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EFFICACY OF SPIRULINA ON PRODUCTION INDICES OF ADVANCED AGE LAYER CHICKEN

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Abstract

The present study was designed to evaluate the productive performance and hematological parameter of advanced age laying hens fed with spirulina supplemented diets and water. The experiment was conducted on 30 commercial laying hens of 60-65 weeks of age. The birds were randomly divided into 3 equal groups designated as Group A, B and C. After one week of adaptation period, along with the commercial layer diet twice daily and adlibitum drinking water the group B and C were supplemented with spirulina at a level of 4 and 2 g per Kg of feed and water, respectively. There was no spirulina supplemented in group A and was considered as control. The feed and water intake, egg production, egg weight and hematological parameters (TEC, PCV and Hb) were recorded during the experimental period of 30 days. The average feed intake was significantly ($p<0.05$) decreased in group B and C as compared to control. Conversely, average egg production and body weight were significantly increased ($p<0.05$) in spirulina supplemented groups. However, a steady increase pattern in egg production was observed when spirulina supplemented at a level of 2 g/Kg of feed and water. This result suggests the higher egg production and body weight gain with less feed consumption which was further reflected in calculated Feed Conversion Ratio. Hematological parameters of Group B and C were found significantly ($p<0.05$) higher than control group. Water consumption and egg weight did not differ significantly among the experimental groups. Conclusively, it could be recommended to supplement layer diets with spirulina at a level of 2 to 4 g/kg of feed and water for better productivity as well as improved hematological responses during the advanced laying period when the production goes decline normally.

Keywords: Diet, FCR, egg, production indices, hematology, aged-chicken.

Introduction

The recent trend in the feed business is currently directed toward the use of natural ingredients as alternatives to antibiotics, synthetic colors, and other chemicals. Typically, commercial layer start laying eggs from 18-19 weeks of age and remain laying eggs continuously till their 72-78 weeks of age and egg production rises rapidly and then starts to fall after 31 weeks of age. Modern industrial farms have taken measures to increase egg production rates that go far beyond what we in the eco-agriculture movement would consider normal or humane. But even ecologically-conscious egg producers, whether at the commercial or homestead level, can implement measures to safely increase laying rates. The three most important factors for increasing the productions of eggs are breeding, nutrition,

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and bird comfort and well-being. In this study spirulina is a blue green microalgae (cyanobacterium) is selected for increase the production rate and better physiology for the laying hen during their advanced age. Spirulina is considered to be the ancestor from which the higher plants evolved (Desai and Sivakami, 2004), found naturally in lakes and grown commercially. Spirulina was originally harvested from lakes in parts of Africa and Mexico, dried and used as a food may also have medicinal uses. It gained prominence more recently after it was used as a dietary supplement for astronauts on space missions. NASA has stated that the nutritional value of 1000 kg of fruits and vegetables equals to one kg of spirulina (Ravi *et al.,* 2002). In Africa, spirulina has served as the sole source of nutrition in certain communities in times of famine and the entire native populations have existed eating only spirulina for over a month at a time. (Mohan *et al.,* 2014). Spirulina has been determined as a highly energy content source (2.50- 3.29 kcal/gram) and its phosphorous availability is 41% (Yoshida and Hoshi, 1980). It is very much potential to enhance immune function, reproduction and growth and supplementation in chicken diets can significantly enhance the defense systems for increased antigen processing, greater microbial killing and T-cell activity (Qureshi *et al.,* 1994). Addition of spirulina less than 1% can improve egg fertility from 87% to over 96% (Ross and Dominy, 1990) but it's supplementation did not have any adverse effect on egg production, egg weight, body weight or mortality rate (Ross *et al.,* 1994). Spirulina provides the majority of essential and nonessential amino acids. It has a fairly well-balanced amino acid pattern and contains the highest amount of beta-carotene, a precursor of vitamin A. It is the only vegetable source of vitamin B12 having two and half times the amount in liver. It is also the source of the essential fatty acid γ-linolenic acid which is the precursor of hormones involved in regulation of body functions (Desai *et al.,* 2009). The constituents of spirulina include protein (50-70%) including all essential amino-acids, essential fatty acids, polysaccharides, B vitamins particularly vitamin B12, beta-carotene and minerals particularly iron (Mohan *et al.,* 2014). It contains antioxidants such as beta carotene and zeaxanthin. Results of animal studies appear to show that spirulina may increase the production of anti-inflammatory chemicals known as interferons and interleukins. Spirulina has been shown as an effective chelating agent for removing toxins such as, lead, mercury and radioactive substances from the body (Islam *et al.,* 2009). It has also been used to remove lead and cadmium from waste water. Chelation therapy, the conventional treatment for heavy metal toxicity, is reported to have a number of safety and efficacy issues (Ponce-Canchihuamán *et al.,* 2010). Spirulina may be helpful for reducing the tissue burden of arsenic in ducks (Islam *et al.,* 2009). Aim of this study was to investigate the effects of dietary supplementation of spirulina on productive performances and hematological status during and after 60-65 weeks of laying period.

