plant⁻¹) but significantly different with bio pesticide (7.33plant⁻¹) and clothianidin (11.33plant⁻¹). However, after 14 days of spray, maximum number of whitefly was recorded in control plot (29.00plant⁻¹) while minimum number of whitefly was recorded in imidacloprid treated plot (5.00plant⁻¹) which was statistically significant with acetamiprid treated plot (7.00plant⁻¹) but significantly different with bio pesticide (10.33plant⁻¹) and clothianidin (13.33plant⁻¹). The overall mean showed that maximum number of *Bemisia tabaci* was recorded in control treated plot (30.88plant⁻¹) while minimum number of *Bemisia tabaci* was recorded in imidacloprid treated plot (7.55plant⁻¹) followed by acetamiprid (8.88plant⁻¹), bio pesticide (11.99plant⁻¹) and clothianidin (14.66plant⁻¹) respectively.

Efficacy of different insecticides against whiteflies (*Bemisia tabaci*) after 2nd application

Table 2 showed that, before spraying showed significant difference among all the treatments. After 3 days of spray, highest number of whitefly was recorded in control plot (33.00plant⁻¹) while lowest number of whitefly was recorded in imidacloprid treated plot (5.67plant⁻¹) followed by acetamiprid treated plot (7.00plant⁻¹) which was significantly different with each other but significantly different with bio pesticides (10.00plant⁻¹) and clothianidin (17.00plant⁻¹) respectively. Similarly, after 7 days of spray, highest number of whitefly was recorded in control plot (35.00plant⁻¹) while lowest number of whitefly was recorded in imidacloprid treated plot (2.00plant⁻¹) which was significant different with acetamiprid treated plot (4.33plant⁻¹) but significantly different with bio pesticide (7.00plant⁻¹) and clothianidin (10.00plant⁻¹). However, after 14 days of spray, highest number of whitefly was recorded in control plot (28.00plant⁻¹) while lowest number of whitefly was recorded in imidacloprid treated plot (4.00plant⁻¹) which

was significantly different with acetamiprid treated plot (7.00plant⁻¹), bio pesticide (10.00plant⁻¹) and clothianidin (15.00plant⁻¹). The overall mean showed that highest number of *Bemisia tabaci* was recorded in control treated plot (30.88plant⁻¹) while lowest number of *Bemisia tabaci* was recorded in imidacloprid treated plot (3.89plant⁻¹) followed by acetamiprid (6.11plant⁻¹), bio pesticide (9.00plant⁻¹) and clothianidin (14.00plant⁻¹) respectively. The current findings are in line with findings of Begum and Patil (2016), indicated that imidacloprid (17.8 SL) was effectively reduced against the leafhoppers and whiteflies. Similar observation was also recorded by Pawar et al., (2016) and Preetha et al., (2009). Pathania et al. (2020) observed similar results, which supports our findings. Kadam (2014) also reported that dinotefuran 20% SG and clothianidin 50% WDG were effective in suppressing *B. tabaci* in cotton, which is consistent with our findings. Said (2011) reported that Rani 20 SL and acetamiprid 20 SP were showed most effectiveness against sucking insect pest.

Economic analysis of different insecticides against whiteflies (*Bemisia tabaci*)

Similarly, as from the result, it is obvious that all the treatments showed significant maximum net return was recorded compare to control treatments. Among different tested treatments, imidacloprid treated plot showed maximum return (1: 15.80) followed by acetamiprid (1: 13.25), bio pesticide (1: 11.64) while minimum return was recorded in clothianidin treated plot (1: 6.59) over control plot. Similarly Said (2011) reported that Rani 20 SL and acetamiprid 20 SP were showed maximum yield as compared to other treatments.

4. CONCLUSION

Satisfactory control of *Bemisia tabaci* was achieved with imidacloprid, followed by acetamiprid, both of which are systemic neonicotinoids. Bio-pesticides exhibited intermediate effectiveness, while clothianidin was the least effective.

5. CONFLICT OF INTEREST

All authors have declared that there is no conflict of interests regarding the publication of this article.

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Table 1. Population reduction of *Bemisia tabaci* after 1st application in okra during 2020

Treatments	Before Spraying	After Spraying			Overall mean
		3DAS	7DAS	14DAS	
Acetamiprid	29.33 ^d	14.33 ^b	5.33 ^d	7.00 ^d	8.88
Imidacloprid	31.33 ^c	13.66 ^d	4.00 ^d	5.00 ^d	7.55
Clothianidin	30.00 ^d	19.33 ^e	11.33 ^b	13.33 ^b	14.66
Bio pesticide	32.33 ^b	18.33 ^c	7.33 ^c	10.33 ^c	11.99
Control	34.33 ^a	33.00 ^a	30.66 ^a	29.00 ^a	30.88
LSD	0.97	0.76	0.97	0.72	-

Mean in columns followed by same letters are non-significant 5% level of probability

DAS: Days after spraying

Neonicotinoids and Bio-pesticides for Bemisia tabaci Control in Okra

Table 2. Population reduction of *Bemisia tabaci* after 2nd application in okra during 2020

Treatments	Before Spraying	After Spraying			Overall Mean
		3DAS	7DAS	14DAS	
Acetamiprid	15.03 ^d	7.00 ^d	4.33 ^{cd}	7.00 ^d	6.11
Imidacloprid	10.66 ^e	5.67 ^e	2.00 ^d	4.00 ^e	3.89
Clothianidin	22.0 ^b	17.0 ^b	10.00 ^b	15.00 ^b	14.00
Bio pesticide	19.03 ^c	10.0 ^c	7.00 ^{bc}	10.00 ^c	9.00
Control	35.0 ^a	33.0 ^a	35.00 ^a	28.00 ^a	32.00
LSD	0.64	0.97	3.01	1.45	-

Mean in columns followed by same letters are non-significant 5% level of probability

DAS: Days after spraying

Table 3. Cost benefit analysis of different neonicotinoids and bio pesticides during 2020

Treatments	Marketable yield kg/ha A	Gross income Rs. B	Cost of control /ha C	Return over control Rs./ha D	Estimated net benefit Rs. /ha E=(D-C)	C: B F=(D/C)
Acetamiprid	1600	80000	3300	43750	40450	1:13.25
Imidacloprid	1800	90000	3400	53750	50350	1:15.80
Clothianidin	1200	60000	3600	23750	20150	1:6.59
Bio Pesticide	1500	75000	3300	38750	38420	1:11.64
Control	725	36250	-	-	-	-

Average price per kg=50/-

CORRELATION OF SERUM CALCIUM WITH ENZYMES ASPARTATE AMINOTRANSFERASE, CREATINE KINASE AND MASTITIS IN POSTPARTUM PERIOD IN DAIRY CATTLE

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UA designed and conducted research plan, QN reviewed the article, WA wrote manuscript, MR applied statistics

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ABSTRACT

Mastitis is inflammation of udder and its chances increases in hypocalcemia cases because sphincters of teat are loosened due to calcium-ion channel blockage. Cows those suffered milk fever have 8 times more chances of developing mastitis than other cows which haven't suffered hypocalcaemia. The values of AST and CK are high in downer cows due to hypocalcemia as compared to healthy cows. Subclinical hypocalcaemia is one of the major complication occurring just after parturition in local and exotic breeds of cattle in Pakistan. It weakens the immune system of dairy animal and also decreases the production potential of animals thus causing serious economic losses in terms of decrease milk production of animal, decrease earning of animal and increased treatment cost due to secondary infection like mastitis occurred due to weakened immune function. The current study was aimed to the correlation of serum calcium with enzymes Creatine Kinase and Aspartate amino transferase and mastitis in postpartum period in dairy cattle. A total of 50 cattle (n=50) just after parturition were included in this study. The cattle were sampled in two ways. Firstly, 10ml blood was drawn aseptically into clot activator vacutainers from jugular vein. Secondly milk samples were taken from all four teats. First 2 milk strips were discarded and next strips were collected in aseptic test tubes. The samples were transported to Pathology laboratory, Department of Pathology, University of Veterinary and Animal Sciences, Lahore maintaining the cold chain and stored at -20 °C till further analysis. Correlation between calcium and creatin kinase comes out to be 0.279 which is weak positive correlation. Correlation between calcium and aspartate aminotransferase is also weak and comes out to be 0.111. Similarly, correlation between calcium and SCC also weak and comes out to be 0.193. This is the first study of correlation of calcium with CK, AST and SCC in dairy cattle after postpartum period.

1. INTRODUCTION

Calcium is a macro-mineral that plays an important function in the body which are bone matrix formation, process of muscle contraction and transmission of nerve impulses. Ionized calcium is also important for essential cellular functions like signaling, neurotransmission, muscle contraction, metabolism, growth and proliferation and triggering of immune responses (Saris & Carafoli 2005), (Vig & Kinet 2009).

Normal blood calcium in the adult cows is maintained between 8.5 and 10 mg/dl (Goff 2008). Maintenance of blood calcium with acceptable is a balancing act between calcium demand of milk production and the cow's homeostatic mechanism to maintain blood calcium. During the last trimester in pregnancy supply of calcium through diet is more than adequate to maintain homeostasis without the activating calcium mobilization system which is activated after parturition (Taylor et al. 2001)

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Hypocalcemia is one of the most common mineral related metabolic disorders of milking animals which occurs at low level of blood calcium. It happens immediately after parturition or just before parturition. A mild degree of hypocalcaemia also leads to conditions like uterine prolapse, retained placenta, metritis, mastitis, ruminal stasis, decrease of immune response and reproductive performance (Khan et al. 2015).

AST activity is widely studied in both animals and humans. It is found that variation of AST level had relations with the liver functioning, skeletal muscle performance and heart muscles of cows (Kaneko et al. 2008). The values of AST and CK are high in downer cows as compared to healthy ones. Ischaemic necrosis results in higher values of AST and CK in circulation due to prolonged recumbancy (Cox et al. 1982). In these factors one is calcium level, if its value drops from normal level in blood or serum then calcium-ion channels providing help in muscle contraction are affected and results in muscle damage. As muscle cell degradation occurs in forced calving or dystocia cases and if level of calcium is also low from normal value it will result in muscle damage and level of CK will be high in serum. Hypocalcemia patient without signs of heart muscle damage and other dystrophies show elevated signs of serum creatinine kinase and high chances of elevation are there in cases of severe hypocalcemia.

Subclinical hypocalcaemia is one of the major complications occurring just after parturition in local and exotic breeds of cattle in Pakistan. It weakens the immune system of dairy animal and also decrease the production potential of animals thus causing serious economic losses in terms of decrease milk production of animal, decrease earning of animal and increased treatment cost due to secondary infection like mastitis occurred due to weakened immune function. Keeping in view the importance of this health malady of cattle and its serious economic concerns the current study has been designed.

2. MATERIALS AND METHODS

Study area and Study animal

The study was carried out in district Sheikhpura at various private and corporate dairy farms and small households. A total of 50 cattle (n=50) just after parturition were included in this study.

Inclusion Criteria was those cattle just after parturition or showing any of following clinical signs like inflammation of udder, anorexia, depression, weakness and subnormal temperature were included in this study.

Digestion of blood sample for calcium analysis

1ml sample was taken for the analysis. 7ml HNO₃ (nitric acid) was added. Then 3ml of HClO₄ (per chloric acid) was added. Sample was then kept in digestion block at 150°C for 30 minutes. After this temperature was raised to 250°C and it was kept for 15 minutes till point is achieved i.e wine-green/crystal clear. Sample was then cooled and final volume 25ml with distilled water was made. Calcium analysis on Flame Photometer Standardize against standard. 1ppm, 5ppm, 10ppm, 15ppm, 20ppm, 25ppm, 30ppm, 35ppm, 40ppm.

Sampling procedure

The cattle were sampled in two ways. Firstly, 10ml blood was drawn aseptically into clot activator vacutainers from jugular vein. The samples were transported to the Pathology laboratory in Department of Pathology, University of Veterinary and Animal Sciences, Lahore maintaining the cold chain. The serum in clot activator vacutainers was stored at -20°C till the further analyses. The data regarding owner, animal, management and environmental factors was recorded on data capture form for each sampling unit.

Secondly milk samples were taken from all four teats First 2 milk strips were discarded and next strips were collected in aseptic test tubes. The samples were transported to Pathology laboratory in Department of Pathology, University of Veterinary and Animal Sciences, Lahore maintaining the cold chain.

Serum analysis

Serum was analyzed for Aspartate aminotransferase (AST) and creatinine phosphokinase (CPK) using semiautomatic biochemical serum analyzer following manufacturer's direction.

Milk analysis

Milk was analyzed for somatic cell count (SCC) through California Mastitis Kit (CMT). Milk sample were collected from all 4 quarters in a clean CMT paddle which has 4 cups marked A, B, C, D for all four quarters. Take a tea spoon of milk (2cc) in 4 cups from four quarters. Equal amount of CMT solution was then added in each cup of paddle. CMT paddle was rotated in circular motion to mix

thoroughly. Sample was not mixed for more than 10 seconds. Visible reaction disintegrates after 20 seconds. Reaction is stored visually. More the gel formation, higher is the score.

3. RESULTS AND DISCUSSION

The mean of calcium in 50 dairy cattle suffering from sub clinical hypocalcemia was 7.042mg/dl in current study with standard deviation of 0.88158 while the mean of creatine kinase (CK) in these animals was 243.02 U/L with standard deviation of 55.356

Calcium and CK has statistically non-significant relationship ($p>0.05$).

The direction of relationship is positive (i.e calcium and CK are positively correlated) meanings that these variables tends to increase together.

The strength of correlation between two variables is approximately weak.

The mean of calcium in 50 dairy cattle suffering from sub clinical hypocalcemia was 7.042mg/dl in current study with standard deviation of 0.88158 while the mean of aspartate aminotransferase (AST) in these animals was 183.30 U/L with standard deviation of 34.195.

Calcium and AST has statistically non-significant relationship ($p>0.05$).

The direction of relationship is positive (i.e calcium and AST are positively correlated) meanings that these variables tends to increase together.

The strength of correlation between two variables is very weak.

The mean of calcium in 50 dairy cattle suffering from sub clinical hypocalcemia was 7.042mg/dl in current study with standard deviation of 0.88158 while the mean of somatic cell count in these animals was 274426 with standard deviation of 400012.52981.

Calcium and Somatic cell count has statistically non-significant relationship ($p>0.05$).

The direction of relationship is positive (i.e calcium and AST are positively correlated) meanings that these variables tends to increase together.

The strength of correlation between two variables is very weak.

Livestock plays an important role for the uplift of rural economy by contributing 11.8% in national GDP and 55.5% in the agriculture sector's share of GDP. It is considered a source of employment generation in rural

areas, helping reduce variation in income. It is central to the livelihood of the rural poor and can play an important role in poverty alleviation.

Milk fever or subclinical hypocalcemia is a very fatal disease in dairy animals which is caused by decrease of calcium level in the body of dairy animals immediately after parturition or just before parturition. About 50% or more animals in second or third lactation are more prone to milk fever due to increase of milk production in second or third lactation. It causes severe economic losses to farmers in terms of decrease milk production and medicate on costs. It also causes production to decrease throughout the lactation period. (Fikadu et al. 2016).

The Government believes that increase in livestock production can have a strong impact on the incomes of the rural poor. Productivity increase in livestock and dairy can have significant positive impact on total income in the sector as well as exports. There is substantial scope in increasing the export of high-value livestock and dairy value-added products. At this time, some of the best strategies for us to avoid losses due to infectious disease are to pay strict attention to the details of close-up and fresh cow management such that metabolic insults to the immune system are avoided. Further research elucidating endocrine, metabolic, and immune interactions around the time of calving are warranted. In addition to metabolites, many dietary nutrients are involved in immune protection and may play roles in immunosuppression.

Dairy cows under stress condition use fat reserves which results in cell structure change and damage to cell structure and disturbance in values of AST and CK. (Rukkamsuk et al. 2000). Decrease in calcium level in the body effects certain functions in the body metabolism and functioning like liver lipid infiltration, effect on enzymes values in serum, deformities and osteoporosis in bones and muscles contraction and relaxation are also highly effected from this.

Moreover, cows effected with milk fever are more prone approximately 9% more to ketosis then those who are not effected with milk fever. The samples are processed for checking the levels of enzymes and calcium to check the effect of decrease calcium on body cells and functioning. The skeleton of a 600-kg cow contains approximately 8.5 kg of Ca. There are 3 g Ca in the plasma pool and only 8 to 9 g Ca in all the extracellular fluids of a 600-kg cow.

Blood Ca in the adult cow is maintained between 2.1 and 2.5 mmol/L (8.5 and 10 mg/dL) and is slightly higher in young animals. About 50% of the blood Ca is bound to proteins such as albumin, less than 10% is in mineral complexes with inorganic phosphates and the remainder exists in the ionized form. The ionized Ca concentration is the biologically active form of the Ca in blood and is most important for immediate metabolic function. (Breves 1995).

As calcium is a macro mineral which also plays a role in transmission of nerve impulses due to which a decrease in its level causes effects on neurotransmission, muscle functioning and immune cell activation. In the current study 50 samples were taken from cattles in different house holding areas in district Sheikhpura and samples were taken from those cattles which were freshly parturated and these samples were taken in clot activator vacutainers and were transported to laboratory by maintaining proper cold chain.

The study done is to find out the relation between calcium and enzymes. Milk samples were also taken from the same cows and were sent to the laboratory by maintaining cold chain for the somatic cell count and serum was extracted from blood samples for checking the levels of AST and CK. (Kimura et al. 2006).

The correlation in the current studies is weak mostly similar to the previous researches but a bit high as compared to the researches in foreign which clearly indicates managerial issues in feeding and cleaning for enzymes and mastitis respectively. This study findings are quiet similar to the findings of the researches done before and have a little bit variation.

The outcome of study done yield data about the status of sub-clinical hypocalcemia in cattle population of district Sheikhpura and its effect on serum enzymes like AST and CPK and its relationship with mastitis. The study findings helped in suggesting some strict measures for minimizing economic losses in dairy industry of Pakistan.

4. CONCLUSION

The study reveals that the requirement of calcium in the body should be maintained by a proper diet containing an adequate amount of calcium to fulfill the body's needs and ensure the proper functioning of enzymes and muscles. If

the diet is low in calcium, it should be supplemented with external calcium, such as Dicalcium phosphate in the diet. Proper management of affected animals through treatment is also essential.

5. CONFLICT OF INTEREST

All authors have declared that there is no conflict of interests regarding the publication of this article.