Materials and Methods

The experiment was carried out in the Post Graduate Laboratory-1, Department of Pharmacology of Bangladesh Agricultural University, Mymensingh 2202 in collaboration with Department of Physiology and

Pharmacology of Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur 1706.

Preparation of birds

A number of 30 White Leghorn layer chicken of 60-65 of age and 1.4-1.5 Kg/bird body weight were purchased from local standard farm near Gazipur. The birds were reared in a well-ventilated room of 70-80% relative humidity and they were supplied a standard layer feed formulated by company protocol (Nourish poultry feed) and adlibitum amount of drinking water.

Collection of spirulina sample

A 100 g spirulina powder packet (Eskalina®-100% organic) was collected form SK+F Bangladesh Ltd. According to the company instruction the composition of the spirulina was carbohydrate-15.1%, proten-60%, fat-8.2%, Amino acid- met, lys, thr, trp, ileu, leu, phe, val,ala, arg, asp, cys, glu, gly, his, pro, ser, tyr; vitamin-B1, B2, B3, B5, B6, B9, B12, C, D, E, bita-carotine, inositol; minarals- Ca, P, Na, K,Mg, Fe, I, Cu, Mn, Zn, Cr, Se, Al.

Lighting

An average 250 lux light intensity were used to maintain temperature of birds due to the fluctuation of weather. Two 20 watt bulbs were used to maintain temperature depending upon the weather. A period of 13-hours light and 11-hours dark period was maintained for a 24 hours lighting cycle. The cages of birds were kept in a well-ventilated house with a relative humidity of 70-80%.

Feeding and drinking

The birds were given feed and water twice a day in the morning and in the afternoon. Each group was placed in different cage and feed and water were supplied separately. During administration of feed and water; water vessel were cleaned with adequate fresh water and feeder were cleaned once in a week with disinfectant (Timsen[r]) and then with fresh water. Feed and water were measured separately with a weighing balance before administration to the birds. Spirulina for individual group were measured with electric balance carefully and mixed manually with the feed and water.

Design of the experiment

All the birds were kept under close observation in order to maintain good health for conducting experiment properly for a period of thirty days. According to the groups, all the birds were kept in separate cage rearing system. The experiment was performed on 30 layer birds to investigate the effect of spirulina on the productivity performance and hematological parameter in layer chicken. Birds were randomly divided into three experimental groups (A, B and C) containing 10 birds in each group. Birds were subjected to treatments with the feed and drinking water twice daily for a period of 30 days as per following schedule: Group-A was kept as control where ready feed was supplied; ensuring from the company that they didn't include spirulina or any other equivalent ingredients that could function as like as the test ingredient. Group-B: was treated with spirulina @ 4 g/Kg feed and 4 g/L water. Group-C: was treated with spirulina @ 2 g/Kg feed and 2 g/L water.

Hematological parameters

Blood was collected aseptically with sterile syringe and needle either from heart or from the wing vein of different groups of chickens. Total 4 ml blood sample were collected from each birds of the groups and total erythrocyte count (TLC), hemoglobin content (Hb), pack cell volume (PCV) were analyzed. For analysis of blood parameters blood from each bird were collected from the wing vein using a number of sterile test tubes containing anticoagulant (3.8% Trisodium citrate solution) at a ratio of 1:10 were taken. About 4.0 ml of blood to be collected for hematological studies. The hematological studies were performed within two hours of collection. Serum sample were collected from the individual blood sample according to the standard procedure and stored at -20^{o}c till analysis. Hb and PCV was determined as per method by Lamberg and Rothstein (1977). The counting and calculation of RBC and WBC was performed as per methods described by Ghai (1999).

Statistical analyses

Results were analyzed by one-way ANOVA using SPSS for Windows. Differences among all treatments were separated by Duncan's Multiple Range Tests (DMRT), and probability values of less than 0.05 were considered as significant. Results were expressed as mean±SE.

Results

Hen-Day Egg Production (HDEP-For 27 days)

Result from Fig. 1, showed that highest HDEP found in spirulina 4 gm and lowest HDEP was found in control group. But The HDEP of spirulina 2 g/kg group was lower than the HDEP of spirulina 4 g/kg group.

Layer production indices

Data presented in Table1 showed that Hen-Housed Egg Production (HHEP) was much higher in group B than control group or group A. Similarly, HHEP was higher in group C than group A. But group B or spirulina 4 g/kg had the higher HHEP than spirulina 2 g/kg or group C (Table 1). Similarly, Egg Mass (EM) was highest in group B followed by group C and group A respectively (Table 1). Highest feed consumption was recorded in group B which was (1159.5±9.0) gm/day/10 birds followed by (1152.1 ± 8.6) gm/day/10 birds in group C and (1069.8±16.3) gm/day/10 birds in group A (Table 1). Water intake was recorded 3604.1 ± 146.9 ml/day/10 birds in group C, followed by 3584.6 ± 138.5 ml/day/10 birds in group A and 3505.5 ± 164.6 ml/day/10 birds in group B (Table 1). Difference in the water intake among the groups of the experiment was statistically significant. Average egg weight of the group A (64.9 ± 1.2 gm); group B (62.8 ± 0.8 gm); and group C (63.8 ± 0.7 gm) were not statistically significant (Table 1). Compare to the groups of the experiment it is evident that feed conversion ratio in group B was 2.06 followed by group C (2.27) and group A (2.74) (Table 1).

Total erythrocyte count (TEC), pack cell volume (PCV), hemoglobin content (Hb)

From the evident of the present finding TEC was highest in spirulina 4gm group than spirulina 2 gm and control group respectively (Fig. 2A). PCV percentage was also higher in spirulina 4 gm or group B followed by group C and group A (Fig. 2B). The trend found similar for hemoglobin concertation % which was also higher in spirulina 4 gm group than spirulina 2gm group and control group respectively (Fig. 2C).

Table 1. Layer production indices

Groups	HHEP% (For 27 days)	EM% (For 27 days)	FCR	Feed Consumption (g/day/group)	Water Intake (ml/ day/group)	Egg Weight (g)
Control (A)	62.6 ± 0.3[a]	42.8 ± 0.5[a]	2.74	1069.8 ± 16.3[a]	3584.6 ± 138.5	64.9 ± 1.2
Spirulina 4gm (B)	93.0 ± 1.2[b]	60.3 ±0.8[b]	2.06	1159.5 ± 9.0[b]	3505.5 ± 164.6	62.8 ± 0.8
Spirulina 2gm (C)	86.3 ± 0.9[c]	55.9 ± 0.7[c]	2.27	1152.1 ± 8.6[b]	3604.1 ± 146.9	63.2 ± 0.7

Here, HHEP-Hen-Housed Egg Production; EM-Egg Mass; FCR-Feed Conversion Ratio; HHEP -Total number of eggs laid during the period/ Total number of hens housed at the beginning of laying period. Average egg mass (Per hen per day in grams) - Percent HDEP X Average egg weight in grams. Data are presented as Mean±SEM; SEM- Standard error of mean. Values with different superscripts in the same column denotes significant difference (*p<0.05*).