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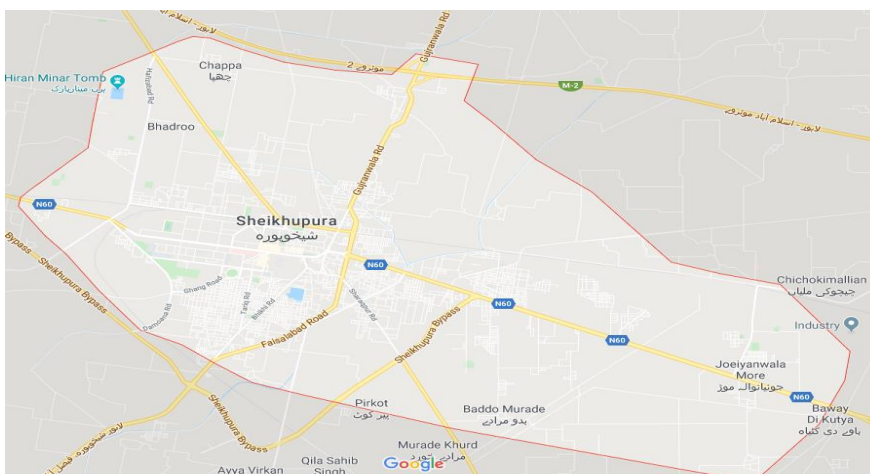


Figure 1. Map of District Sheikhupura

Table 1. Summary Statistics of Calcium and CK Level

	Mean	Std. Deviation	N
Calcium	7.0420	.88158	50
CK	243.02	55.356	50

Table 2. Correlation of Calcium with CK

		Calcium	CK
Calcium	Pearson Correlation	1	0.279
	p-value		0.050
	N	50	50
CK	Pearson Correlation	0.279	1
	p-value	0.050	
	N	50	50

Table 3. Summary Statistics of Calcium and SCC Levels

	Mean	Std. Deviation	N
Calcium	7.0420	0.88158	50
SCC	274426.000	400012.52981	50

Table 4. Summary Statistics of Calcium and AST Levels

	Mean	Std. Deviation	N
Calcium	7.0420	0.88158	50
AST	183.30	34.195	50

Table 5. Correlation of Calcium with AST

		Calcium	AST
Calcium	Pearson Correlation	1	0.111
	p-value		0.442
	N	50	50
AST	Pearson Correlation	0.111	1
	p-value	0.442	
	N	50	50

Table 6. Correlation of Calcium with Mastitis (Somatic Cell Count)

		Calcium	SCC
Calcium	Pearson Correlation	1	.193
	p-value		.178
	N	50	50
SCC	Pearson Correlation	.193	1
	p-value	.178	
	N	50	50

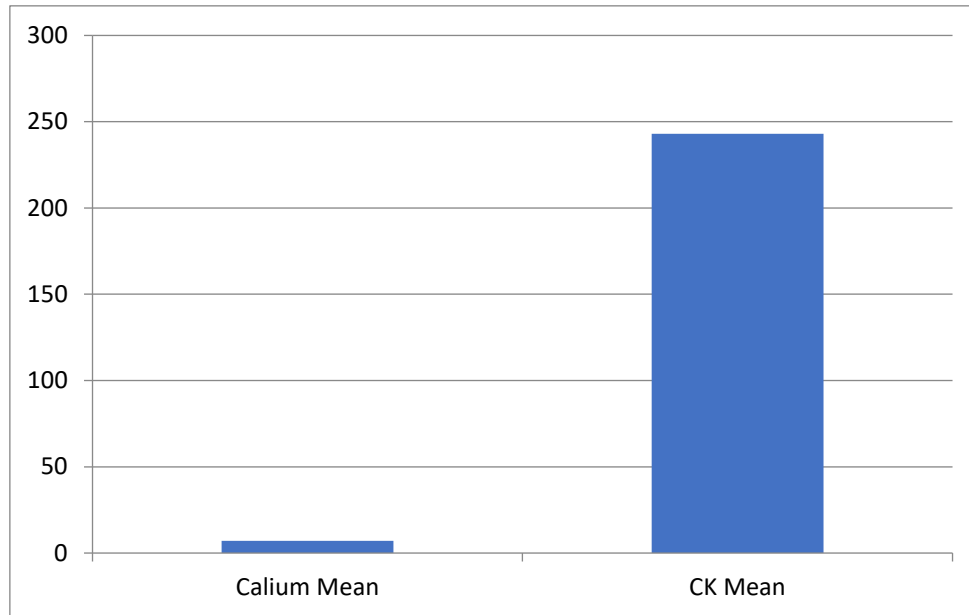


Figure 2. Comparison of mean of calcium and creatine kinase (CK) in infected animals.

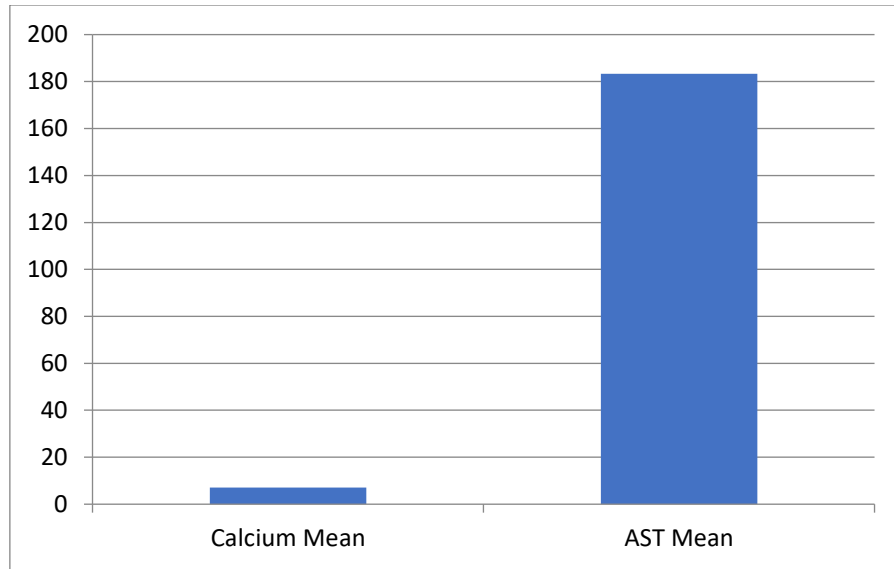


Figure 3. Comparison of mean of calcium and aspartate aminotransferase (AST) in infected animals

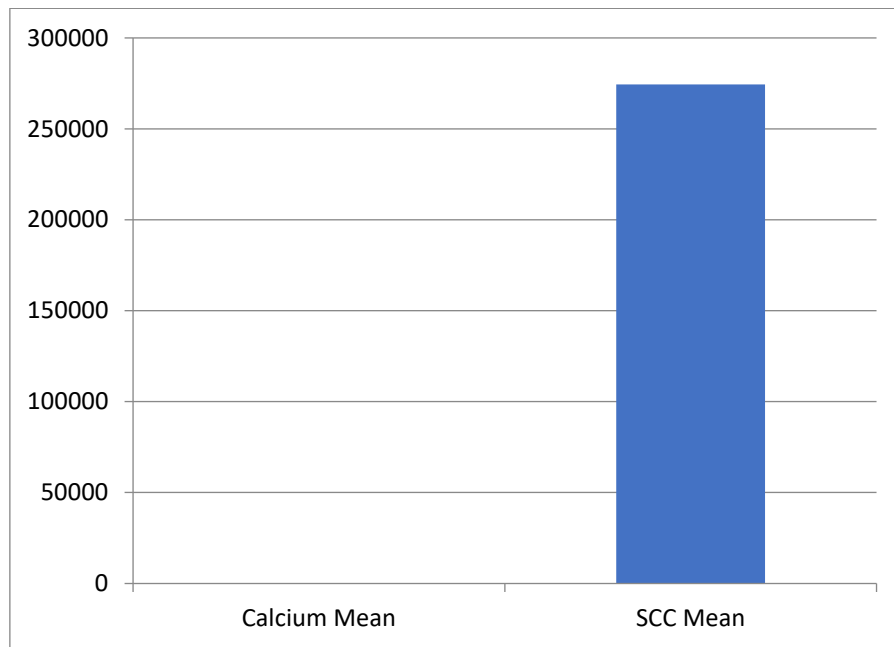


Figure 4. Comparison of mean of calcium and somatic cell count (SCC) in infected animals

COMPARATIVE EFFICACY OF MEDROXYPROGESTERONE ACETATE (PROGESTIN) SPONGES WITH CO-SYNCH PROTOCOL FOR ESTRUS SYNCHRONIZATION IN HOLSTEIN FRIESIAN CATTLE

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ABSTRACT

The study involved forty non-pregnant, first-parity Holstein Friesian cows housed under semi-intensive management conditions. They were divided into four groups: Group A received a 7-day CIDR followed by a PGF2 α injection, Group B received a 12-day CIDR followed by a PGF2 α injection, Group C underwent a 10-day Co-Synch protocol, and Group D served as the control. Estrus symptoms were weak in all groups, while strong signs of standing heat were observed. Group B showed the highest estrus response, earliest onset of estrus, longest duration, and highest conception rate, resulting in positive pregnancies. In contrast, Group D had no pregnancies. These findings suggest that the extended CIDR treatment in Group B led to improved reproductive outcomes, indicating its potential as an effective method for estrus synchronization in Holstein Friesian cattle.

1. INTRODUCTION

The livestock sector is the backbone of agriculture in Pakistan. It engages about 30 to 35 million people in rural areas, who derive more than 40% of their incomes from livestock sector (Faisal et al., 2021; Memon et al., 2022). Milk is the most important commodity derived from livestock sector. The estimated annual milk production in 2021-2022 was 63 million tons (GOP, 2021). But the demand of milk is increasing rapidly due to increase in human population and urbanization. Most of the indigenous animals are non-descriptive type having low productivity compared with developed countries.

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Pakistan has three times more animals but have low yields than Germany, New Zealand's, and USA. They are producing 94.5 billion liters of milk annually from 3.4 million animals representing a significant loss in economic and social value (Tahir et al., 2019).

The increase in milk production in Pakistan was 3%, while the demand was projected 5% leaving a significant gap in the supply and demand chain (Shabir et al., 2021). This gap was tried to be met by the import of Holstein Friesian cows which are well known worldwide for their high milk yield and reproductive performance. Holstein Friesian cow produces 10,000-12,000 litres milk per lactation period. The Holstein Friesian calf gets mature

within two years and start producing milk, while local breeds take three years (Wondossen et al., 2018).

The milk demand in summer (April to July) is higher in Pakistan than its production (Ayaz et al., 2011; Channo et al., 2022). To address this issue, reproductive cycle is manipulated using various estrus synchronization protocols in order to get animal conceived and thus flow of milk supply may be maintained (Jelani et al., 2022). Reproductive cycle of the animals play important role in productivity of the animals in terms of milk yield and meat. Earlier attempts were focused on the control of follicle growth and luteal span (Hryciuk et al., 2021; Channo et al., 2022). There are two methods of manipulating corpus luteum (CL) using either Progestins or injecting with luteolytic agents. Several hormones have been used to synchronize animals such as progestins, prostaglandins, gonadotropins and their analogues (Szymanska&Blitek, 2020; Iqbal et al., 2022).

Commercially, available intra-vaginal devices are expensive and are not easily approachable for dairy farmers. A local prototype prepared by Nuclear Institute of Agriculture and Biology (NIAB) from polyurethane sponges were purchased that was impregnated with medroxyprogesterone acetate (Rihana et al., 2013). Moreover, little work on Holstein Friesian cattle using progesterone-based protocols in local environment were reported. Therefore, the present study was designed to compare efficacy of medroxyprogesterone acetate (Progesterin) sponges with co-synch protocol for estrus synchronization and their effect on conception rate in Holstein Friesian cattle.

2. MATERIALS AND METHODS

Animals and management

A total of forty (n=40) non-pregnant 1st parity and 60-120 days post-parturition Holstein Friesian cows were housed under semi-intensive management condition at National Dairy Farm, Kotri near Hyderabad. The routine farm practices were followed as recommended by farm policies. Duration of experiment was 2 months (February to March, 2022).

Experimental design

The all animals were randomly allocated into four different groups.

Group - A Progesterin sponges group with 7th Day Prostaglandin n=10:

In this group, animals (n = 10) were inserted intra vaginal with Progesterin sponges. Polyurethane sponges (diameter 6 cm & length 10 cm) were impregnated with 250 mg Progesterin (Depo Provera; The Pfizer, Belgium). The prepared Progesterin sponges were dusted with streptomycin, packed, and sealed in sterilized polythene bags. The prepared sponges were purchased form Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad, Punjab. The Progesterin sponges were deposited in cranial part of the vagina for 7 days with the help of sterilized applicator lubricated with paraffin oil. On day 7 all the animals were given an injection of PGF2 α (Dalmazin, cloprostenol sodium, 250 μ g, Fatro Laboratories, Italy), and progesterin-based sponges were removed. The experimental group was monitored for onset of estrus within 2-5 days after prostaglandin injection (Singh, 2003). The estrus was confirmed through behavioural signs, and examination of reproductive organs per rectum. Estrus cows were inseminated with Ghazi brother's imported semen (0.2ml) of Holstein Friesian bull.

Group - B Progesterin sponges group with 12th Day Prostaglandin n=10:

In this group, animals (n = 10) Progesterin sponges were inserted on day 0. The prepared sponges were placed in vagina for 12 days. On the day 12 of Progesterin sponges removed and an injection of prostaglandin analogue were given. The experimental group was monitored for onset of estrus within 2-5 days after prostaglandin injection (Singh, 2003). The estrus was confirmed through behavioural signs, examination of reproductive organs per rectum and then inseminated using Ghazi brother's imported semen of Holstein Friesian bull.

Group - C Ovsynchprotocol n=10:

In this group, animals (n = 10) co-synch protocol were used. In this protocol, non-pregnant animals an injection of GnRH, Dalmarin (Burserelein acetate 0.0042 mg, Fatro Laboratories Italy) were given on day 0, and an injection prostaglandin analogue, Dalmazin, (cloprostenol sodium, 250 μ g, Fatro Laboratories Italy) were given on day 7. Second dose of GnRH injected on day 10, Darmarin (Burserelein acetate 0.0042 mg, Fatro Laboratories Italy) and Fixed time AI were followed using Ghazi brother's imported semen of Holstein Friesian bull.

Group - D Control Group n=10

The control group consist of (n= 10) animals they were injected with 2ml Intra muscular normal saline (FDL Pakistan) on days 0, 7 and 10 of the experiment. The estrus was confirmed through behavioural signs and examination of reproductive organs per rectum Later on inseminated using Ghazi brother's imported semen of Holstein Friesian bull.

Behavioural signs of estrus

Sign of estrus were recorded three times a day and night (4 AM, 12 PM and 8 PM). The estrus behaviour was recorded according the scale developed by Van Eerdenburg et al., (1996) (Table-3.2). Each time signs were observed and if the sum of the points exceeded 100 during a whole day, estrus response was considered strong. If the sum of the points is below 100, the estrus response was considered weak. *Onset of estrus.* The females of all groups were watched closely after 2nd injection of PGF_{2α} for behavioural signs of heat. The animals were artificially inseminated two times at 12 and 24 hours, after the last injection of GnRH in group A and C. However, females of B and D group were artificially inseminated at 72 and 96 hours after the last injection of PGF_{2α} with frozen-thawed semen.

Duration of estrus

Duration of estrus was recorded by observing estrus signs visually from onset of estrus signs till cessation of estrus signs.

Fertility/ Pregnancy rate

The pregnancy diagnosis was performed on day 30th of post artificial insemination through ultrasonography.

Statistical Analysis

Graph pad instate 3.05 versions of statistical package was used to analyse the data. Analysis of variance (ANOVA). To determine significant difference between means of groups chi square test was used.

3. RESULTS AND DISCUSSION

Behavioural signs of estrus

Results on the behavioural signs of estrus in Holstein Friesian cows administered with different protocols is mentioned in Table 4.1. Data indicates that estrus signs were observed as weak in all groups except group B,

while standing heat was observed as strong in all groups. The observed overall chi-square between the groups was 61.91 and the estimated p-value among the groups were 0.0001, which indicates significant difference (P<0.05).

Estrus response

Results on the estrus response in Holstein Friesian cows administered with different protocols is mentioned in Table 4.2. This data indicates that the maximum estrus response was recorded in group B followed by A and C, while the minimum estrus response was recorded in group D. Significant (P<0.05) variation in estrus response were analyzed among the groups.

Onset of estrus (hrs)

Results on the onset of estrus in Holstein Friesian cows administered with different protocols is mentioned in Table 4.3. This data indicates that minimum time for onset of estrus was recorded in group A followed by B and C, while maximum time for onset of estrus was recorded in group D. Significant (P<0.05) variation in onset of estrus were analyzed among the groups.

Duration of estrus

Results on the duration of estrus in Holstein Friesian cows administered with different protocols is mentioned in Table 4.4. This data indicates that maximum time for duration of estrus was recorded in group C followed by B and A, while minimum time for duration of estrus was recorded in group D. Significant (P<0.05) variation in duration of estrus was analyzed among the groups.

Artificial insemination & Pregnancy diagnosis

Results on the artificial insemination and pregnancy diagnosis in Holstein Friesian cows administered with different protocols is mentioned in Table 4.5. Pregnancy was found positive six6 animals in group A, four4 animals in group B, and two2 animals in group C respectively. Pregnancy was found negative in all cows of group D.

Conception rate

Results on the conception rate in Holstein Friesian cows administered with different protocols is mentioned in Table 4.6. Data indicates that the conception rate of 75.00%, 66.66% and 50.00% was recorded in group A (CIDR for 7 days), group B (CIDR for 12 days) and

group C (Co-Synch). Zero percent conception rate was observed in group D (Control).

To get more of females into heat at once, estrus synchronisation entails manipulating the estrous cycle. For dairy cows, estrus synchronisation is a crucial reproductive control strategy. This method aids in determining the optimal time for mating and timing the time of delivery when the environment is most conducive to the development and survival of the infants. Higher fertility is attained using these reproductive techniques throughout both breeding and non-breeding seasons. Estrus synchronisation increases animal productivity and reproductive effectiveness, which has greater economic advantages (Prusley et al., 2004). *Bos indicus* cows often have a lower rate of estrus detection than *Bos taurus* cows (Mukasa-Mugerwa, 1989). As a result, estrus induction therapies that provide either a TAI chance or insemination after estrus detection have a great deal of promise for increasing the reproductive effectiveness of both dairy and meat cattle.

In the current investigation, standing heat was noted to be strong in each of the groups (A, B, C, and D). According to Deshmukh et al. (2015), who observed the incidence of strong, moderate, and mild estrus to be 40%, 40%, and 20%, the current results of percent intensity of estrus are consistent with their observations. Similar results were reported by Bhoraniya et al. (2012), who reported intensity levels of 50, 33.33, and 16.66% high, medium, and mild, respectively. The results of the double Prostaglandin (PG) technique show that the strength of estrus is comparable to those from Makode (1990), who determined that the percentages of intense, moderate, and weak estrus were 37.5, 37.5, and 25.0%, respectively. Maximum estrus response in this research was substantially reported in group B (Co-Synch), followed by groups A (CIDR for 12 days) and C (CIDR for 7 days), and smallest estrus response in group D. (control). Variation in estrus response might be associated with the estrus synchronisation protocol used in different groups. Results are consistent with those of Shahid et al. (2021), who found that the estrus response in Sahiwal cows was 87% (40/46), while Hassan et al. (2017) found that the estrus response in nondescript indigenous cows was 95.45% when recorded with Ovsynch therapy in the field. The outcomes of the CIDR CO-Synch treatments for estrus response are consistent with those of Haider et al. (2017), who found 82.5% and

90% estrus responses in CIDR alone and CIDR combined with GnRH treated nondescript cows of Punjab (Pakistan). The breed, breeding season, genetic make-up, and environmental factors may all affect how an estrus responds differently. In the research, the smallest time for the onset of estrus (CIDR for 7 days) was recorded in group B, followed by groups A (CIDR for 12 days) and C (Co-Synch), while the highest time for the onset of estrus (CIDR for 14 days) was reported in group D. (control). The breed, breeding season, genetic make-up, and environmental factors may all have a role in the variation in estrus onset.

The findings of Shahid et al. (2021); Shah et al. (2023) and Hassan et al. (2017), which demonstrated that the beginning of estrus response was greater in the CIDR protocol compared to Ovsynch therapy, are consistent with the results. The results of the present research show that group B (Co-Synch) recorded the longest time for estrus duration, followed by groups A (CIDR for 12 days) and C (CIDR for 7 days), while group D (CIDR for 7 days) recorded the least time for estrus duration (control). The procedure utilised and duration are related to the difference in duration. The findings of Shahid et al. (2021) and Hassan et al. (2017), which demonstrated that the beginning of estrus response was greater in the CIDR protocol compared to Ovsynch therapy, are consistent with the results. In this investigation, the pregnant status of 6, 4 and 2 cows from groups B (CIDR for 7 days) and A (CIDR for 12 days) and Group C (Co-Synch), respectively, was determined to be positive. None of the cows in groups D were pregnant (control). Results are consistent with Shahid et al. (2021), who found that the 7-day CIDR procedure had a greater pregnancy rate. When heifers were given the CO-Synch+CIDR treatment, Estrada et al. (2002) saw a 60% pregnancy rate. Kim et al. (2005) showed that Holstein cows treated with CIDR plus GnRH had a conception rate of 52.3-53.9%. The different pregnancy rates might be the result of different protocols. In our research, group A (CIDR for 7 days) and group B both had conception rates of 75.00% and 66.66%, respectively (CIDR for 12 days). 50.00% conception rate was recorded in group C (Co-Synch) and in group D (Control) Zero percent conception rate was recorded.