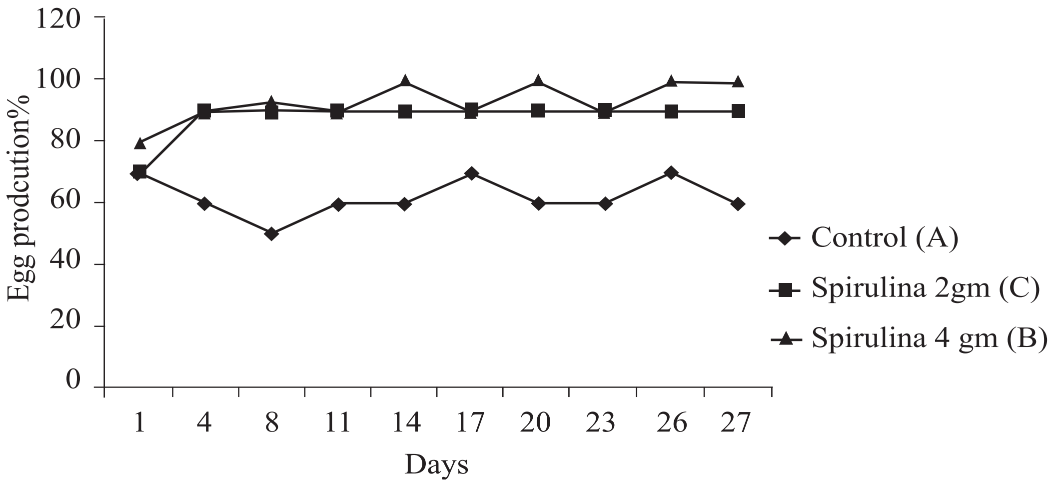

Fig. 1. Hen-Days Egg Production (For a long period). The data lines showing 27 days continuous production in the experimental birds. Data are presented as mean value which finally converted to % production. n=10, each group. Here, HDEP -Total number of eggs produced during the period/ Total number of hen-days in the same period x 100

Effect of spirulina on egg production

The highest number of egg production was found in group B which was (9.3±0.17 n/ group). Egg production in group C and Group A was (8.63±0.16 n/group) and (6.26±0.17n/ group) respectively. Differences among the means were statistically significant (p <0.05). Difference in the egg production between the groups are portrayed in Fig. 3.

Effect of spirulina on average body weight between the groups of the experiment

Average body weight in group B was higher than group C and group A respectively in comparison with the body weight before and after of the experiment. Difference between group of the experiment was statistically significant (p <0.05). Difference in the body weight between the groups are shown in Fig. 4.

Discussion

Experiment showed that highest HDEP found in spirulina 4 g/kg and lowest HDEP was found in control group. But The HDEP of spirulina 2 g/kg group was lower than the HDEP of spirulina 4 g/kg group. Hen-Housed Egg Production (HHEP) was much higher

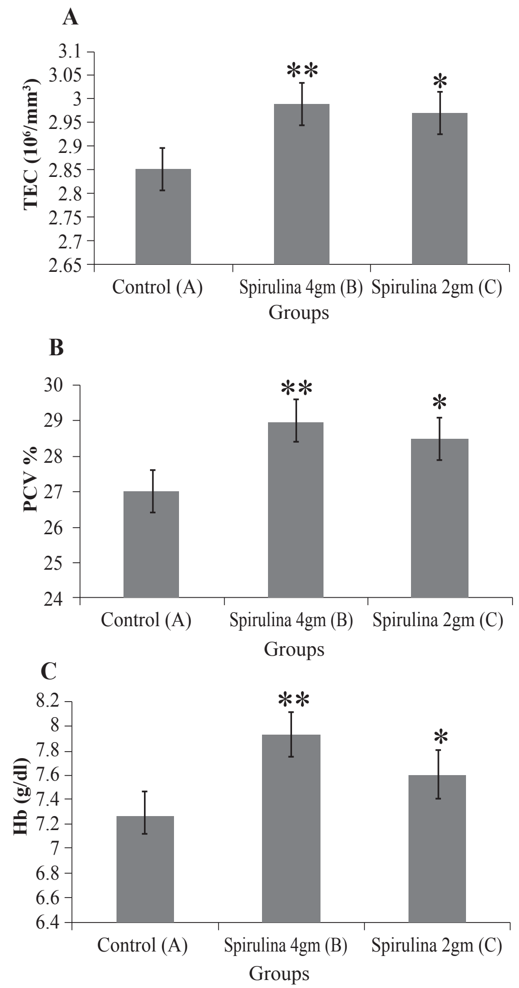

Fig. 2. Effect of spirulina on blood parameters-A) TEC (million/dl), B) % of PCV and C) Hb (g %) in layer chicken after one month treatment with spirulina. Data are presented as Mean±SEM;*, *p<0.05*) and, *p<0.01* considered as significant difference vs control, n=5 from each group.**