The findings of the current investigation correspond to the findings published by Hassan et al. (2017) revealed that the pregnancy rate in Sahiwal cows was 43%

(17/40), however Shahid et al. (2021) stated that conception rate/AI (50%) with Ovsynch therapy in the unremarkable indigenous cows was higher. The results of the CIDR CO-Synch treatments' conception rate are consistent with those of Haider et al. (2017), who reported 52% and 58% in CIDR alone and CIDR along with GnRH treated nondescript cows of Punjab (Pakistan). Similarly, Naikoo et al. (2016) observed that 33.33% of cows who had CIDR therapy became pregnant. However, Sathiamoorthy and Kathirchelvan (2010) observed significantly lower conception rates of 26.00 to 42.74 with CIDR, while greater conception rates of 50 to 80% have also been recorded in crossbred cows by Savalia et al. (2014) and Dhami et al. (2015). According to Pursley et al. (1997a), the Ovsynch protocol increased the rate of conception. The early timing of AI may be the cause of the reduced conception rates in the CIDR implanted groups. Therefore, more precise insemination time may lead to higher conception rates. The effectiveness of CIDR treatments may be impacted by a number of variables, including parity, suckling, breed composition, postpartum interval, variations in pasture and food, BCS, and location (Lamb et al., 2001). The variation in conception rate may be influenced by the mating season, breed, genetic make-up, and environmental factors.

4. CONCLUSION

The current study revealed that the treatment of Group B with CIDR + 12 days (Prostaglandin) resulted in the development of strong signs of estrus, including standing heat. Furthermore, this group exhibited an early onset of estrus, the longest duration of estrus, and a higher conception rate compared to other treatment groups.

5. CONFLICT OF INTEREST

All authors have declared that there is no conflict of interests regarding the publication of this article.

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Table 1. Behavioral Signs of Estrus in Different Treatment Groups

Estrus signs	Group A (CIDR for 7 days)	Group B (CIDR for 12 days)	Group C (Co-Synch for 10 days)	Group D (Control)	Chi-square (p-value)
Mucous vulvular discharge	3%	10%	5%	3%	61.91 (0.0001)
Flehmen	3%	5%	5%	3%	
Restlessness	5%	15%	10%	5%	
Sniffing the vagina of another cow	10%	20%	15%	5%	
Chin resting	15%	30%	20%	10%	
Mounted but not standing	35%	40%	30%	5%	
Mounting (or attempt) other cows	45%	60%	50%	10%	
Standing heat	100%	100%	100%	100%	

Overall Chi-Square 61.91, P-Value 0.0001, Degrees of Freedom 21

Table 2. Estrus Response in Different Treatment Groups

Group (10 animals in each)	No. of animals	No. of animals showing estrus response	Percentage (%)
A (CIDR for 7 days)	10	6	60(%)
B (CIDR for 12 days)	10	8	80(%)
C (Co-Synch for 10 days)	10	4	40(%)
D (Control)	10	2	20(%)

Overall P-value = 0.0305, a,b,c,d= indicates significant (p<0.05) difference among groups.

Table 3. Onset of Estrus (hours) in Different Treatment Groups

Group (10 animals in each)	No. of animals exhibited estrus	Onset of estrus (hrs)
A (CIDR for 7 days)	6	52.30 \pm 3.55 ^d
B (CIDR for 12 days)	8	66.41 \pm 5.44 ^a
C (Co-Synch)	4	63.88 \pm 3.61 ^b
D (Control)	2	56.54 \pm 2.88 ^c

P-value = 0.0004, ^{a,b,c,d} indicates significant (p<0.05) difference among the groups.

Table 4. Duration of Estrus (hours) in Different Treatment Groups

Group (10 animals in each)	No. of animals exhibited estrus	Duration of estrus (hrs)
A (CIDR for 7 days)	6	21.14±2.10 ^c
B (CIDR for 12 days)	8	25.33±2.18 ^a
C (Co-Synch)	4	23.20±1.41 ^b
D (Control)	2	16.54±1.24 ^d

Overall P-value = 0.0014, ^{a,b,c,d} indicates significant (p<0.05) difference among the groups.

Table 5. Artificial Insemination and Pregnancy Diagnosis in Different Treatment Groups

Group (10 animals in each)	No of animals comes in heat	AI performed animals	Pregnant animals
A (CIDR for 7 days) 10	6	6	4 ^a
B(CIDR for 12 days) 10	8	8	6 ^b
C (Co-Synch) 10	4	4	2 ^c
D (Control) 10	2	2	0 ^d

Overall P-value = 0.0001, ^{a,b,c,d} indicates significant (p<0.05) difference among the groups.

Table 6. Conception Rate (%) in Different Treatment Groups

Group	No. of animals conceived	Conception rate (%)
A (CIDR for 7 days)	4	66.66%
B (CIDR for 12 days)	6	75.00%
C (Co-Synch)	2	50.00%
D (Control)	0	0%

P-value = 0.0001, ^{a,b,c,d} indicates significant (p<0.05) difference among the groups.

BREEDING ASSESSMENT OF *SCHIZOTHORAX PLAGIOSTOMUS* IN RIVER INDUS, DISTRICT KOHISTAN, NORTHERN PAKISTAN

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ABSTRACT

In this study, the relationship of total body length (TBL) and total body weight (TBW), Fulton's condition factor (FCF), gonado somatic index (GSI) and absolute fecundity were determined in snow trout (*Schizothorax plagiostomus*) from river Indus at district Kohistan, Northern Pakistan. A total of 540 matured specimens (male=277, female= 263) were collected between January and December 2016 by using a gill net. A curvilinear relationship of fecundity and GSI with TBL and TBW were investigated by the quadratic model fit. The study revealed the fact that *S. plagiostomus* spawned twice in a year, during spring and autumn. The growth coefficient *b* value and mean condition factor were estimated at 3.42 and 1.75 in both sexes respectively. The GSI in males and females was found 4.4% and 10.96% respectively. In females mean absolute fecundity was 4578.16 eggs. The present study has significance in providing baseline information for the management and conservation of this species of economic importance through captivity breeding.

1. INTRODUCTION

Pakistan is blessed with a diversity of natural water resources in the form of rivers, streams, lakes, and springs (Soomro *et al.* 2011), fed mainly by largest mass and collection of glaciers in north comprising of Karakorum range and Himalaya (Hayat *et al.* 2019). Indus is the major river of Pakistan with a total length of 3058 Km, originating in the Mansorawar Lake in Tibet and draining an area of 963,480 Km² before it falls into the Arabian Sea (Sehgal, 1988). Indus River and its tributaries are the major sources for the largest irrigation network of Pakistan which serves forty-two million acres of cultivated land (Ahmad *et al.* 2007). Tarbela dam and six barrages (Jinnah, Chasma, Taunsa, Guddu, Sukkur, and Kotri) are also constructed by the flowing water of the Indus River (Muhammad *et al.* 2017).

Almost one hundred eighty species of fishes have been reported from different parts of the Indus River and its tributaries in Pakistan which further endorse the concern of researchers on the fish fauna of this river (Mirza & Mirza 2014). Many ichthyologists have investigated the fish fauna of river Indus and its tributaries, (Jayaram, 1981; Talwar & Jhingran, 1991; Rafique, 2000; Iqbal *et al.* 2013). However, a brief description is still needed as several serious threats due to the climate change and hydal power projects are critical. Meristic and morphometric analysis constitute an important tool to differentiate closely related organisms. Length weight relationship (LWRs) and relative condition factor (K) are very important tools in the fisheries sciences since it provides valuable information about the growth, fitness, and maturation of the target fish.

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Like any other morphometric character, the length-weight relationship can be used as a character for the differentiation of taxonomic units and the relationship changes with various developmental events in life such as metamorphosis, growth and the onset of maturity (Negi & Nautiyal, 2002). This length weight relationship is helpful for estimating the wellbeing of a fish and can be used in studies of gonad development, rate of feeding, metamorphosis, maturity, and condition (Le Cren, 1951). The spawning season has a temporal scale, with most fishes exhibiting one or two spawning seasons in a year. Reproduction has key components of gonadal development (maturity stages), gonadosomatic index (GSI) and fecundity, which are vital demographic characteristics essential to an understanding of a species' life history (Hussain et al. 2018). *S. sinuatus*, *S. plagiostomus* and *S. richardsoni* had exhibited intermittent spawning from July to January (Badola & Singh, 1984). *S. plagiostomus* spawns twice a year (March to April and September to October) and becomes sexually mature at 18-24 cm total length both in a natural and artificial environment (Jan et al. 2016).

Fecundity is known to vary within species with latitude and location and with spawning time (Hussain et al. 2018). Along with these, the Condition factor (CF) is also a useful index for the monitoring of feeding intensity, age and growth rates in fish (Goel et al. 2011). Condition factors can be used as an indicator of relative health (Brown and Murphy, 1991). Previously the reproductive behavior in fishes has shown a strong relationship with total body length (TBL), total body weight (TBW), gonad weight, gonad length, ovary length and ovary weight (Sehgal, 1988), fecundity with TBL and TBW, and GSI (Amtyaz et al. 2013) and hepatosomatic index in *S. plagiostomus* has been examined (Jan & Ahmed, 2016).

Several studies have been done to explore the fish species composition and distribution of the ichthyofauna in the River Indus. However, the reproductive information of *S. plagiostomus* inhabiting River Indus is meager. For the same purpose, the present study sort to investigate the breeding behavior, seasonal variations, correlation of total body length (TBL) with total body weight (TBW), gonado-somatic index (GSI) and absolute fecundity (F_{ab}) of *S. plagiostomus*, along river Indus and its tributaries in Kohistan district of northern Pakistan. The study would be useful in understanding the relationship of fecundity with various parameters in the target fish for its seed

production in captivity. Captivity breeding will facilitate the stocking process of this economically important fish species into natural water resources for the purpose of its conservation.

2. MATERIALS AND METHODS

The present study was conducted in Kohistan District of Khyber Pakhtunkhwa (KP), northern Pakistan, situated between 34° 54' and 35° 52' north latitudes and 72° 43' and 73° 57' east longitudes, covering an area of 7492 (Jayaram, 1981). A total of 540 fish samples (45 specimens each month) were collected from Jalkot, Palas, Keyal, Barseen, Kandia and Darel streams along River Indus as described in table 1. The coordinates for the collection site were extracted from google earth PRO (version 7.3) and the site map was constructed in ArcGIS software version 10.3.1 (Figure 1).

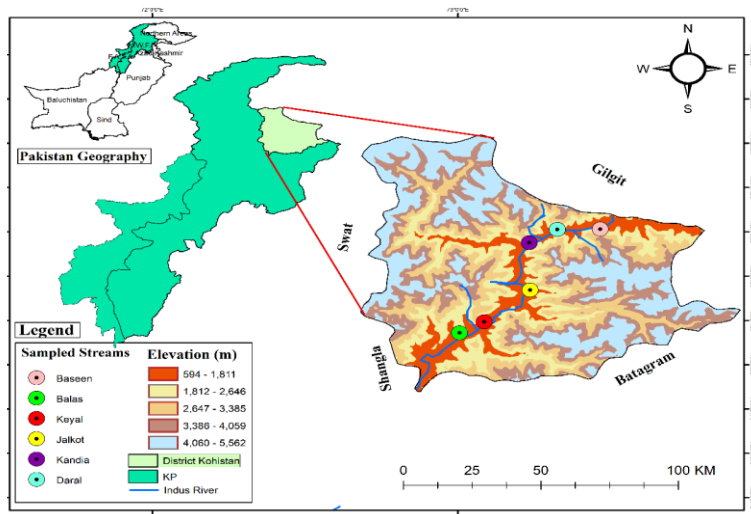


Figure 1. Sampling sites at different streams along River Indus in Indus Kohistan, KP, Pakistan.

Gill nets of 1, 1.5, 2, 2.5 and 3cm stretched bar mesh having a length of 25 feet and depth of 6 feet were used for fish sampling. Fish samples were identified by using the previously described keys (Jayaram, 1981; Kullander et al. 1999).

The total body length (TBL) in centimeter and total body weight (TBW) in gram of each specimen were recorded using line gauge and balancer respectively. Each specimen was dissected carefully, and the gonads were separated for the weight and length measurement. The maturity stages were also identified by using a seven-point maturity scale as described by (Muhammad et al.

2017) as described in table 1, followed by preservation in 5% formalin solution.

The relationship between TBL and TBW of each male and female *S. plagiostomus* was calculated by using the equation (Bagenal & Tesch, 1978).

$$W = aTL^b$$

where W is the total body weight and TL is the total body length, a and b are the intercept and slope of the regression line, respectively.

The Fulton's Condition Factor was also calculated by using the following equation (Bagenal and Tesch, 1978).

$$FCF (K - value) = \frac{TBW}{TBL^3} \times 100$$

where FCF is a Fulton's condition factor.

During this study, the monthly GSI of *S. plagiostomus* was calculated to determine its period and frequency of spawning round the year (Bariche, 2017).

$$GSI(\%) = \frac{Gonad\ Weight}{TBW}$$

where GSI denotes for gonado somatic index and TBW is total body weight.

The fecundity was calculated by using a total of 263 ovaries extracted from female *S. plagiostomus*. Gravid females were easily recognized from enlarge abdomen, after careful dissection each lobe of the matured ovaries were cut down into three sections (anterior, middle and posterior) and preserved in Gilson's fluid in order to loosen ova from the ovarian wall.

Following the same method, the two lobes of ovaries were dried up with blotting paper and weighed. Then 0.01g of each ovary was taken separately from the anterior, middle and posterior parts of each lobe. The matured ova were easily identified and distinguished from their reddish color. Only the number of ripe oocytes in 0.01 g (reddish in color) were counted with the help of the magnifying lens and then multiplied by the total weight of the ovary.

The gravimetric method was applied to determine the total fecundity of gravid fish (Yeldan & Avşar, 2000).

$$F_a = \frac{Gonad\ weight\ (g) \times no\ of\ oocytes\ in\ sub - sample}{Sub - sample\ weight\ (g)}$$

$$F_a = \frac{F_1 + F_2 + F_3}{3}$$

where F_a is the absolute fecundity and F_1 , F_2 and F_3 are the fecundity count in the sub-sections

Statistical analysis

The recorded observations were entered into the Microsoft Excel sheet (version 2013) and each variable was assorted. Furthermore, the statistical analysis was performed by transferring the excel sheet to the SPSS version 21 for the chi-square test considered <0.05 a constant significant value. A curvilinear quadratic regression analysis was used for the estimation of the regression curve between TBL correlated with TBW and each with GSI.

3. RESULTS AND DISCUSSION

A total of 540 *S. plagiostomus* each 277 (51%) males and 263 (49%) females were examined in this study. The collection of *S. plagiostomus* was also recorded such as 94 (17%) were collected in each Palas, Keyal, Barseen and Kandia streams followed by 86 (16%) and 82 (15%) from Jalkot and Darel stream respectively as described in table 2. A high significant difference was observed in the distribution of male and female *S. plagiostomus* along collection sites (<0.0001).

A maximum of seven maturity stages in the gonads of *S. plagiostomus* were identified i.e., Stage-I immature, stage-II unambiguous sex, stage-III developing, stage-IV maturing, stage- V mature, stage-VI running, and stage-VII spent as described in table 1 (Nagelkerke, 1997).

A synchronized breeding condition with slight variations in the maturity stages of male *S. plagiostomus* was observed figure 2a. As compared to females the gonad developing stage in males started a bit earlier from late July. In August the developing gonads illustrated slight reddish color followed by lobed and white color gonads in late August in both sexes however the developing stage extends to early September in males (Autumn spawning). The maturity of gonads begins in September ranged till November followed by lobed and white gonads (maturing stage IV) in January and February (Spring spawning).

Stages I, II and III were also found missing during the same breeding season where both gonads were recorded in stage IV directly during January. In line with the literature reviewed, the present study has confirmed that *S. plagostomus* spawn twice a year, March, and April. The spent stage was quite extended in males compared to females during the month of May (Figure 2).

The scatter plot of the curvilinear relationship between TBL and TBW in both males and females was calculated. The quadratic curve shows the initial slight increase of TBW in response to TBL in both male and female *S. plagostomus* however it increases exponentially when the total body length exceeds 30 cm. The overall model of weight-length relationship was highly significant with R^2 value of 0.902 for male and 0.900 for female *S. plagostomus* (Figure 3 A-B).

To calculate the condition factor K-value, the month-wise line graph for both male and female *S. plagostomus* was plotted. During the present study length weight relationship and condition factor of *S. plagostomus* showed some variation throughout the year. In male *S. plagostomus* the highest mean K-value of 1.99 was recorded during June while the lowest K-value of 1.52 and 1.47 were recorded during February and January respectively (Figure 4). The variation in the K-value was less significant (0.046) for male *S. plagostomus*. In female *S. plagostomus* the highest mean K-value of 1.98 was observed during July and November whereas the lowest K-value of 1.41 was recorded during February. The variations were significant for female *S. plagostomus*.

The mean GSI calculated for male *S. plagostomus* during spring and autumn breeding cycles was 17.84 and 15.41 respectively. Similarly, the mean GSI estimated for female *S. plagostomus* following the same breeding cycles was 17.77 and 16.06 respectively (Figure 5).

The monthly regression analysis record of total body length (TBL) correlated with GSI shows the highly significant effect of TBL on GSI in male *S. plagostomus* (0.0001). The scatter plot shows an early increase of GSI between 20 cm to 25 cm of TBL, however, a rapid decline was observed in the GSI of male *S. plagostomus* when body length has increased as shown in figure 6A. An initial increase of GSI in response to TBL increase up to 25 cm followed by the rapid decline in GSI against TBL

increase was recorded among female *S. plagostomus*. The declining pattern of GSI versus TBL is evident in the scatter plot as shown in figure 6B.

The initial increase of GSI among male *S. plagostomus* weighing up to 250 g of TBW, however, it was significantly decreased when body weight increased. The minimum GSI was observed in individuals around 1250 g of total body weight. The minimum GSI was recorded in female *S. plagostomus* having TBL between 40 cm to 45 cm. Female *S. plagostomus* gain maximum GSI at 250 g of total body weight. The minimum GSI was recorded in specimens having TBW of 1250 g as shown in figure 7.

The effect of TBL on absolute fecundity was significant in individuals having a TBL between 20 cm and 25 cm. The scatter plot shows an initial increase of absolute fecundity in specimens having 25 cm of TBL and then a declining pattern of absolute fecundity. The minimum absolute fecundity was recorded in individual fish specimens having TBL around 45 cm (Figure 8A).

The regression analysis shows the initial increase of absolute fecundity in individuals weighing between 250 g and 500 g of TBW and then a rapid decline when TBW increases. The minimum absolute fecundity was estimated in individual specimens weighing between 750 g and 1000 g above which a slight increase in the absolute fecundity was observed in individuals at 1250 g of TBW (Figure 8B).

A total of 263 female *S. plagostomus* having 20.5 cm to 43.8 cm matured female fish were used to estimate fecundity. The estimated fecundity during the present study was ranged from 659.89 to 20474.96 eggs Kg^{-1} . The maximum mean absolute fecundity of 14079.92 (± 648.93) eggs Kg^{-1} were estimated during November with a mean TBL of 27.5 cm and mean TBW of 406 g whereas the minimum mean absolute fecundity of 2046.93 (± 288.66) eggs Kg^{-1} were recorded during February in fish having mean TBL 32.7 cm and mean TBW of 484 g (Figure 9).

Studies on the reproductive behavior of fishes involve morphometry (TBL and TBW), fecundity estimation, reproductive season cycle, spawning behavior and spawning fraction which contributes a greater value in quantifying the reproductive capacity of fish species. The absolute health of fishes is based on the TBL, TBW, and gonadal weight which enhances the fecundity quality of

fishes in addition to the maturational cycle and monthly variation in GSI provided a good indication of the development of gonads (Akhtar *et al.* 2016). The economically important fish species need special concerns over the reproductive strategies, seasonal fecundity rate and determination of physical growth.

Fish fauna are the economically important sector which plays a vital role in the economy of Pakistan (Shah *et al.* 2018). Among these the *S. plagiostomus* are also important and mostly confined to the northern Himalaya region (Jan *et al.* 2016). Various studies have confirmed the existence of *S. plagiostomus* in different regions of KP (Khatak *et al.* 2015). In present study a total of 540 comprising of 277 (%) male and 263 (%) female *S. plagiostomus* were collected from various streams of District Kohistan and their physical morphometry (TBL and TBW) were correlated with the GSI and fecundity rate. The K-value and maturity stages throughout the year were also estimated.