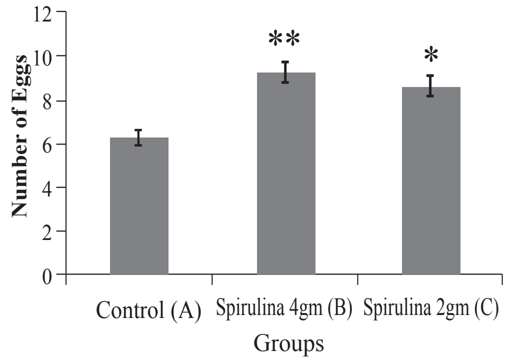

Fig. 3. Effect of spirulina on egg production. Data are presented as Mean±SEM; *, *p<0.05*) and **, *p<0.01* considered as significant difference vs control, n=10 from each group.

in group B than control group or group A. Similarly, HHEP was higher in group C than group A. But group B or spirulina 4 g/kg had the higher HHEP than spirulina 2 g/kg or group C. There is no previous research showing the effect of spirulina on Hen Day Egg Production (HDEP) and Hen Housed Egg Production (HHEP). Experiment showed that the highest egg production was found in group B which was (9.3±0.17 n/group). Egg production in group C and Group A were (8.63±0.16 n/group) and (6.26±0.17 n/group) respectively. This present finding was agreed with Mariey *et al.* (2012) who found that egg production was significantly ($p<0.05$) improved by dietary treatment with spirulina. At a level of 0.3% spirulina can be used as an effective feed additive to improve egg production, egg quality and hepatoprotective activity (Selim *et al.,* 2018). Few other authors also reported that laying hens fed with spirulina containing diets attained the highest rate of egg production and feed conversion compared with those of the control groups (Ross *et al.,* 1994; Nikodémusz *et al.,* 2010). In other hand Halle *et al.* (2009) observed decrease in feed intake and no effect on the overall egg production with higher levels of spirulina. Present evident showed that spirulina didn't have any positive correlation with the egg weight. The present finding is similar to the finding of Zahroojian *et al.* (2013) who found that spirulina addition had no effect on egg quality parameters, Yolk index, egg yolk cholesterol, yolk color, shell thickness, shell weight and specific gravity except for yolk color were

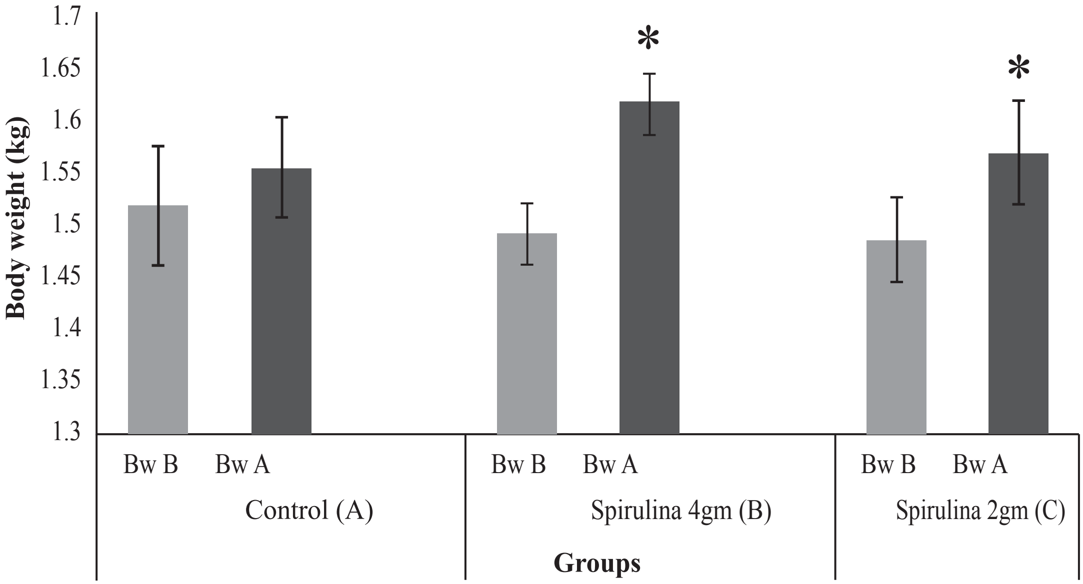

Fig. 4. Effect of spirulina on average body weight during the period of thirty days between the groups of the experiment. Data are presented as Mean±SEM; *, $p<0.05$) considered as significant difference vs control, n=10 from each group. Here, BwB- Body weight before; BwA- Body weight after.