Almost synchronized period of spawning was observed for both male and female *S. plagiostomus* during spring (February to April) and autumn seasons (September to November). Various reports confirmed that *S. plagiostomus* spawn two time each year (Singh & Sharma, 2016), while contrary to that Akhtar *et al.* showed that *S. plagiostomus* spawn once annually (Akhtar *et al.* 2016). In this study it was observed that *S. plagiostomus* spawn in both spring and Autumn season, and their gonads developing stages includes seven stages of maturity in both sexes of *S. plagiostomus* including Stage-I immature, stage-II unambiguous sex, stage-III developing, stage-IV maturing, stage-V mature, stage-VI running, and stage-VII spent. Similar reports were also provided by (Negi & Nautiyal, 2002). However, there are six stages of maturity in *Schizothorax richardsonii* (Agarwal *et al.* 2018), five maturity stages in *Schizothorax niger* and *Schizothorax esocinus* (Raina, 1976). The International Council for Exploration of the Seas has recognized seven maturity stages for fish in temperate waters (Qadri *et al.* 2019).

Month wise variation showed immaturity of gonads in June while maturity was noticed two times each in September to November and February to April. The structural changes were observed in July and August which were not observed in Autumn breeding cycle and the matured gonads were followed by a spent stage in

May. Similar consequences were showed by Munro, (1990). The sudden increase in water temperature during May seems to inhibit the breeding response where gonads were observed totally in spent condition. Exteroceptive factors such as water temperature, food availability, and duration of photoperiod were positively correlated with the spawning behavior of *S. richardsonii* (Negi & Nautiyal, 2002).

The statistical analysis between TBL and TBW has a diverse application in fisheries sciences which is very useful for the evaluation of condition factor in fishes. The quadratic curve shows an initial slight increase of TBW in response to TBL in both male and female *S. plagiostomus* showing R² value of 0.900 and 0.900 respectively. The exponential increases in the growth were observed by increases in the total length. Similarly, the TBL and TBW was ranged from 9.6 cm to 52 cm and 7 to 948 g respectively with the maximum growth with respect to total length was found 0.908 (Bhat *et al.* 2010), 0.992 (Jan *et al.* 2016), and linear regression 0.92 and non-linear 0.95 in *Schizothorax richardsonii*. Thus, in length weight relationship of Snow trout, weight increases in length throughout its life.

The slope coefficient b₂ for TBL correlated with TBW in males and females are within the range of 0.825 to 3.138 and 1.15 to 2.68 respectively. The highest b₂ value in males *S. plagiostomus* indicates the rapid weight gain in males than females. The slight difference of minimum TBL in male shows that it gains maturity a bit earlier than female owing to the consequences of (Jan *et al.* 2014; Khan *et al.* 2018), and contrary to the findings of (Dar *et al.* 2012). The slope value of regression line less than 3 has also been reported in *Cyprinus carpio communis* and *Ctenopharyngodon idella* (Dhanze & Dhanse, 1997).

The CF in both male and female *S. plagiostomus* shows variations in different months. The highest K-value of 1.99 ± 0.13 for male *S. plagiostomus* was noticed in the month of June while for females it was 1.98 ± 0.12 in July and 1.98 ± 0.09 in November. The K-value recorded for both sex of *S. plagiostomus* during the present study is slightly higher when compared to the K-values recorded for *S. plagiostomus* by (Jan *et al.* 2014). from Lidder River Kashmir. Maximum K value found in the month of September (1.05) and least in July (1.03) (Kumar *et al.* 2014). The average CF for *S. richardsonii* was found 0.73 (Goel *et al.* 2011). The seasonal variation in CF seems to

be attributed to high feeding activity, fat deposition and gonadal development as a preparation for coming breeding season. Maguire & Mace (1993) reported that increase in K-values indicates the accumulation of fat and sometimes it reflects the gonadal development. CF revealed that the fluctuations in K values can be attributed to the spawning cycle as well as feeding intensity.

The present study revealed the fact that all male and female *S. plagiostomus* gain maximum GSI at 25 cm of total body length. The smaller fish specimens have greater GSI when compared to larger ones. In both male and female *S. plagiostomus* the GSI was found to decrease curve-linearly with the increase in TBL and TBW. A linear relationship between these variables were observed by (Bahuguna & Khatri, 2009) and (Muhammad and Pathak, 2010). Monthly variations in GSI index provided a very good indication of gonads development round the year. The suddenly decreased in the GSI were indicating the post-spawning season of *S. plagiostomus*. Stage-wise calculation of GSI in the present study revealed that highest GSI were observed in ripe stages (Stage-V and VI) while the lowest values were recorded in spent (stage-VII), immature (Stage-I) and maturing (stage-II), respectively.

Fecundity of *S. plagiostomus* calculated in the present study, did not show allometric pattern of growth with TBL and TBW which is contradictory to the findings of (Goel et al. 2011) and (Soomro et al. 2011). In this study, the fecundity was at the peak in fishes of 20 cm to 25 cm in TBL and 250 g and 500 g TBW. Generally, all teleost fishes have a linear relationship of fecundity with TBW and ovary weight, however Bagenal, (1957) reported a non-linear relationship of these parameters. The number of ova varied from 3437 to 34800 for a fish varied in TBL 26.2 to 45.3 cm and TBW 176.5 g to 1150 g. The regression equation was found to be linear for both TBL ($r = 0.96$) and TBW ($r = 0.961$). In *S. plagiostomus* the fecundity was ranged from 1695 ± 524.44 to 3297 ± 282.99 eggs with TBL 22.52 ± 2.84 to 27.90 ± 1.88 cm and TBW 172.33 ± 10.9 to 240.9 ± 7.59 g [4]. Similarly, the Study determined that fecundity of *S. plagiostomus* ranged from 1695 ± 524.44 and $3,297 \pm 282.99$ on the average length of fish ranging 22.52 ± 2.8 to 27.90 ± 1.8 cm and average fish weight ranging 172.3 ± 10.9 to 240.9 ± 7.5 g (Akhtar et al. 2016).

In the present study the highest absolute fecundity of 8504.59 ± 614.62 (2386.67 - 13610.19) was recorded in the month of April during spring cycle in gravid specimen having total body length of 29.24 (13.80-52) cm. During autumn the mean absolute fecundity of 14079.92 ± 648.93 and range (9998.88 - 20474.96) was recorded in the gravid specimens having total body length of 27.5 (21.5-39.5) cm in the month of November. Same relationship between absolute fecundity and TBL in *S. phulo* were reported by (Pishka et al. 1997). It was also observed that gravid females were more fecund in November than April. The mean value of fecundity was estimated as 14599 eggs with a mean TBL (34.340 cm) and a mean TBW (440.60 g). This seasonal variability may be due to changes in food availability and the resulting scope of individual fish to allocate energy either to reproduction or to somatic growth and maintenance. Similar seasonal changes were observed in the gonads of *S. richardsonii* (Qadri et al. 2013).

4. CONCLUSION

Being economically important species in the county, conservation, and management of *S. plagiostomus* are necessary in the river Indus, especially during its breeding season. The results of the study provide important insights about the fecundity and reproductive parameters that will be helpful to conserve this species through breeding in captivity and then seed stocking into the natural water resources.

5. CONFLICT OF INTEREST

All authors have declared that there is no conflict of interest regarding this publication.

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Table 1. Locality wise distribution of Male and Female *S. platiostomus*.

Locality/Stream	Total %	Male Frequency %	Female Frequency %	Cumulative %
Jalkot	86 (16)	52 (60)	34 (40)	16
Palas	94 (17)	41 (43)	53 (57)	33
Keyal	94 (17)	56 (60)	38 (40)	51
Barseen	94 (17)	48 (51)	46 (49)	68
Kandia	90 (17)	38 (42)	52 (58)	84
Darel	82 (15)	42 (51)	40 (49)	100
Total	540 (100)	277 (51)	263 (49)	100

Table 2. Locality wise distribution of male and female *S. plagiostomus*

Locality (Streams)	Total (%)	Male frequency (%)	Female frequency (%)	Cumulative %	P value
Jalkot	86 (16)	52 (60)	34 (40)	16	
Palas	94 (17)	41 (43)	53 (57)	33	
Keyal	94 (17)	56 (60)	38 (40)	51	
Barseen	94 (17)	48 (51)	46 (49)	68	<0.0001
Kandia	90 (17)	38 (42)	52 (58)	84	
Darel	82 (15)	42 (51)	40 (49)	100	
Total	540 (100)	277 (51)	263 (49)	100	

Table 1. Gonad maturation stages for cyprinids (Nagelkerke, 1997).

Gonad Stages	Male	Female
I	Immature, indistinguishable male from female, gonads are a pair of elongated transparent strings running along the body cavity.	Immature, indistinguishable male from female, gonads are a pair of elongated transparent strings running along the body cavity.
II	Unambiguously male. Testes verysmall, tube shaped white-reddish andnon-lobed.	Unambiguously female. Ovaries verysmall, tube shaped white-reddish and invisible eggs.
III	Testes larger and white reddish incolor. Lobed up to some extent and starting flattened to sideways.	Ovaries larger and white reddish in color. Starting flattened to sideways. Eggs very small.
IV	Large testes white in color, lobed andflattened to sideways	Large Ovaries, flattened to sideways and covering body cavity wall. Eggs yellowish.
V	Large whitish testes, sperm run outwhen testes cutoff.	Large and full ovary and completely covering the body cavity. Eggs yellowish and run out when ovaries cut.
VI	Running, large white testes	Running, eggs yellow and extruded out through stripping.
VII	Spent, testes became empty, wrinkled, and reddish in color.	Spent, ovaries wrinkled reddish in color which contain few yellow eggs.

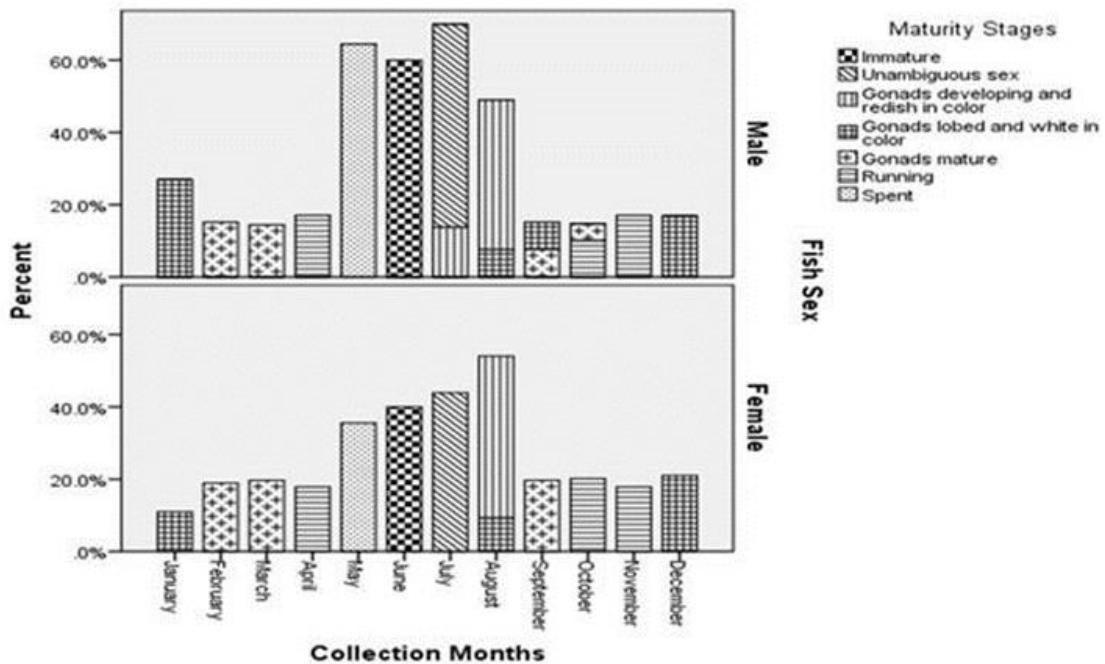


Figure 2. Variations in the maturity stages of male and female *S. plagiostomus*

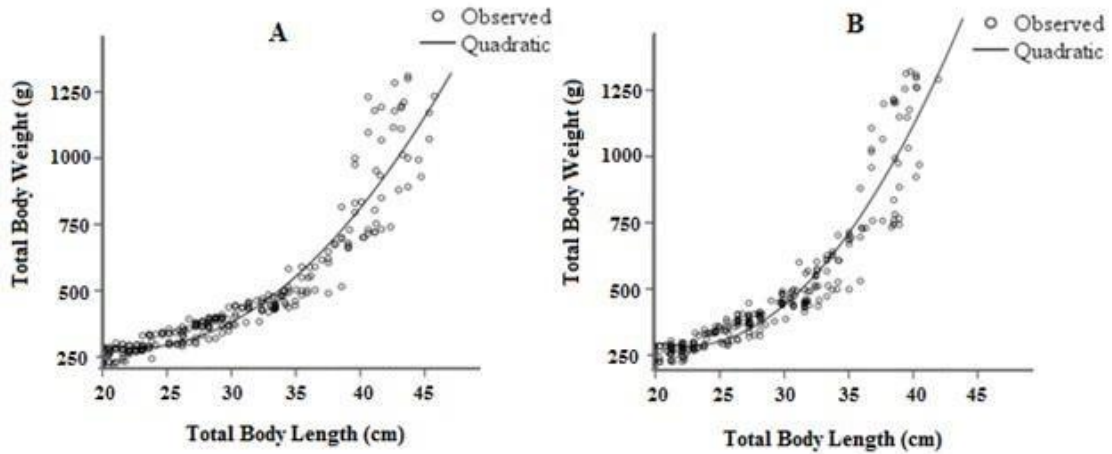


Figure 3. Scatter plot of the curvilinear relationship between TBL and TBW in both male (A) and female (B) *S. plagiostomus*.

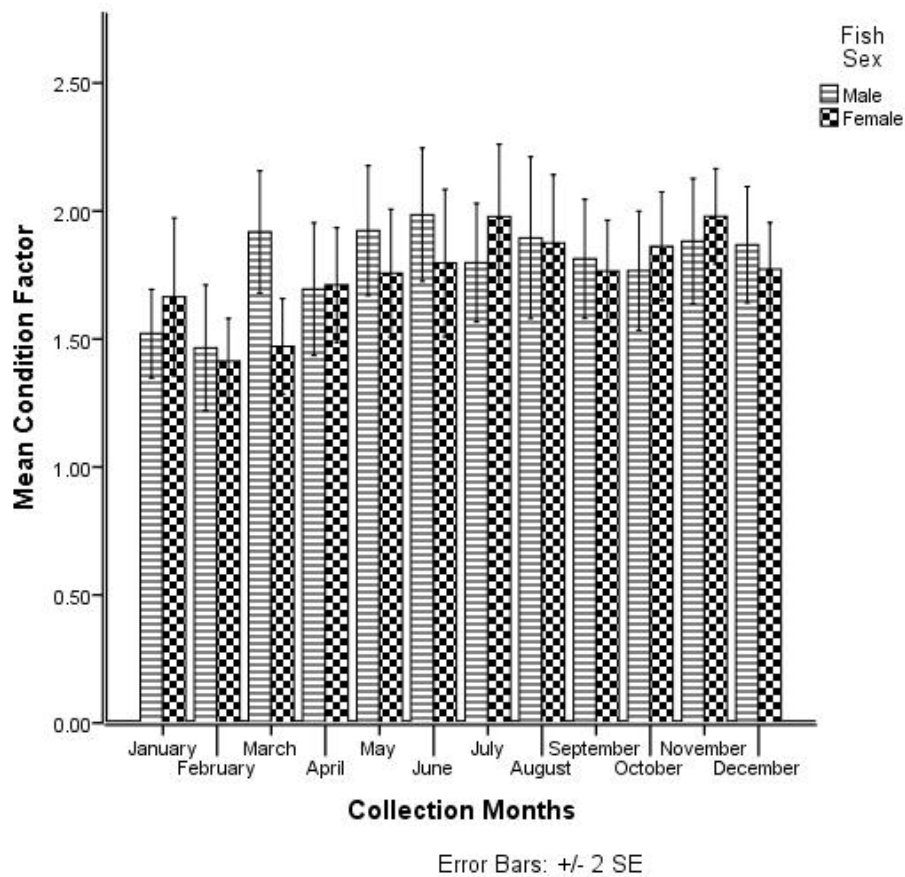


Figure 4. Bar graph showing monthly variation in the mean Condition Factor (K) with standard error bars, of male and female *S. plagiostomus* caught from river Indus in northern Pakistan during 2016.

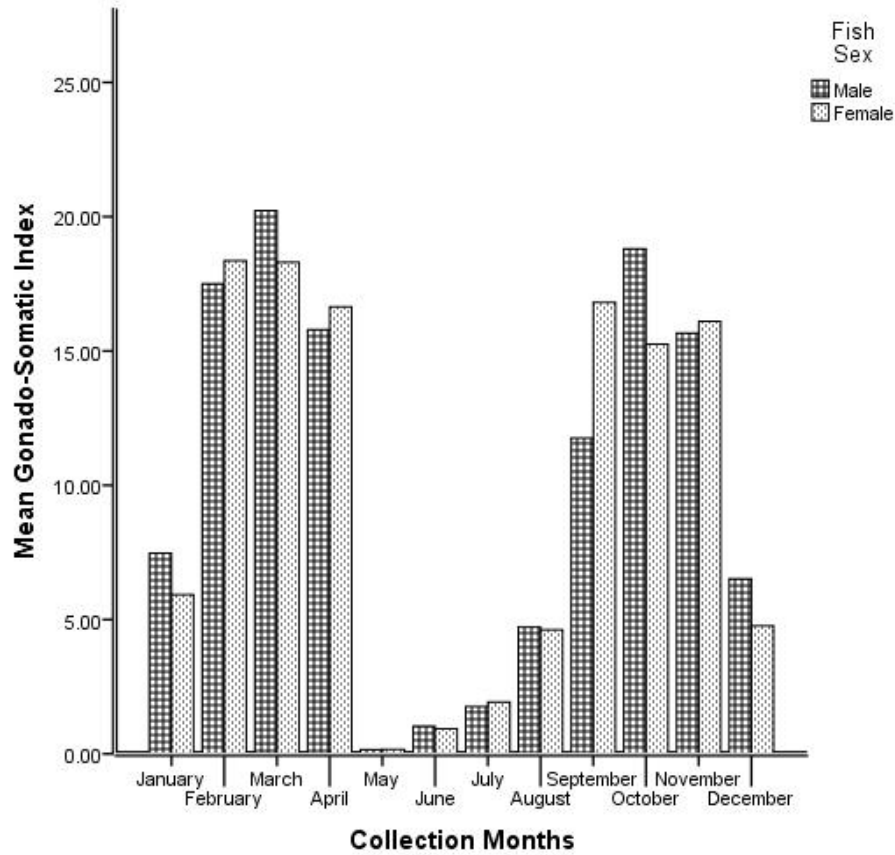


Figure 5. Bar graph showing monthly variation in the mean GSI of male and female *S. plagiostomus* caught from river Indus in northern Pakistan during 2016.

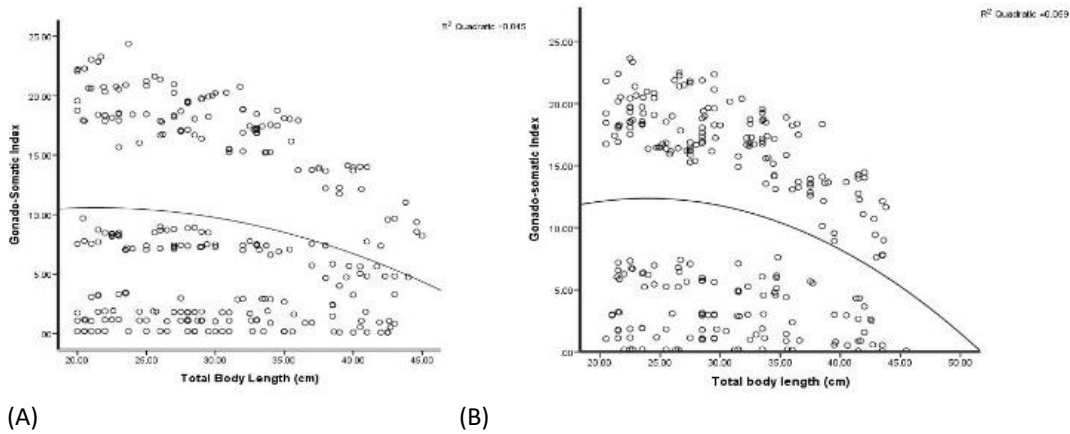


Figure 6. Scatter plot showing curvilinear relationship negative (quadratic model fit) between total body length TL (cm) and GSI of male *S. plagiostomus* (A) and female *S. plagiostomus* (B) caught from river Indus in northern Pakistan during 2016.