compared with the control group. Mariey *et al.* (2012) who also agreed with our finding that there were no significant differences in egg weight of feeding experimental diets containing spirulina. This experiment showed that dietary supplementation of spirulina increased the feed consumption in group B than the other groups of the experiment and there was no significant result on water intake between the groups of the experiment. We didn't find any authors who experimented on this parameter of spirulina however Tokai *et al.* (1997) reported that spirulina containing diets may increase the lactobacillus population and enhance the absorbability of dietary vitamins. Average body weight in group B was higher than group C and group A respectively in comparison with the body weight before and after of the experiment. This finding were supported by Jamil *et al.* (2015) who found that the body weight was significantly ($p<0.05$) increased in the treatment groups fed with spirulina diet. The supplementation of Spirulina powder at levels of 1% in the Japanese quail diet or at 0.25% in the drinking water sustained its quail's growth performance, improved the fertility and decreased the serum cholesterol and free fatty acids, while it did not show any effect on egg production or egg quality (Abouelezz *et al.,* 2017). Body weight gains of laying hens increased insignificantly with spirulina diets as compared to that of the control group, but fed with 0.20% spirulina had the significant result (Mariey *et al.,* 2012). Few other authors finding were also similar to this finding where Danny *et al.* (2016) found that the body weight increased significantly in Japanese quail with the spirulina treatment and Kanagaraju and

Omprakash *et al.* (2016) who showed that the quails fed with spirulina at 1, 2, and 3% of the diet had better FCRs than that of the control birds. Similarly, Shanmugapriya *et al.* (2015) reported that the body weight gain, feed conversion ratio and villi length were significantly increased by the dietary inclusion of the 1% of *Spirulina platensis* as compared to the control fed broilers. Experiment showed that TEC, PCV and Hb were significantly increased in spirulina treated group compared with control group. A research result found that dietary supplementing of spirulina microalgae alone and in combination with chlorella significantly increased the reduced levels of RBC, WBC, PCV and selenium in diabetic rats (Emami & Olfati, 2017) and spirulina have the ability to increase immune function by increasing hematological parameters (Yeganeh *et al.,* 2015). Nasirian *et al.* (2017) reported that oral supplement of spirulina (30 mg/kg body weight) significantly improve TEC, WBC, MCHC, PCV and MCV in rats. Spirulina or blue green algae are well known for their antioxidant properties which may help to increase the activity of antioxidant enzymes and subsequently improve hematological markers (Elmalawany *et al.,* 2014). In conclusion the present study shows that spirulina supplementation with feed (4 g/kg) and water (4 g/L) in advance aged layer increases the egg production rate compared to the control.

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Footnotes to tables should be indicated by superscript lower-case letters (or asterisks for significance values and other statistical data) and included beneath the table body. A unit of measure should be reported as the actual quantity multiplied by a power of 10 to give the reported quantity (the unit may be changed by the use of m or μ).

Table 1. Range, mean, standard error and co-efficient of variation of different characters of 31 inbred lines of sunflower

Characters	Range	Mean	SE(±)	Coefficient of Variation (%)
DM (day)	88-101	96	2.39	3.04
PH (cm)	48.29-137.67	97.25	8.50	10.71
HD (cm)	6.68-17.87	13.23	2.81	25.98
SD (cm)	1.12-2.27	1.60	0.19	14.96
SH (no.)	37-250	118.81	44.11	45.43
SW (g)	2.23-21.42	10.58	5.22	60.48
SY (g)	30.23-541.20	266.65	65.89	30.26

DM= days to maturity, PH= plant height (cm), HD= head diameter (cm), SD= stem diameter (cm), SH= number of seeds per head, SW= seed weight per head (g), SY= Seed yield (g)

Source : Rashid *et al.* (2018)

Figures

All figures are to be numbered using Arabic numerals. Figure parts should be denoted by lowercase letters (a, b, c). If illustrations are supplied with uppercase labeling, lowercase letters will still be used in the figure legends and citations. Figures should always be cited in text in consecutive numerical order (Fig. 1 and 2). For each figure, please supply a figure legend. Make sure to identify all elements found in the figure in the legend. Identify any previously published material by giving the original source in the form of a reference at the end of the legend. The publisher reserves the right to reduce or enlarge figures. Figure legends should be grouped together in text. Figure should submit in TIF or GIF format.