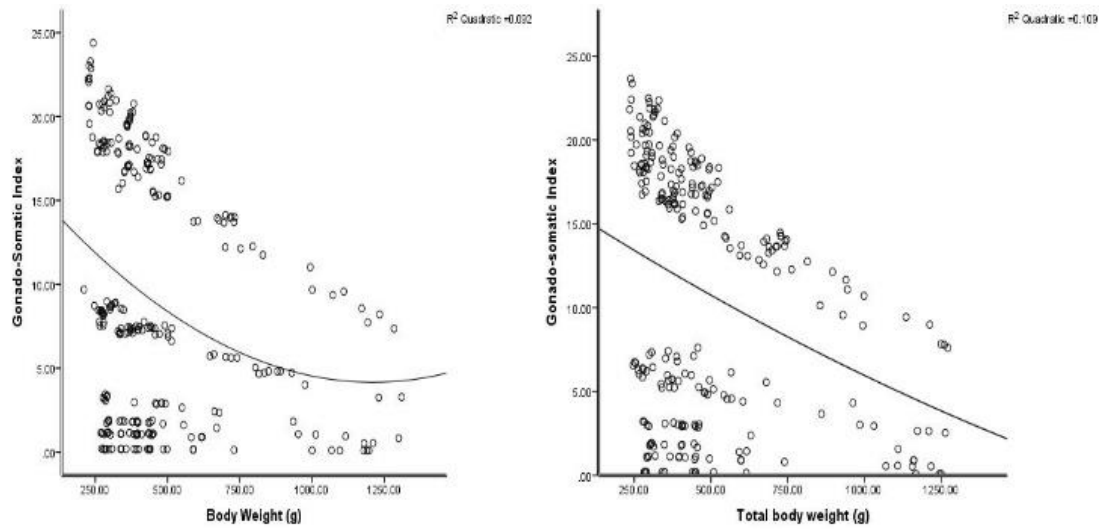


Figure 7. Scatter plot showing curvilinear relationship negative (quadratic model fit) between total body Weight (g) and GSI of male *S. plagiostomus* (A) and female *S. plagiostomus* (B) caught from river Indus in northern Pakistan during 2016.

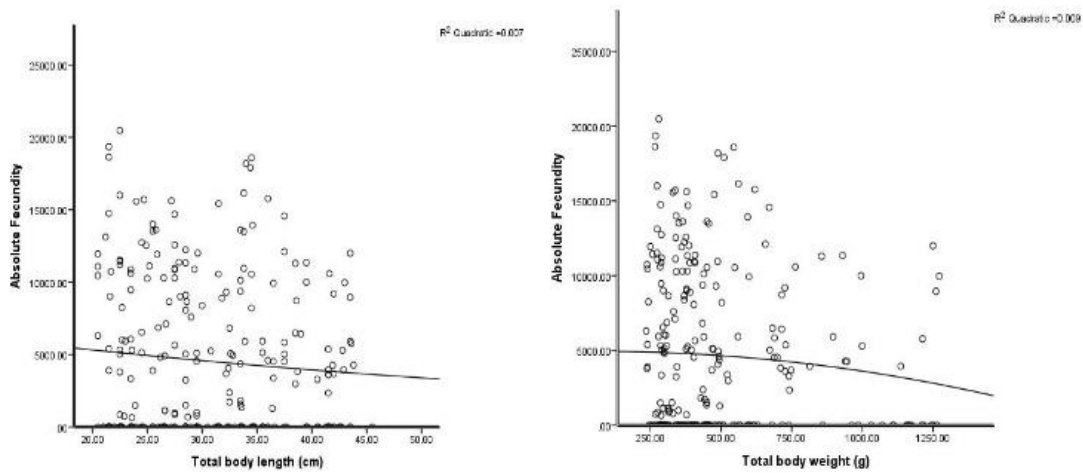


Figure 8. Scatter plot showing curvilinear relationship negative (quadratic model fit) total body length TL (cm) vs fecundity (A) and total body Weight (g) vs fecundity (B) in female *S. plagiostomus* caught from river Indus in northern Pakistan during 2016.

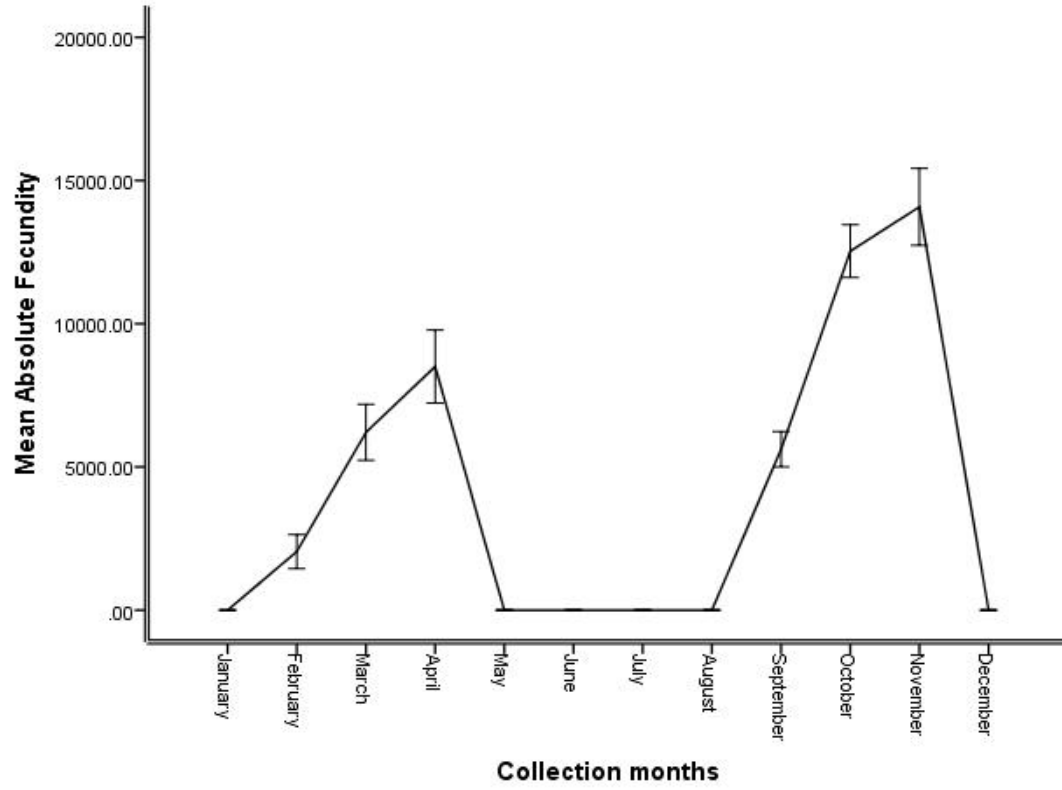


Figure 9. Line graph showing monthly variations in the mean absolute fecundity with standard error bars of female *S. plagiostomus*.

EVALUATION OF DIFFERENT SALINITY LEVELS ON GROWTH PERFORMANCE AND PROXIMATE COMPOSITION OF *WALLAGO ATTU*

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Wallago attu, Salinity levels, Growth performance, Proximate composition, River Chenab, Muzaffargarh

ABSTRACT

The study was conducted to determine the optimum level of salinity tolerance on the growth performance and proximate composition of *Wallago attu* fed with tilapia meat. The experimental fish were collected from River Chenab district Muzaffargarh and acclimatized for 14 days in freshwater before the experiment began. The fish were divided into five treatments: T1, T2, and T3 with salinity levels 4, 8 and 12-ppt respectively with a control group (T0) in freshwater. Each treatment had one replicate and fish were stocked at a density of 5 fish's/aquarium in the Laboratory. Fish were fed 5% of their body weight with tilapia meat and water was exchanged regularly after every alternate day. The present study indicates the highest survival rate at 12-ppt and mortality was observed beyond this point. Non-significant changes in growth parameters viz; Feed intake, Feed conversion ratio, and Weight gain, were found in treatments and the control group. Additionally, proximate composition (moisture, ash content, protein and fat) showed significant changes ($P < 0.005$) in groups. Water quality parameters were found within the acceptable range during the study period. The result suggests that *W. attu* is a suitable species for rearing in harsh water earthen lakes and emphasizes the importance of considering salinity levels and leads to innovation in sustainable aquaculture practices.

1. INTRODUCTION

Aquaculture plays an important role in solving global food crisis and practiced especially in the production of 60% protein from Fresh, brackish and saline waters which are considered as suitable for aquaculture development (Jarwar, 2008; Minfal, 2012). Fish is a complete diet and help to improve malnutrition, it contains high proportion of protein, water and lipid soluble vitamins, minerals, polyunsaturated fatty acids and Omega-3 fatty acids. Fish reduce the risk of heart diseases, blood pressure and improve blood clotting regulation (Nettleton, 1995). The intensive and non-intensive farming systems have created an immersive pressure to produce healthy and low-cost fish feed ingredients (Flefil et al., 2021).

Freshwater ecosystem facing salinization, leading to severe impacts on aquatic communities and food webs. However, in aquaculture and fisheries, salinity tolerance has been practiced and are limited but catfish are major targeted species (Sahoo and Ferosekhan, 2018; Thorslund et al., 2021; Astorg et al., 2022). *W. attu* is a new potential catfish in aquaculture belonging to family Siluridae due to its fast-growing nature, high protein content in its flesh, high nutritional and good market demand (Lilabati and Vishwanath, 1996; Azam et al., 2004). It is known as freshwater shark or Asian silurid catfish, found in South-east Asian rivers reservoirs and survive in a wide range of ecological environment (Halls and Johns, 2013; Quyen et al., 2017; Jahan et al., 2019).

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The current study is conducted to present an opportunity for innovation and sustainable aquaculture practices. By using information, farmers can adjust their cultivation method of *W. attu* and improve the survival and growth rates, ultimately leading to more sustainable and profitable aquaculture industry. With connected research and innovation aquaculture can play a critical role in addressing environmental challenges and meet the growing demand for high quality food. Few published literatures are available on salinity tolerance of *W. attu* commercially important fish. The aims of this study to determine the optimum tolerance level of salinity of threaten species *W. attu* and to determine the effects of salinity on proximate composition and growth parameters while making suggestions for its conservational strategies to culture even in salt effected areas.

2. MATERIALS AND METHODS

The freshwater catfish (*W. attu*) were collected from River Chenab and transported to Saline Water Aquaculture Research Centre (SWARC) Muzaffargarh. Fish were acclimatized in freshwater for 7 days in glass aquaria before the experiment began. The experiment was designed into five treatments (T1, T2 and T3) with 4, 8 and 12 and 14 salinity levels respectively with a control group (T0) was on freshwater. Each treatment had one replicate and fish were stocked as 5 fish/aquarium. Fish were fed 5% of their body weight with tilapia meat and water was exchanged regularly after every alternate day. The levels of salinities selected in this experiment was based on various salinities of Punjab in District Muzaffargarh because huge resources of Brackish and saline water forming is present. The continuous aeration was provided to maintain dissolved oxygen, salinity was maintained and tested on daily basis during the experiment lasted 60 days. Detritus, uneaten feed was removed every morning by siphoning out from the tank (Soundarapandian et al., 2009). All the physicochemical parameters were maintained by using Apera 8500 EC meter, Apera 8500 pH meter and P-512 dissolved oxygen meter on daily basis.

Growth Parameters

Fish having average weight of 29 g were stocked at 5 fish per aquaria in each treatment groups. The growth parameters viz., feed intake weight gain, growth rate percentage, and FCR was measured (Batool et al. 2018). Fish was regularly fed twice a day up to sixty days at the

end of experiment. All of the growth parameters were calculated as per following formulas:

$$\begin{aligned} \text{Weight gain} &= \text{Final weight} - \text{Initial weight} \\ \text{FCR} &= \text{feed given (g)} / \text{Weight gain (g)} \\ \text{Growth Rate (\%)} &= \text{WG (g)} / \text{Wi (g)} \times 100 \\ \text{Feed Intake; FCR} &= \text{FCR} \times \text{Weight gain (g)} \end{aligned}$$

Proximate composition

The proximate composition was analyzed by following AOAC (1990). The 5g of fish meat was dried in an oven at 105 °C. After complete dryness, loss of moisture was calculated as percent moisture content. The dried samples were finely crushed by mortar and pestle and then content of lipid was determined by using chloroform-methanol method. Crude protein contents were determined using standard micro-Kjeldahl method. The dried sample of 2g was burnt in a muffle furnace at 550 °C until its complete combustion and then white residue was evaluated as ash content.

Statistical analysis

The data obtained was analyzed statistically by performing Analysis of Variance (ANOVA) technique and means were compared by Duncan's Multiple Range Test.

3. RESULTS AND DISCUSSION

The experiment was specially designed for sixty days to assess the growth performance (final weight, feed intake, growth rate, weight gain and feed conversion ratio) and proximate composition (Crude Protein, Crude fat, Moisture, Ash) of *Wallago attu* at different level of salinities (4-ppt, 8-ppt, 12-ppt and 14-ppt along with their replicates and control group 0-ppt).

The growth parameters under all the treatments are presented in Table I. The initial weight of fish at the time of stocking was T0 (29.26±0.36g), T1 (29.68±.152g), T2 (29.60±.20g) and T3 (29.88±0.120g). The final weight after the end of trial was T0 (58.06±0.50g), T1(58.10±0.361) T2(58.06±.369) and T3 (59.32±0.10). The weight gain was T0 (28.80±0.68), T1(28.42±0.36) T2(28.46±.3826) and T3 (29.44±0.10). The growth rate was T0 (98.57±3.33), T1(95.77±1.40), T2 (96.18±1.64) and T3 (98.53±0.68). The feed intake was T0 (37.71±0.52), T1 (39.60±0.86) T2 (43.05±0.39) and T3 (47.49±0.64). The feed conversion ratio was T0

(1.31±0.02), T1 (1.3±0.03), T2 (1.5±0.02) and T3 (1.6±0.02) respectively. The statistical analysis of growth performance shows no significant changes ($P\leq 0.05$) between treatments and control group in term of final weight, weight gain, growth rate, feed intake, feed conversion ratio as shown in Table 1 and Figure 1.

Proximate composition is an important factor that determines fish flesh quality which may be changed due to different salinity exposure. The proximate composition (mean \pm SEM) of *Wallago attu* was determined at different salinity levels the proximate composition of Crude protein was T0 (67.60±0.17), T1(68.28±0.24), T2(70.44±0.74) and T3 (71.54±0.15); Crude fat was T0 (4.62±0.07), T1(5.32±0.11), T2(6.30±0.15) and T3 (7.26±0.10); Moisture was T0 (73.30±0.40), T1(71.84±0.28), T2(70.74±0.25) and T3 (69.54±0.28); Ash was T0 (1.12±0.02), T1(1.07±0.09), T2(1.03±0.03) and T3 (1.02±0.02). The highest amount of moisture was 73.30±0.40 at 0-ppt while lowest was 69.54±0.28 at 12-ppt. Maximum crude protein was 71.54±0.15 at 12-ppt while minimum was 67.60±0.17 at 0-ppt. The highest crude fat was 7.26±0.10 at 12-ppt and lowest was 4.62±0.07 at 0-ppt. Maximum ash content was 1.12±0.02 observed at 0-ppt and minimum was 1.02±0.02 at 12-ppt. Each parameter of Proximate composition (crude protein, crude fat, moisture and ash) were statistically significant ($P<0.005$) between treatments and control group. However, Moisture and ash content in the fish significantly decreases as salinity increases and Protein and fat content in the fish significantly increase as salinity increases as shown in (Table 2 and Figure 2).

Active and healthy fish feeding behavior remains normal up to 12-ppt during the 60-days trial with further increase in salinity signs of stress, poor feeding behavior, sluggish and mortality were also observed.

(a, b, c and d) Average in the same row having different superscripts significantly different at level ($P\leq 0.05$)

Throughout the experiments no negative variations in water Quality parameters viz; temperature, dissolved oxygen, pH, and TDS. During the whole experimental period, water temperature varied from 20.42 -28.67 °C in T0; 21.51-28.59 °C in T1; 19.56 -28.45 °C in T2 and 20.56 -28.55 °C in T3, Dissolved oxygen (DO) varied from 6.5 -7.4 mg/L in T0; 6.6-7.3 mg/L in T1; 6.2 -7.1 mg/L in T2 and 6.5 -7.2 mg/L in T3, pH varied from 7.1-7.6 in T0; 7.2-7.8 in T1; 7.4-7.8 in T2 and 7.1-7.6 in T3, total

dissolved solids (TDS) varied from 900-1000mg/L in T0; 1334-1450 mg/L in T1; 1835-1843 mg/L in T2 and 2135-2243 mg/L in T3. These water quality parameters were found to be optimum for culture of *W. attu* in brackish water.

The study was conducted to investigate the growth and proximate composition of *Wallago attu* cultured at different salinities (0, 4, 8, 12-ppt) in Laboratory aquarium. It is accepted that salinity is an important key factor in monitoring growth that shows better performance in brackish water. The growth performance in terms of weight gain (g), Food conversion ratio (FCR), Feed intake (FI), Growth rate (%) were seen no significant changes in treatments and in control group. Food conversion ratio (FCR) and Feed intake were found to be increase with increase of salinity up to 12-ppt, with further increase of salinity cause mortality (Table 1). [Bocuf and Payan, \(2001\)](#) and [Sparks et al. \(2003\)](#) described accelerated growth performance of tilapia reared in seawater. Similarly, [McElwee et al. \(2002\)](#) also reported faster growth of tilapia (*Oreochromis shiranus chilwae*, *O. shiranus chilwae* and *O. karongae*) in 10-ppt declaring potential candidate for brackish water aquaculture. [Kang'ombe and Joseph, \(2008\)](#) reported improved growth of *Tilapia rendalli* reared in 5-15-ppt than freshwater. [Phuc, \(2015\)](#) reported better weight gain of *P. hypophthalmus* at 0 to 10-ppt while lowest at 14 to 18-ppt.

Fish's biochemical constituents are affected by reared medium in which salinity are maintained. The results of proximate composition shown significant differences in treatments and control group. Highest moisture content in the control group (73.30±0.40), which gradually reduced with the increase in the salinity (69.54±0.28) in T3 group. Highest Ash content in the control group (1.12±0.02), which gradually reduced with the increase in the salinity (1.02±0.02) in T3 group. Similarly, protein from 67.60±0.17 to 71.54±0.15 and fat from 4.62±0.07 to 7.26±0.10, showing higher values at 0 to 12 ppt. [Dempson et al., \(2004\)](#) described remarkable decrease in the percentage of ash and increase protein and lipid. Similarly, [Barman, \(2012\)](#) studied Milkfish, reported significant reduction of moisture (%) while protein and fat increased from 0-15-ppt.

The present findings regarding the physicochemical parameters measured in this study such as temperature, dissolved oxygen (DO), pH and total dissolved solids

(TDS) agreed with the findings of Rehman et al. (2006), Dixit et al. (2015) and Okomoda et al. (2016) who also recorded all physicochemical attributes water containing different species of fish. The water quality parameter viz; DO, pH, TDS and Temperature are also considered important factors for fish culture (Buentello et al. 2000; Uzoka et al., 2012, 2015). The water quality parameters were kept suitable for catfish culture in brackish water. The overall results indicate baseline information of improved body composition, survival and cultivation of *W. attu* at various salinities.

4. CONCLUSION

The current study demonstrates *Wallago attu* economically important fish in aquaculture that tolerate salinity up to 12-ppt in Laboratory conditions and shows greatest growth performance with improved body composition. The present study provides base line literature for growth and proximate composition of *W. attu* in brackish water.

5. CONFLICT OF INTERESTS

All authors have declared that there is no conflict of interest regarding this publication.

6. ACKNOWLEDGEMENTS

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Table 1. Showing growth performance (Mean \pm SEM) of *Wallago attu* at different salinity levels (0-ppt, 4-ppt, 8-ppt and 12-ppt) during the 60-days experiment.

Groups	Initial weight	Final weight	Weight gain	Feed intake	Growth Rate	FCR
0-ppt	29.26 \pm 0.36	58.06 \pm 0.50	28.80 \pm 0.68	37.71 \pm 0.52	98.57 \pm 3.33	1.31 \pm 0.02
4-ppt	29.68 \pm .152	58.10 \pm 0.361	28.42 \pm 0.36	39.60 \pm 0.86	95.77 \pm 1.40	1.3 \pm .03
8-ppt	29.60 \pm .20	58.06 \pm .369	28.46 \pm .3826	43.05 \pm 0.39	96.18 \pm 1.64	1.5 \pm 0.02
12-pp	29.88 \pm 0.120	59.32 \pm 0.10	29.44 \pm 0.10	47.49 \pm 0.64	98.53 \pm 0.68	1.6 \pm 0.02

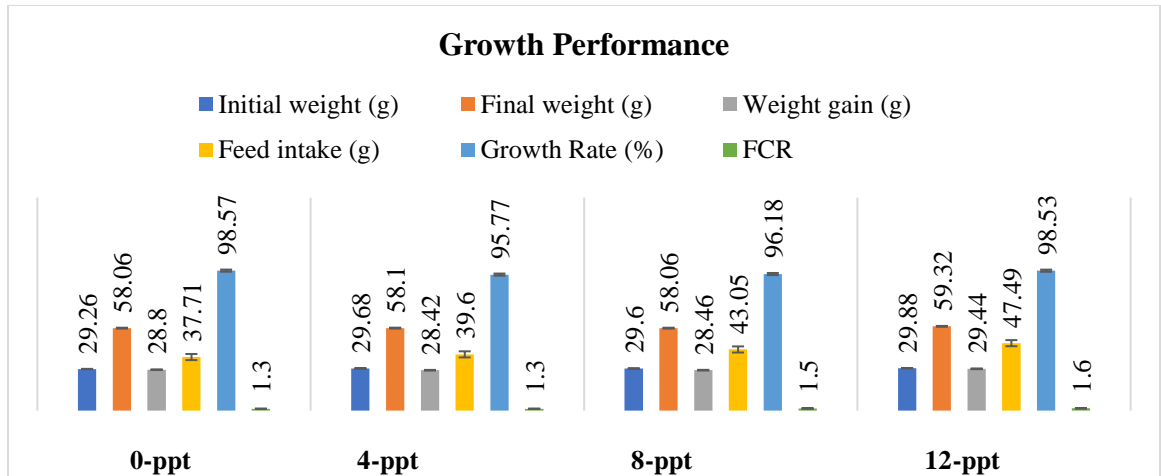


Figure 1. Clustered column bar graph showing growth performance of *Wallago attu* at different salinity levels (0-ppt, 4-ppt, 8-ppt and 12-ppt) during the 60-days experiment.

Table 2. Effect of different salinity levels on proximate composition of freshwater *Wallago attu* at different salinity levels (0-ppt, 4-ppt, 8-ppt and 12-ppt) during the 60-days experiment.

Parameters	Treatments			
	0-ppt	4-ppt	8-ppt	12-ppt
Crude Protein	67.60±0.17 ^a	68.28±0.24 ^a	70.44±0.74 ^b	71.54±0.15 ^b
Crude fat	4.62±0.07 ^a	5.32±0.11 ^b	6.30±0.15 ^c	7.26±0.10 ^d
Moisture	73.30±0.40 ^d	71.84±0.28 ^c	70.74±0.25 ^b	69.54±0.28 ^a
Ash	1.12±0.02 ^c	1.07±0.09 ^b	1.03±0.03 ^a	1.02±0.02 ^a

(a, b, c and d) Average in the same row having different superscripts significantly different at level (P≤0.05)

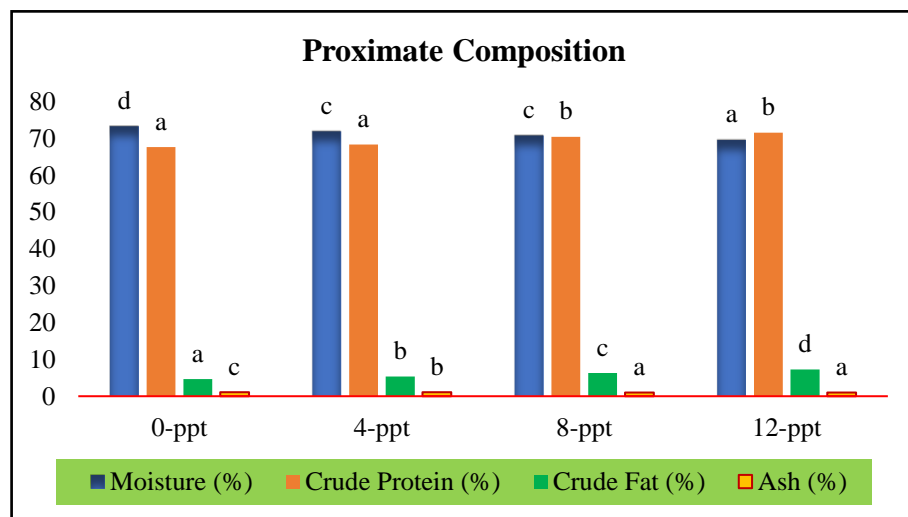


Figure 2. Proximate Composition Analysis of *Wallago attu* under Different Salinity Levels

EXPLORING THE IMPACT OF WEATHER FACTORS ON THE POPULATION TREND OF OXYCARENUS LAETUS KIRBY (DUSKY COTTON BUG) AND NATURAL ENEMIES IN DIFFERENT AGRO-ECOLOGICAL ZONES OF SINDH, PAKISTAN

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ABSTRACT

The research work was carried out in different agro-eco-zones of Sindh, Pakistan at the experimental field area of Cotton Agriculture Research Station at Tandojam, Sakrand, Kotdiji and Sarhad, 2019. The peak population of the Dusky cotton bug (DCB) was recorded at Tandojam. The high population of natural enemies like Chrysoperla, Orius, and Mirid bug was observed from July to September. The peak pest of pest observed averagely at Tandojam and Sakrand (49 to 23%). The abiotic factors like temperature and humidity averagely at Tandojam and Sakrand (32 to 50%) followed by Kotdiji and Sarhad (41 to 18%) recorded ideal for Chrysoperla, Orius, and Mirid bug. The abiotic factors were recorded as favorable for natural population multiplication like temperature and respectively. The weather factors noted optimum for spider population at Tandojam. It was concluded that the peak population of the pest was observed in different agro-eco-zones from September and October.

1. INTRODUCTION

The fiber of cotton is considered the best in the globe. The cotton-based textile industries are the world's biggest and are spread all over the world. It has been a yearly minimum of \$600 of an economic impact overall across the globe. It is a major crop and significant role in Pakistan's economy and enjoyed the position of "king of fibers" (Ashraf *et al.*, 2018). It has high-quality fiber and is cultivated in more than fifty countries of the world. (Azad *et al.*, 2011). It is a profitable fiber throughout the globe. Cotton is a perennial crop that is grown in the warm temperate region. The seeds are produced by cotton with different products like lint, oil, hulls, and animal food. (Ozyigit *et al.*, 2007).

The Dusky cotton bug has been observed on various plants throughout the year and survived on various alternate host plants and no cotton varieties were found resistant to this pest (Rind *et al.*, 2021). The *Oxycarenus laetus* is considered a critical pest of cotton and feeds on seeds of host plants (Sarmad *et al.*, 2020).

The cotton was infested by various insect pests which affected the yield and export quality. The DCB was firstly considered a minor pest and reached the status of a major insect (Henry, 1983). The *Oxycarenus* species was recorded as a major insect pest of cotton and caused an emerging threat to transgenic cotton. No cotton variety was recorded free from this pest. (Iqbal *et al.*, 2017). The DCB caused damage to the unripened seeds. The systemic insecticides were more used on Bt. cotton and the application of contact insecticides were reduced. As a result, the DCB population reached the major insect pest status of the cotton crop. Cottonseed weight, germination, and oil content were reduced by the Dusky cotton bug (Henry, 1983).

The lint quality of the cotton crop was declined by DCB and losses occurred from 10 to 40% in the production of the crop (Gahukar, 2006). Lint quality was Discoloration during the process. The cotton quality, as well as quantity both, were affected due to the *O. laetus* attack. (Srinivas *et al.*, 2004; Ahmed *et al.*, 2015). When sever attacked by DCB, its adults produced an unpleasant odor and also reduced the quality of the seed (Srinivas *et al.*,

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2004; Thangavlu, 2007). It was considered that *O. laetus* disturbed the lint as a result of discoloration during the ginning process. When heavy attacks happened by DCB; as a result, a reduction occurred in seed germination; oil content, and lint quality. When DCB caused a severe attack on the cotton crop, the adults of DCB were given an unpleasant smell during feeding on cotton seeds (Nakache, 1992; Thangavelu, 2007).

The Dusky cotton bug has attained the position of major insect pest of cotton because of its high growth rate which affected the rate and quality of the cotton crop. It also damaged the growth of the buds and flowers and the cash crop was badly affected by this pest. Sometimes it caused problems for cotton pickers due to itching. This pest should be controlled during these peak months and might cause considerable losses in the lint of cotton (Jawaid *et al.*, 2019).

The Dusky cotton bug has been reached major pest of the cotton crop which effected the value of lint and also seed. (Abbas *et al.*, 2015). It was considered cotton insect-like as the Dusky cotton bug was a new emerging pest and threatened the cotton crop in Pakistan. (Saleem *et al.*, (2018). The pest has reached the status of major and the Dusky cotton bug affected the lint and seed quality of cotton (Abbas *et al.*, 2015). *Oxycarenus laetus* has become recorded as an economic insect pest of cotton throughout the globe (Henry, 1983).

The main factors which kept the pest population below the economic threshold level by the natural enemies (Evans, 1998). The meaning of natural enemies is “natural balance” and the natural regulation effect on all insect populations to either smaller or larger levels. The natural enemies up setting the insect pest's population density out breaks forecasting. (Solangi *et al.*, 2005).

The outcomes revealed that the optimum temperature (20-36⁰C) and humidity (24-32%) were recorded as ideal for DCB multiplication on transgenic and non-transgenic cotton varieties (Rind, *et al.*, 2021). The pest numbers increased at the time of maturing of the crop. The temperature was noted positive correlation, whereas, rainfall and humidity were shown negative correlation with the Dusky cotton bug population (Qayyoom *et al.*, 2014). The temperature has importantly affected the infestation caused by this pest (Patil *et al.*, 1992). However, the temperature positively and rainfall

negatively correlated with pest infestation (Chaudhary *et al.*, 1999).

Natural enemies such as the Spider, *Dictyna* species were recorded in the cotton crop. The high numbers of pests and their natural enemies were noted from June to October. (Ashfaq *et al.*, 2011). The population fluctuation of natural enemies such as *Chrysoperla carnea* population was recorded in range (0.0 – 1.07/ plant) and touched the highest pest population on seventh 7th July population on 7th July with the low population on ninth June and twelfth August respectively. The population of Orius was observed in a range of (0 – 0.21/ plant) and the highest population was observed on 28th July and low on third and ninth September respectively. It touched the highest significant correlation between environmental factors and a positive correlation of temperature and humidity with natural enemies (Bhutto *et al.*, 2012).

Keeping in view the importance of the crop and the losses caused by different insect pests to it, the present research was aimed at studying the population dynamics of insect pests and their natural enemies on cotton. *Dictyna* sp. was observed on 10th July and a high population was noted from 19 to 29, September (Ashfaq, *et al.*, 2011).

2. MATERIALS AND METHODS

The research work was carried out at the experimental field area of Cotton Agriculture Research Station in different agro-eco-zones of Sindh, Pakistan Tandojam, Sakrand, Kotdiji, and Sarhad in 2019. The cotton variety (Sindh-1) was sown in April at (Tandojam, Sakrand) and in May at (Kotdiji and Sarhad) respectively. All possible agronomic practices were conducted as per routine. There was no application of insecticide practiced during the overall cropping period. The seed rate was used at 20 kg per hectare along with row to row and plant to plant distance of 2.5ft. X 1.0 ft. was maintained. The crop was raised under canal irrigation conditions. The data were taken in each district from one acre land for the cotton crop.

Sampling method

The observations were taken at fortnightly intervals from the first appearance of the pest till the harvesting of the crop. (Qayyoom, *et al.*, 2014). Thirty plants were selected randomly from different locations six plants

from each corner, and six plants from the center. The adults and nymphs were counted from the bottom, middle, and top bolls of the cotton crop early in the morning (Sanghi, *et al.*, 2014). The natural enemies were counted from the whole cotton plant. The weather data were taken from Accu weather mobile software.

Statistical analysis of the collected data

The data of the insect pest population was analyzed by using analysis of variance for significant difference and least significant difference (LSD) were tested and applied to compare the mean through statistical software student package Statistics-8.1 the USA.

3. RESULTS AND DISCUSSION

The results of the present research work (Table-1) showed that the first appearance of the pest was recorded on 13th July (0.47DCB/plant) in the Agro-eco zone of Hyderabad (Tandojam). The peak population of the pest was recorded on 19th October (31.30 DCB/ plant). The high population of natural enemies like Chrysoperla (24th August, 0.73/ plant), Orius (13th July, 1.17/ plant), Mirid bug (24th August, 1.30/ plant), and Spider (7th September, 0.79/ plant). The ideal weather factors like temperature (34.50 to 27^{0C}) and humidity (49 to 26%) were observed for pest multiplication. Likewise, the favorable abiotic factors were noted as favorable for natural enemies population like (Chrysoperla, Orius, and Mirid bug) such as temperature (33.50 to 35^{0C}) and humidity (58 to 39%). The weather factors were recorded as ideal for the spider population at Tandojam Temperature (34.50 to 26^{0C}) and humidity (49 to 26%). The data were statistically analyzed (table-1) and have shown significant effects on the Dusky cotton bug and natural enemies ($F=8/233$; $P1.12E-05$). Whereas, the pest was significantly correlated with temperature and humidity ($F=18.125$; $P=1.6E-05$).

The results of the research work (Table-2) have shown that the first appearance of the pest (0.23/ plant) was noted on 26th July with an average temperature (36.00^{0C}) and humidity (47.00%). The high pest population was observed on 20th September (30.80 DCB/ plant). The natural enemies were present throughout the crop period and high numbers were observed like Chrysoperla (23rd August, 30.80 DCB/ plant), Orius (23rd August, 1.43 DCB/ plant), Mirid bug (23rd August, 0.97DCB/ plant), and Spider (15th November, 0.95 DCB/ plant). The favorable abiotic factors for pest multiplication were temperature (33.50^{0C}) and humidity (32.00%). The ideal abiotic factors for natural enemies (Chrysoperla, Orius, and

Mirid bug) were noted as temperature (33 to 36^{0C}) and humidity (50 to 32%) and for spiders (19.50 to 30^{0C}; 20 to 28%) respectively. The analysis data (Table 4.2) revealed that the natural enemies were a significant effect on pests ($F=11.88$; $P>0.0001$). However, weather factors such as Temperature and humidity were significantly effective with the Dusky cotton bug ($F=9.85$; $P=0.00075$).

The results of the present research work (Table 3) indicated that the first appearance of the pest was recorded on (22nd August, 0.50 DCB/plant) and the peak population was noted on (17th October, 29.07 DCB/plant). Whereas, natural enemies were present overall crop period. A high population of natural enemies were recorded like Chrysoperla (22nd August 0.60/ plant), Orius (22nd August 1.13/ plant), and Spider (17th October 0.93/ plant). The abiotic factors like temperature ((34 to 27^{0C}), and humidity ((28 to 21%) have remained suitable for pest multiplication. The weather factors noted optimum for spider population averagely temperature and humidity at Kotdiji (37 to 29.50^{0C}; 41 to 19%), and for spiders (20.50 to 29.50^{0C}; 19 to 41%). The analyzed data (Table-3) depicted that the natural enemies were significantly ($F=16.63$; $P>0.0004$) effective with the Dusky cotton bug. While abiotic components like temperature and humidity were significantly ($F=6.19$; $P>0.0147$) effective with pests.

The research work results (Table-4) showed that the first emergence of pests (0.43 DCB/ plant) was observed on 21st August with an average temperature (35.50^{0C}) and humidity (40%). The high Pest (27.50 DCB/ plant) population was observed on 16th October followed by 2nd October (21.17 DCB/ plant), and 30th October (18.37 DCB/ plant) respectively. The natural enemies overall population and the high numbers were recorded like Chrysoperla (4th September 0.63/plant), Orius bug (18th September 1.20/ plant), Mirid bug (18th September 0.90/plant), and Spider (27th November 0.89/plant) on with average temperature (33.00^{0C}) and humidity (26.00%). The pest's overall seasonal average population (13.41/ plant) with temperature (29.44^{0C}) and humidity (26.50%) were recorded. The weather factors noted optimum for spider population averagely temperature and humidity at Sarhad Sarhad (35.50 to 31^{0C}; 40 to 18 %) and for spiders ((20 to 31^{0C}; 21 to 23%)

The analysis data (table4) were revealed that abiotic factors like as temperature and humidity ($F=9.82$; $P=0.0009$) significantly effective on the Dusky bug population and natural enemies were also effective with pest numbers ($F=15.03$; $P>0.00001$) were also

The Dusky cotton bug was found all over the year and survived in different host plants. Now, it attained the status of a major pest of the cotton crop. Iqbal *et al.*, (2017) reported that *Oxycarenus* species were noted as the key pest of cotton in Pakistan. Sarmad *et al.*, (2020) reported that *Oxycarenus laetus* has become an important pest of fiber crops. It also feeds on seeds, leaves, and stems of host plants. Henry (1983) evaluated that *Oxycarenus* species has reached the major pest position. Past time this pest appeared on cotton once when most of the cotton bolls were opened. The current results indicated that the peak population of the Dusky cotton bug was recorded at different Agro-eco-zones of Sindh like Tandojam (19, October, 31.30 DCB/plant) followed by Sakrand (20th September, 30.80 DCB/plant), Kotdiji (17th October, 29.07DCB/plant), Sarhad (16th October, 27.50 DCB/plant) respectively. The results were supported by Rind *et al.*, (2021) reported that the first appearance of the Dusky cotton bug was recorded on the cotton crop during the first week of September, and high numbers were recorded in October. The outcomes were also supported by Khan *et al.*, (2017) found that the peak population of the bug was observed in October. Shah *et al.*, (2016) investigated the Dusky cotton bug huge numbers were observed in September and October months. The pest population trend increased from September up to November months. Ashfaq *et al.*, (2011) evaluated that the maximum population of insect pests and natural enemies was recorded from June to October.

The natural enemies peak population were recorded in different agro-eco-zones of Sindh like Tandojam (*Chrysoperla* 24th August 0.73/plant, *Orius* 13th July 1.17/plant, Mirid bug 8th July 0.73/plant) followed by Sakrand (*Chrysoperla* 9th August 0.73/plant, *Orius* 23rd August 1.43/plant, Mirid bug 23rd August 0.97/plant), Kotdiji (*Chrysoperla* 22nd August 0.60/ plant, *Orius* 22th August, 1.13/plant, Mirid bug 5th September, 0.80/plant), Sarhad (*Chrysoperla* 4th September, 0.63/plant, *Orius* 18th September 1.20/plant, Mirid bug 4th September, 0.97/plant) respectively.

Whereas, a high spider population was recorded in different agro-eco-zones of Sindh like Tandojam (7th September 0.79/plant), Sakrand (15th November 0.95/plant), Kotdiji (14th November, 0.93/plant), Sarhad (*Spider* 27th November 0.89/plant). The findings were supported by Evans, (1998) said that the natural enemies maintained the pest population below the economic threshold level. Dhaka *et al.*, (2007) reported that natural enemies such as *Chrysoperla* sp., and the spider were observed in the cotton crop and played important role in the cotton agro eco-system (Dhaka *et al.*, 2007). Wang, *et al.*, (1996) observed that beneficial insects like Green lacewing insect, and *Orius* have been checked in the insect pests population in cotton crop. Ashfaq *et al.*, (2011) evaluated that the maximum population of insect pests and natural enemies was recorded from June to October. Dhaken *et al.*, (2007) described that *Chrysoperla carnea* Stephens and spiders were noted as natural enemies of major insect pests of the cotton crop and played a vital role in the cotton ecosystem.