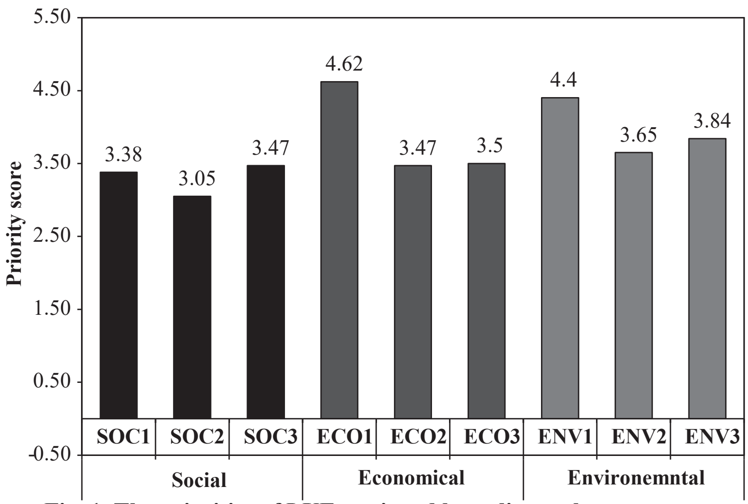

Fig. 1. The priorities of LUFs assigned by policy makers at present.

Source : Miah *et al.* (2018)

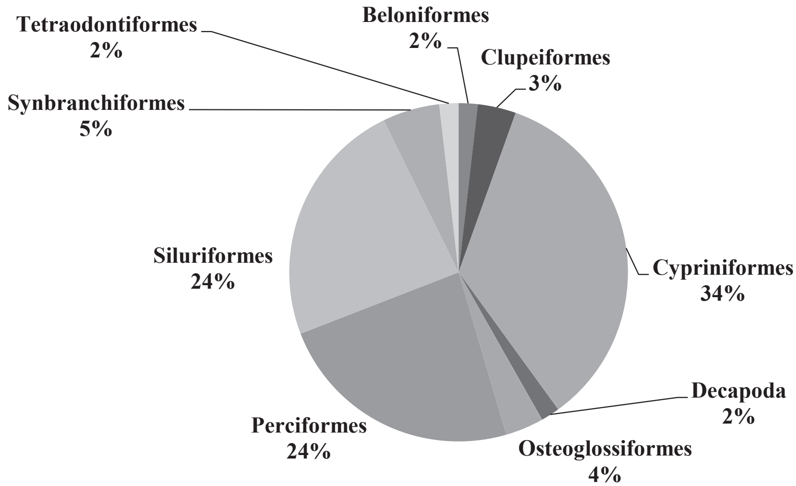

Fig. 2. Percentage of fish species according to the fish order in Ghaghat river.

Source : Islam *et al.* (2018)

Acknowledgments

Acknowledgments of people, grants, funds, etc. should be placed in a separate section before the reference list. The names of funding organizations should be written in full.

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Citation in Text

Cite references in the text by name and year in parentheses. Some examples:

Negotiation research spans many disciplines (Thompson, 1990).

This result was later contradicted (Becker and Seligman, 1996).

This effect has been widely studied (Shils, 1991; Liakat *et al.,* 1995; Hossain and Smith, 1998; Medvec *et al.,* 1993).

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Book

Hill, D. S. 1787. Agricultural Insect Pests of the Ttropics and Their Control. 2nd ed. Cambridge Univ. Press, New York, USA 746 P.

Book Chapter
Troughton, J. H. 1975. Phytoshynthetic Mechanisms in Higher Plants. Pp. 57-91. In Cooper. J.P. (ed.) Photosynthesis and Productivity in Different Environments. Cambridge Univ. Press, Cambridge, UK.

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