The weather factors in different Agro-eco-zones of Sindh were recorded as ideal for the Dusky cotton bug multiplication like the temperature at Tandojam (34.50 to 27^oC), Sakrand (34 to 29.50^oC), Kotdiji (34 to 27^oC), Sarhad (33 to 21.50^oC). Whereas, humidity like Tandojam humidity (26 to 49%), Sakrand (47 to 23%), Kotdiji (28 to 21%), and Sarhad (26 to 23 %) respectively. The findings were supported by Rind *et al.*, (2021) who observed that the favorable temperature (20-36^oC) and humidity (24-32%) for the reproduction of the Dusky cotton bug was observed both on non-Bt. and Bt. cotton. Qayyoun *et al.*, (2014) reported that humidity negatively correlated with the Dusky cotton bug and temperature positively correlated with it. Ashfaq *et al.*, (2011) reported that *Dictyna* sp was observed on 10th July and a high population was noted from 19 to 29, September. (Ashfaq, *et al.*, 2011)

The abiotic factors in different agro-eco-zones of Sindh recorded favorable for natural enemies population multiplication like temperature and humidity at Tandojam (32 to 36^oC; 49 to 58%), Sakrand (36 to 33^oC; 50 to 32%), Kotdiji (37 to 29.50^oC; 41 to 19%), and Sarhad (35.50 to 31^oC; 40 to 18 %) respectively. The findings were supported by the weather factors noted optimum for spider population averagely temperature and humidity at Tandojam (34.5 to 26^oC; 49 to 26%) followed by Sakrand (19.50 to 30^oC; 20 to 28%), Kotdiji

(20.50 to 29.50^{OC}: 19 to 41%), and Sarhad (20 to 31^{OC}: 21 to 23%) respectively.

4. CONCLUSION

It was concluded that the Dusky cotton bug population was recorded during July on cotton crop in agro-eco-zones of Sindh like Tandojam and Sakrand with low population due to high temperature and humidity. The peak population of the pest was observed during September and October. The Dusky cotton bug was negatively correlated with weather factors like temperature and humidity. The high population of natural enemies was observed in different agro-eco-zones of Sindh from July to September. Whereas, Spider's maximum population was noted from September to November. The natural enemies were positively correlated with weather factors like temperature and humidity except for the spider which showed a negative correlation.

5. CONFLICT OF INTERESTS

All authors have declared that there is no conflict of interest regarding this publication.

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Figure 1. Map of Sindh province showed the different agro-eco zones of Sindh.

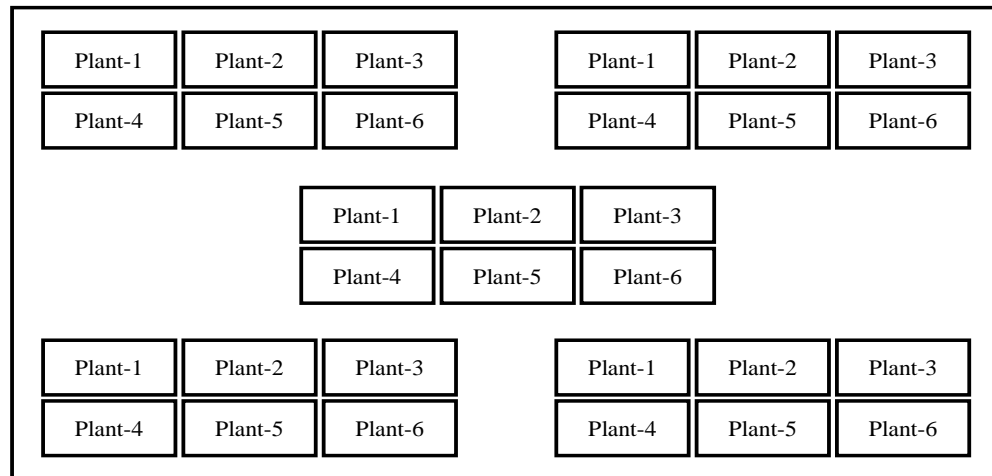


Figure 2. Mario method used for DCB sampling described by (Carolyn *et al.*, 2004)
Statistical analysis of the collected data

Table 1. Population dynamics of dusky cotton bug and effect of bio-ecological factors on its population in Agro-eco zone of Hyderabad (Tandojam)

Dated	No. of DCB/ plant	Natural enemies/plant				Overall Natural enemies	Av. Temp.	Abiotic factors
		Chrysopa	Orius	Mirid bug	Spider	Mean±S.E		Humidity%
13.7.2019	0.47	0.53	1.17	0.87	0.40	0.81±0.02	35.50	50.00
27-07-2019	0.60	0.43	0.90	0.60	0.90	0.81±0.06	36.00	58.00
08-07-20 ⁹	1.00	0.67	1.07	0.73	0.40	0.81±0.03	32.00	57.00
24-08-2019	2.63	0.73	0.87	0.6	0.50	0.8±0.02	33.00	54.00
07-09-2019	13.48	0.72	0.92	0.65	0.79	0.74±0.03	34.50	49.00
21-09-2019	27.67	0.49	0.65	0.47	0.41	0.54±0.03	33.00	39.00
05-10-2019	29.07	0.30	0.6	0.37	0.67	0.54±0.27	30.00	33.00
19-10-2019	31.30	0.35	0.68	0.51	0.68	0.61±0.02	27.00	26.00
02-11-2019	12.77	0.36	0.51	0.49	0.83	0.6±0.02	26.00	26.00
Mean±S.E	13.22±4.36	0.51±0.05	0.82±0.07	0.59±0.05	0.64±0.07	0.71±0.04	31.89±1.19	43.56±4.28

Table 2. Population dynamics of Dusky cotton bug and effect of bio-ecological factors on its population in Agro eco zone of Shaheed Benazirabad (Sakrand)

Dated	No. of DCB/plant	No. of Natural enemies/ plant				Overall Natural enemies	Abiotic factors	
		Chrysopa	Orius	Mirid bug	Spider	Mean±S.E	Av. Temp.	Humidity%
26/07/2019	0.23	0.60	1.23	0.77	0.40	0.90±0.02	36.00	47.00
09-08-2019	0.73	0.43	1.33	0.80	0.23	0.87±0.02	34.00	50.00
23-08-2019	1.60	0.57	1.43	0.97	0.33	0.93±0.03	33.00	44.00
06-09-2019	27.73	0.53	0.76	0.80	0.50	0.80±0.02	34.00	47.00
20-09-2018	30.80	0.43	0.70	0.80	0.37	0.71±0.03	33.50	32.00
04-10-2019	20.10	0.27	0.53	0.29	0.50	0.51±0.3	30.00	28.00
18-10-2019	22.86	0.34	0.62	0.34	0.62	0.61±0.02	29.50	20.00
01-11-2019	14.43	0.31	0.31	0.28	0.79	0.52±0.02	29.00	23.00
15-11-2019	10.37	0.47	0.63	0.63	0.95	0.72±0.06	19.50	20.00
Mean±S.E	14.32±3.94	0.44±0.04	0.84±0.13	0.63±0.09	0.52±0.08	0.73±0.06	30.94±1.64	34.56±4.16

Table 3. Population dynamics of Dusky cotton bug and effect of bio-ecological factors on its population in Agro-eco zone of Khairpur (Kotdiji)

Dated	No. of DCB/ plant	No. of Natural enemies/plant				Overall Natural enemies	Abiotic factors	
		Chrysopa	Orius	Mirid bug	Spider	Mean±S.E	Av. Temp.	Humidity%
22-8-2019	0.50	0.60	1.13	0.73	0.17	0.79±0.24	33.00	41.00
05-09-2019	3.93	0.40	0.77	0.80	0.40	0.69±0.13	37.00	40.00
19-09-2019	16.53	0.30	0.93	0.53	0.67	0.66±0.11	34.00	28.00
03-10-2019	24.77	0.33	0.87	0.73	0.60	0.70±0.11	31.00	27.00
17-10-2019	29.07	0.30	0.70	0.60	0.93	0.69±0.12	29.50	19.00
31-10-2019	26.30	0.27	0.57	0.50	0.90	0.63±0.12	27.00	21.00
14-11-2019	15.33	0.23	0.57	0.50	0.93	0.59±0.12	19.50	18.00
28-11-2019	10.17	0.20	0.47	0.40	0.79	0.48±0.10	20.50	22.00
Mean±S.E	15.83±3.72	0.31±0.03	0.75±0.08	0.60±0.05	0.67±0.10	0.65±0.02	28.94±2.22	27.00±3.20

Table 4. Population dynamics of Dusky cotton bug and effect of bio-ecological factors on its population in Agro-eco zone of Ghotki (Sarhad)

Dated	No.of DCB/ plant	No. of Natural enemies/plant				Overall Natural enemies	Abiotic factors	
		Chrysopa	Orius	Mirid bug	Spider	Mean±S.E	Av. Temp.	Humidity %
21-8-2019	0.43	0.50	1.07	0.67	0.17	0.70±0.03	35.50	40.00
04-09-2019	1.87	0.63	1.10	0.97	0.37	0.83±0.02	34.50	37.00
18-09-2019	10.43	0.47	1.20	0.90	0.47	0.79±0.03	33.00	26.00
02-10-2019	21.17	0.50	0.97	0.73	0.60	0.79±0.04	32.00	24.00
16-10-2019	27.50	0.40	0.70	0.73	0.85	0.76±0.30	31.00	18.00
30-10-2019	18.37	0.27	0.47	0.43	0.77	0.52±0.25	28.00	23.00
13-11-2019	14.07	0.20	0.47	0.40	0.79	0.48±0.02	21.50	23.00
27-11-2019	12.83	0.25	0.56	0.54	0.89	0.63±0.02	20.00	21.00
Mean ±S.E	13.41±3.76	0.42±0.06	0.85±0.12	0.69±0.08	0.57±0.09	0.70±0.05	29.44±2.06	26.50±2.76

Short communication

A CHECKLIST OF THE AVIAN SPECIES OF SAIDPUR UPAZILA OF BANGLADESH

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ABSTRACT

To understand birds' behaviour besides the residence more or less dominant as a whole. Bangalipur union of Saidpur is composed of many medium-sized trees with bushes that are a good shelter for those birds. This survey was carried out from 2019 to 2021. Quantitative, qualitative, and survey methods were carried out for this study. A total of 73 bird species of 39 families were identified, where 33 were very common, 18 common, 8 uncommon, 9 rare, and 5 few. In addition, the behaviour of birds found with winter visitors (7 species), summer visitors (3), local migrants (2), and residents (61). In the Ardeidae family, there was the highest number of birds (6 species) and then Cuculidae (5 species). The species richness of birds in the cantonment (protected) area revealed that it is safe for all resident birds.

1. INTRODUCTION

Bangladesh sustains diverse avian composition compared to its small land area (Khan, 1982; Sarker & Sarker 1988; Khan, 2008; Siddiqui *et al.*, 2008). Siddiqui *et al.* (2008) detected more than 650 bird species (Siddiqui *et al.*, 2008). As a cantonment, Saidpur upazila under the Nilphamari district of Bangladesh is a notably protected bird habitat in the country. This cantonment has a vast area with many long trees, and it has a Cantonment Public School and College with two teachers' quarters which have many bird species (Kabir, 2012; Kabir *et al.*, 2020). There is not enough data on the birds of cantonment areas of Bangladesh. This paper provides the list of all the birds observed during the present study period in the Bangalipur union of Saidpur upazila.

Study area and Ecological features

Bangalipur union under the Nilphamari district of Bangladesh ranges 41.44 square kilometres and has a population of 21,379 (Figure 1). Saidpur Cantonment is nearby this study area and covers ponds, crop fields, grass, and fallow lands.

During observation of the study period, birds consumed nectar, pulp, and ripe fruits of many local and exotic plant species. Since the cantonment is protected, many birds (eg. open-billed stork, Indian pied myna, house crow, and little egret) could play successful breeding performances. Trees, scrub forests, undergrowth, and water bodies constitute unique habitats for the birds in the cantonment area. The profound habitat for the birds in this area is Cantonment Public School and College, EME Centre and School, Bangladesh Army University of Science and Technology (BAUST), and Saidpur upazila.

2. MATERIALS AND METHODS

The present checklist focused on long-time fieldwork from 2019 to 2021 from early in the morning (5:00-8:00 hr) and evening (16:00-18:00 hr). During the observations, walk through the existing roads and trails of the crop fields, and sometimes their singing and call helped for proper identification. Vixen binoculars (palm-sized compact binoculars) (6X18) and field-guides (Ali & Ripley, 1983; Grimmitt *et al.*, 2001) helped in the field for identification of the recorded birds. Photographs from

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a digital camera (600D) assisted in a clear conception of the same type of different species of this area.

The relative frequency of the presence of the species assessed as very common (VC), seen during 80-100% of the field visits; common (C): 50-79% of the field visits; uncommon (UC): 20-49% of the field visit; few (F): met less than 19% and rare (R): found occasionally (Khan, 1982). Scientific and common names followed the IUCN Bangladesh 2015. All species have shown into residents, winter visitors, summer visitors, and local migrants (Table 1).

3. RESULTS AND DISCUSSION

Seventy-three species of birds representing 39 families were observed from the Bangalipur upazila of Saidpur from 2019 to 2021, of which 41 (56.16%) species were non-passerine and 32(43.84%) passerine (Table 1). Resident birds (83.56%) constituted the dominant avifaunal composition in the area, and local migrants (2.74%) were the lowest (Figure 2). Very common and few were the highest (45.21%) and the lowest (6.85%) composition accordingly (Figure 3). Among 73 species, *Pelargopsis capensis*, *Ceryle rudis*, *Butorides striata*, *Terpsiphone paradisi*, *Rhipidura albicollis*, *Upupa epops*, *Micropternus brachyurus*, *Coracina melanoptera*, and *Phoenicurus ochruros* were rare birds of this union. These birds were residents, and only *Upupa epops* and *Phoenicurus ochruros* were winter visitors, and *Coracina melanoptera* was a summer visitor. This union was a natural breeding ground for many bird species especially open-billed stork, Indian pied myna, house crow, and little egret. The breeding ecology and habits of these species were little known, and the population is also declining due to urbanization.

Though the Bangalipur union is a small area its birdlife is quite rich and diverse with 73 species. Many conservation programs especially 'Setubondhon' play a significant role in these birds. Kabir (2012) enlisted 14 species in the cantonment area and its teachers' quarters-2 of the Cantonment Public School and College showed 30 species of birds (Kabir et al., 2020). This study denotes 42 new species of birds than the previous works. This Bangalipur union was in the cantonment area so 18 species of birds were identified previously. Only one species of bird fork-tailed drongo (*Dicrurus adsimilis*) was misidentified due to the more or less the same appearance as the black drongo (*Dicrurus macrocercus*)

(Table 2). From the last study (Kabir et al., 2020) the birds were 30 (though those places were limited) but comparatively at present 73 species around this area are of course remarkable. This list will influence the reconfirmation and new identification of available bird species in this area by other researchers.

4. CONCLUSION

In conclusion, the study reveals a diverse bird species composition in the Bangalipur union of Saidpur, with 73 species identified. The area's vegetation, including medium-sized trees and bushes, provides suitable habitats for birds. The presence of winter and summer visitors, along with resident species, highlights the area's importance for bird conservation. The protected cantonment area plays a crucial role in supporting the richness of resident bird species.

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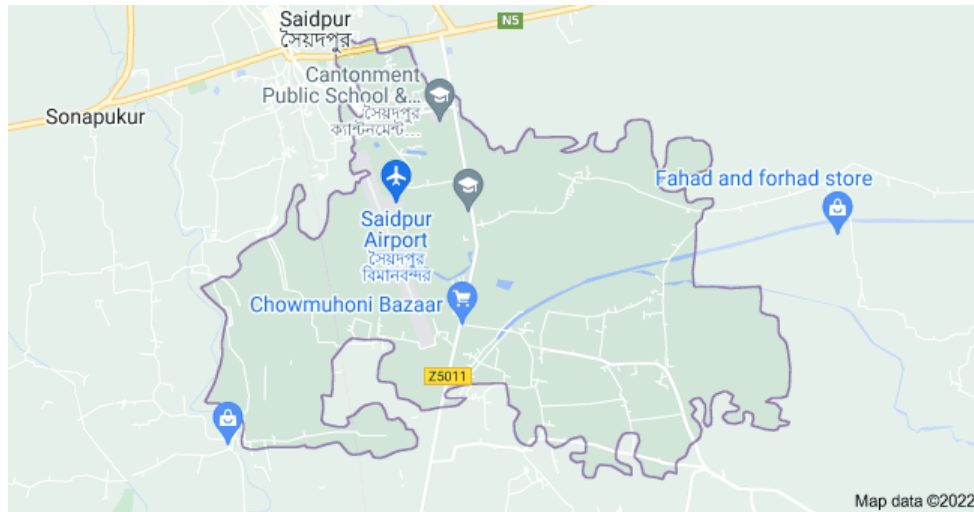


Figure 1. Map of Bangalipur union of Saidpur upazila

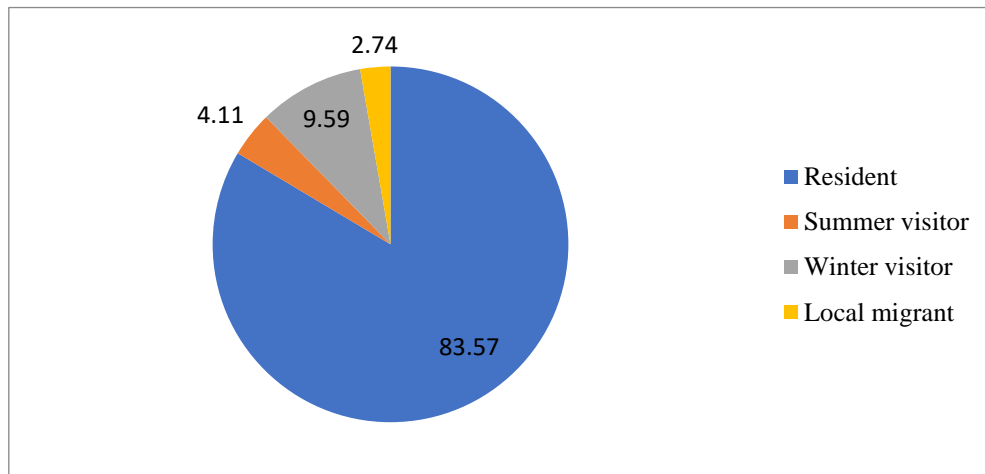


Figure 2. Union status of the birds in Bangalipur, Saidpur

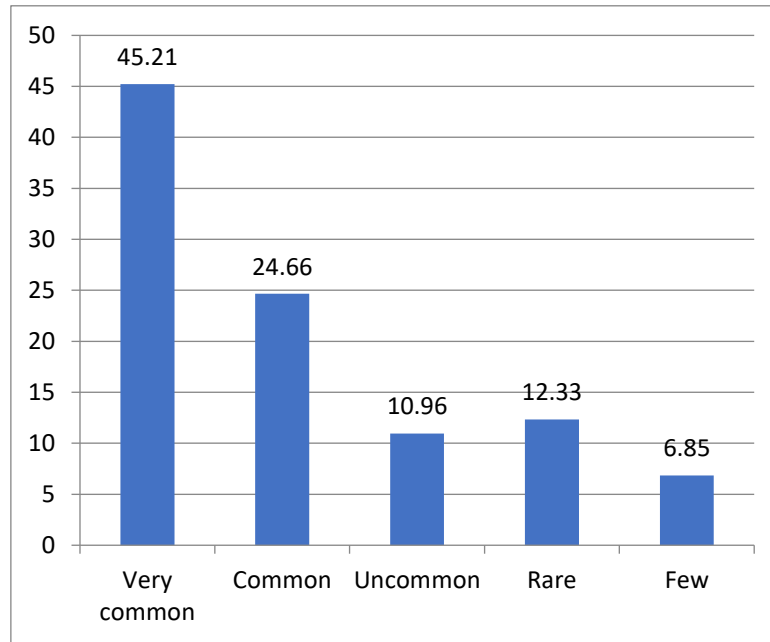


Figure 3. Relative frequency of the presence of the birds in Bangalipur

Table 1. Observed bird species in Bangalipur union of Saidpur, Bangladesh

Sl	Names	Saidpur status	Country Status
	Cuculidae		
1	Asian koel (<i>Eudynamys scolopaceus</i>)	F	Local migrant
2	Pied crested cuckoo (<i>Clamator jacobinus</i>)	F	Summer visitor
3	Indian cuckoo (<i>Cuculus micropterus</i>)	C	Summer visitor
4	Common hawk-cuckoo (<i>Hierococcyx varius</i>)	C	Resident
5	Greater coucal (<i>Centropus sinensis</i>)	VC	Resident
	Alcedinidae		
6	White-throated kingfisher (<i>Halcyon smyrnensis</i>)	VC	Resident
7	Common kingfisher (<i>Alcedo atthis</i>)	VC	Resident
8	Storkbilled kingfisher (<i>Pelargopsis capensis</i>)	R	Resident
9	Pied kingfisher (<i>Ceryle rudis</i>)	R	Resident
	Meropidae		
10	Green bee-eater (<i>Merops orientalis</i>)	C	Resident
11	Blue-tailed bee-eater (<i>Merops philippinus</i>)	C	Local migrant
	Motacillidae		
12	White Wagtail (<i>Motacilla alba</i>)	C	Winter visitor
	Nectariniidae		
13	Purple sunbird (<i>Cinnyris asiaticus</i>)	C	Resident
14	Purple-rumped sunbird (<i>Leptocoma zeylonica</i>)	UC	Resident
	Ciconiidae		
15	Asian openbill stork (<i>Anastomus oscitans</i>)	VC	Resident
	Ardeidae		

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16	Indian pond heron (<i>Ardeola grayii</i>)	VC	Resident
17	Striated heron (<i>Butorides striata</i>)	R	Resident
18	Cinnamon bittern (<i>Ixobrychus cinnamomeus</i>)	C	Resident
19	Little egret (<i>Egretta garzetta</i>)	VC	Resident
20	Cattle egret (<i>Bubulcus ibis</i>)	VC	Resident
21	Black-crowned night heron (<i>Nycticorax nycticorax</i>)	VC	Resident
	Pycnonotidae		
22	Red-vented Bulbul (<i>Pycnonotus cafer</i>)	VC	Resident
23	Red-whiskered Bulbul (<i>Pycnonotus jacosus</i>)	UC	Resident
	Monarchidae		
24	Indian paradise flycatcher (<i>Terpsiphone paradisi</i>)	R	Resident
	Oriolidae		
25	Black-hooded Oriole (<i>Oriolus xanthornus</i>)	VC	Resident
	Cisticolidae		
26	Common tailorbird (<i>Orthotomus sutorius</i>)	VC	Resident
	Paridae		
27	Great tit (<i>Parus major</i>)	C	Resident
	Passeridae		
28	House sparrow (<i>Passer domesticus</i>)	VC	Resident
29	Eurasian tree sparrow (<i>Passer montanus</i>)	VC	Resident
30	Weaver bird (<i>Ploceus philippinus</i>)	C	Resident
	Psittaculidae		
31	Rose-ringed parakeet (<i>Psittacula krameri</i>)	VC	Resident
	Columbidae		
32	Blue rock-pigeon (<i>Columba livia</i>)	VC	Resident
33	Spotted dove (<i>Spilopelia chinensis</i>)	VC	Resident
34	Eurasian collared dove (<i>Streptopelia decaocto</i>)	F	Resident
	Rhipiduridae		
35	White-throated fantail flycatcher (<i>Rhipidura albicollis</i>)	R	Resident
	Sturnidae		
36	Common myna (<i>Acridotheres tristis</i>)	C	Resident
37	Jungle myna (<i>Acridotheres fuscus</i>)	UC	Resident
38	Grey-headed myna (<i>Sturnia malabarica</i>)	VC	Resident
39	Pied myna (<i>Gracupica contra</i>)	VC	Resident
	Leiothrichidae		
40	Jungle babbler (<i>Turdoides striata</i>)	VC	Resident
	Upupidae		
41	Hoopoe (<i>Upupa epops</i>)	R	Winter visitor
	Picidae		
42	Black-rumped flameback woodpecker (<i>Dinopium benghalense</i>)	VC	Resident
43	Rufous woodpecker (<i>Micropternus brachyurus</i>)	R	Resident
44	Fulvous-breasted woodpecker (<i>Dendrocopos macei</i>)	VC	Resident
45	Streak-throated woodpecker (<i>Picus xanthopygaeus</i>)	C	Resident
	Megalaimidae		
46	Blue-throated barbet (<i>Megalaima asiatica</i>)	VC	Resident
47	Coppersmith barbet (<i>Megalaima haemacephala</i>)	VC	Resident
	Apodidae		

48	House swift (<i>Apus nipalensis</i>)	VC	Resident
49	Asian palm swift (<i>Cypsiurus balasiensis</i>)	C	Resident
	Hirundinidae		
50	Barn swallow (<i>Hirundo rustica</i>)	VC	Resident
	Rallidae		
51	White-breasted waterhen (<i>Amaurornis phoenicurus</i>)	VC	Resident
	Anatidae		
52	Lesser whistling duck (<i>Dendrocygna javanica</i>)	F	Resident
	Accipitridae		
53	Black kite (<i>Milvus migrans</i>)	VC	Resident
54	Black-shouldered kite (<i>Elanus caeruleus</i>)	C	Resident
	Falconidae		
55	Common kestrel (<i>Falco tinnunculus</i>)	C	Winter visitor
	Phalacrocoracidae		
56	Little cormorant (<i>Phalacrocorax niger</i>)	VC	Resident
	Scolopacidae		
57	Common sandpiper (<i>Actitis hypoleucos</i>)	UC	Winter visitor
	Rostratulidae		
58	Greater painted snipe (<i>Rostratula benghalensis</i>)	F	Resident
	Laniidae		
59	Brown shrike (<i>Lanius cristatus</i>)	VC	Winter visitor
60	Long-tailed shrike (<i>Lanius schach</i>)	C	Resident
	Aegithinidae		
61	Common iora (<i>Aegithinia tiphia</i>)	UC	Resident
	Campephagidae		
62	Black-headed cuckoo-shrike (<i>Coracina melanoptera</i>)	R	Summer visitor
	Corvidae		
63	House crow (<i>Corvus splendens</i>)	VC	Resident
64	Eastern jungle crow (<i>Corvus leuclantii</i>)	C	Resident
65	Rufous treepie (<i>Dendrocitta vagabunda</i>)	VC	Resident
	Dicruridae		
66	Black drongo (<i>Dicrurus macrocercus</i>)	VC	Resident
	Muscicapidae		
67	Black Redstart (<i>Phoenicurus ochruros</i>)	R	Winter visitor
68	Red-throated flycatcher (<i>Ficedula albicilla</i>)	C	Winter visitor
69	Oriental magpie-robin (<i>Copsychus saularis</i>)	UC	Resident
	Estrildidae		
70	Scaly-breasted munia (<i>Lonchura punctulata</i>)	UC	Resident
71	Indian silverbill (<i>Euodice malabarica</i>)	UC	Resident
	Strigidae		
72	Spotted owlet (<i>Athene brama</i>)	VC	Resident
	Tytonidae		
73	Barn owl (<i>Tyto alba</i>)	C	Resident

VC-very common; C-common; UC-uncommon; F-few; R-rare

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Table 2. Bird species of earlier checklists which are not included in the present list

Sl	Family	Names	Source	Remarks
1	Cuculidae	Asian koel (<i>Eudynamys scolopaceus</i>)	Kabir, 2012; Kabir <i>et al.</i> , 2020	Not observed during the study period
2	Cuculidae	Pied crested cuckoo (<i>Clamator jacobinus</i>)	Kabir, 2012; Kabir <i>et al.</i> , 2020	Not observed during the study period
3	Cuculidae	Indian cuckoo (<i>Cuculus micropterus</i>)	Kabir, 2012; Kabir <i>et al.</i> , 2020	Not observed during the study period
4	Cuculidae	Common hawk-cuckoo (<i>Hierococcyx varius</i>)	Kabir, 2012	Mentioned this species previously
5	Cuculidae	Greater coucal (<i>Centropus sinensis</i>)	Kabir <i>et al.</i> , 2020	Mentioned this species previously
6	Alcedinidae	White-throated kingfisher (<i>Halcyon smyrnensis</i>)	Kabir <i>et al.</i> , 2020	Mentioned this species previously
7	Alcedinidae	Common kingfisher (<i>Alcedo atthis</i>)	Kabir, 2012; Kabir <i>et al.</i> , 2020	Not observed during the study period
8	Alcedinidae	Storkbilled kingfisher (<i>Pelargopsis capensis</i>)	Kabir, 2012; Kabir <i>et al.</i> , 2020	Not observed during the study period
9	Alcedinidae	Pied kingfisher (<i>Ceryle rudis</i>)	Kabir, 2012; Kabir <i>et al.</i> , 2020	Not observed during the study period
10	Meropidae	Green bee-eater (<i>Merops orientalis</i>)	Kabir, 2012; Kabir <i>et al.</i> , 2020	Not observed during the study period
11	Meropidae	Blue-tailed bee-eater (<i>Merops philippinus</i>)	Kabir, 2012; Kabir <i>et al.</i> , 2020	Not observed during the study period
12	Motacillidae	White Wagtail (<i>Motacilla alba</i>)	Kabir, 2012; Kabir <i>et al.</i> , 2020	Not observed during the study period
13	Nectariniidae	Purple sunbird (<i>Cinnyris asiaticus</i>)	Kabir, 2012; Kabir <i>et al.</i> , 2020	Not observed during the study period
14	Nectariniidae	Purple-rumped sunbird (<i>Leptocoma zeylonica</i>)	Kabir, 2012; Kabir <i>et al.</i> , 2020	Not observed during the study period
15	Ciconiidae	Asian openbill stork (<i>Anastomus oscitans</i>)	Kabir, 2012; Kabir <i>et al.</i> , 2020	Not observed during the study period
16	Ardeidae	Indian pond heron (<i>Ardeola grayii</i>)	Kabir <i>et al.</i> , 2020	Mentioned this species previously
17	Ardeidae	Striated heron (<i>Butorides striata</i>)	Kabir, 2012; Kabir <i>et al.</i> , 2020	Not observed during the study period

18	Ardeidae	Cinnamon bittern (<i>Ixobrychus cinnamomeus</i>)	Kabir, 2012; Kabir <i>et al.</i> , 2020	Not observed during the study period
19	Ardeidae	Little egret (<i>Egretta garzetta</i>)	Kabir, 2012; Kabir <i>et al.</i> , 2020	Not observed during the study period
20	Ardeidae	Cattle egret (<i>Bubulcus ibis</i>)	Kabir, 2012; Kabir <i>et al.</i> , 2020	Not observed during the study period
21	Ardeidae	Black-crowned night heron (<i>Nycticorax nycticorax</i>)	Kabir, 2012; Kabir <i>et al.</i> , 2020	Not observed during the study period
22	Pycnonotidae	Red-vented Bulbul (<i>Pycnonotus cafer</i>)	Kabir <i>et al.</i> , 2020	Mentioned this species previously
23	Pycnonotidae	Red-whiskered Bulbul (<i>Pycnonotus jacosus</i>)	Kabir, 2012; Kabir <i>et al.</i> , 2020	Not observed during the study period
24	Monarchidae	Indian paradise flycatcher (<i>Terpsiphone paradisi</i>)	Kabir, 2012; Kabir <i>et al.</i> , 2020	Not observed during the study period
25	Oriolidae	Black-hooded Oriole (<i>Oriolus xanthornus</i>)	Kabir <i>et al.</i> , 2020	Mentioned this species previously
26	Cisticolidae	Common tailorbird (<i>Orthotomus sutorius</i>)	Kabir <i>et al.</i> , 2020	Mentioned this species previously
27	Paridae	Great tit (<i>Parus major</i>)	Kabir <i>et al.</i> , 2020	Mentioned this species previously
28	Passeridae	House sparrow (<i>Passer domesticus</i>)	Kabir, 2012; Kabir <i>et al.</i> , 2020	Mentioned this species previously
29	Passeridae	Eurasian tree sparrow (<i>Passer montanus</i>)	Kabir <i>et al.</i> , 2020	Mentioned this species previously
30	Passeridae	Weaver bird (<i>Ploceus philippinus</i>)	Kabir, 2012; Kabir <i>et al.</i> , 2020	Mentioned this species previously Not observed during the study period
31	Psittaculidae	Rose-ringed parakeet (<i>Psittacula krameri</i>)	Kabir, 2012; Kabir <i>et al.</i> , 2020	Not observed during the study period
32	Columbidae	Blue rock-pigeon (<i>Columba livia</i>)	Kabir, 2012; Kabir <i>et al.</i> , 2020	Not observed during the study period
33	Columbidae	Spotted dove (<i>Spilopelia chinensis</i>)	Kabir, 2012; Kabir <i>et al.</i> , 2020	Mentioned this species previously
34	Columbidae	Eurasian collared dove (<i>Streptopelia decaocto</i>)	Kabir, 2012; Kabir <i>et al.</i> , 2020	Not observed during the study period
35	Rhipiduridae	White-throated fantail flycatcher (<i>Rhipidura albicollis</i>)	Kabir, 2012; Kabir <i>et al.</i> , 2020	Not observed during the study period

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36	Sturnidae	Common myna (<i>Acridotheres tristis</i>)	Kabir, 2012; Kabir <i>et al.</i> , 2020	Mentioned this species previously
37	Sturnidae	Jungle myna (<i>Acridotheres fuscus</i>)	Kabir, 2012; Kabir <i>et al.</i> , 2020	Not observed during the study period Mentioned this species previously
38	Sturnidae	Grey-headed myna (<i>Sturnia malabarica</i>)	Kabir, 2012; Kabir <i>et al.</i> , 2020	Mentioned this species previously
39	Sturnidae	Pied myna (<i>Gracupica contra</i>)	Kabir, 2012; Kabir <i>et al.</i> , 2020	Mentioned this species previously
40	Leiothrichidae	Jungle babbler (<i>Turdoides striata</i>)	Kabir, 2012; Kabir <i>et al.</i> , 2020	Not observed this species previously Mentioned this species previously
41	Upupidae	Hoopoe (<i>Upupa epops</i>)	Kabir, 2012; Kabir <i>et al.</i> , 2020	Not observed during the study period
42	Picidae	Black-rumped flameback woodpecker (<i>Dinopium benghalense</i>)	Kabir, 2012; Kabir <i>et al.</i> , 2020	Not observed this species previously Mentioned this species previously
43	Picidae	Rufous woodpecker (<i>Micropternus brachyurus</i>)	Kabir, 2012; Kabir <i>et al.</i> , 2020	Not observed during the study period
44	Picidae	Fulvous-breasted woodpecker (<i>Dendrocopos macei</i>)	Kabir, 2012; Kabir <i>et al.</i> , 2020	Not observed during the study period
45	Picidae	Streak-throated woodpecker (<i>Picus xanthopygaeus</i>)	Kabir, 2012; Kabir <i>et al.</i> , 2020	Not observed this species previously Mentioned this species previously
46	Megalaimidae	Blue-throated barbet (<i>Megalaima asiatica</i>)	Kabir, 2012; Kabir <i>et al.</i> , 2020	Not observed this species previously Mentioned this species previously
47	Megalaimidae	Coppersmith barbet (<i>Megalaima haemacephala</i>)	Kabir, 2012; Kabir <i>et al.</i> , 2020	Mentioned this species previously Not observed this species previously
48	Apodidae	House swift (<i>Apus nipalensis</i>)	Kabir, 2012; Kabir <i>et al.</i> , 2020	Not observed during the study period
49	Apodidae	Asian palm swift (<i>Cypsiurus balasiensis</i>)	Kabir, 2012; Kabir <i>et al.</i> , 2020	Not observed during the study period
50	Hirundinidae	Barn swallow (<i>Hirundo rustica</i>)	Kabir, 2012;	Not observed during the study period

			Kabir <i>et al.</i> , 2020	
51	Rallidae	White-breasted waterhen (<i>Amaurornis phoenicurus</i>)	Kabir, 2012; Kabir <i>et al.</i> , 2020	Not observed during the study period Mentioned this species previously
52	Anatidae	Lesser whistling duck (<i>Dendrocygna javanica</i>)	Kabir, 2012; Kabir <i>et al.</i> , 2020	Not observed during the study period
53	Accipitridae	Black kite (<i>Milvus migrans</i>)	Kabir, 2012; Kabir <i>et al.</i> , 2020	Not observed during the study period
54	Accipitridae	Black-shouldered kite (<i>Elanus caeruleus</i>)	Kabir, 2012; Kabir <i>et al.</i> , 2020	Not observed during the study period
55	Falconidae	Common kestrel (<i>Falco tinnunculus</i>)	Kabir, 2012; Kabir <i>et al.</i> , 2020	Not observed during the study period
56	Phalacrocoracidae	Little cormorant (<i>Phalacrocorax niger</i>)	Kabir, 2012; Kabir <i>et al.</i> , 2020	Not observed during the study period
57	Scolopacidae	Common sandpiper (<i>Actitis hypoleucos</i>)	Kabir, 2012; Kabir <i>et al.</i> , 2020	Not observed during the study period
58	Rostratulidae	Greater painted snipe (<i>Rostratula benghalensis</i>)	Kabir, 2012; Kabir <i>et al.</i> , 2020	Not observed during the study period
59	Laniidae	Brown shrike (<i>Lanius cristatus</i>)	Kabir, 2012; Kabir <i>et al.</i> , 2020	Not observed during the study period Mentioned this species previously
60	Laniidae	Long-tailed shrike (<i>Lanius schach</i>)	Kabir, 2012; Kabir <i>et al.</i> , 2020	Mentioned this species previously Not observed during the study period
61	Aegithinidae	Common iora (<i>Aegithinia tiphia</i>)	Kabir, 2012; Kabir <i>et al.</i> , 2020	Not observed during the study period Mentioned this species previously
62	Campephagidae	Black-headed cuckoo-shrike (<i>Coracina melanoptera</i>)	Kabir, 2012; Kabir <i>et al.</i> , 2020	Not observed during the study period
63	Corvidae	House crow (<i>Corvus splendens</i>)	Kabir, 2012; Kabir <i>et al.</i> , 2020	Mentioned this species previously
64	Corvidae	Eastern jungle crow (<i>Corvus leuallantii</i>)	Kabir, 2012; Kabir <i>et al.</i> , 2020	Not observed during the study period Mentioned this species previously

Bird Checklist of Saidpur Upazila, Bangladesh

65	Corvidae	Rufous treepie (<i>Dendrocitta vagabunda</i>)	Kabir, 2012; Kabir <i>et al.</i> , 2020	Mentioned this species previously
66	Dicruridae	Black drongo (<i>Dicrurus macrocercus</i>)	Kabir, 2012; Kabir <i>et al.</i> , 2020	Possibly misidentification with black drongo Mentioned this species previously
67	Muscicapidae	Black Redstart (<i>Phoenicurus ochruros</i>)	Kabir, 2012; Kabir <i>et al.</i> , 2020	Not observed during the study period
68	Muscicapidae	Red-throated flycatcher (<i>Ficedula albicilla</i>)	Kabir, 2012; Kabir <i>et al.</i> , 2020	Not observed during the study period
69	Muscicapidae	Oriental magpie-robin (<i>Copsychus saularis</i>)	Kabir, 2012; Kabir <i>et al.</i> , 2020	Mentioned this species previously
70	Estrildidae	Scaly-breasted munia (<i>Lonchura punctulata</i>)	Kabir, 2012; Kabir <i>et al.</i> , 2020	Not observed during the study period Mentioned this species previously
71	Estrildidae	Indian silverbill (<i>Euodice malabarica</i>)	Kabir, 2012; Kabir <i>et al.</i> , 2020	Not observed during the study period
72	Strigidae	Spotted owlet (<i>Athene brama</i>)	Kabir, 2012; Kabir <i>et al.</i> , 2020	Not observed during the study period
73	Tytonidae	Barn owl (<i>Tyto alba</i>)	Kabir, 2012; Kabir <i>et al.</i> , 2020	Not observed during the study period



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