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PATHOGENIC EFFECTS ASSOCIATED WITH *TRYPANOSOMA DANILEWSKYI* STRAIN FCC 1 INFECTION IN JUVENILE COMMON CARP, *CYPRINUS CARPIO* L

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ABSTRACT

Mortality and pathological effects caused by *Trypanosoma danilewskyi* strain F Cc 1 in laboratory-infected single breed juvenile common carp were investigated in this study. The study was divided into two parts. In the first part, four groups (A-D) each containing 20 fish (4 months old) were inoculated intraperitoneally with various size of inocula; 1000, 25,000 and 50,000 live trypanosomes/fish in groups A-C respectively while group D was injected 0.2 ml of PSG (phosphate buffered saline with glucose, pH 7, filter sterilized)/fish as a control. Blood was examined at 10 days intervals to monitor the development of parasitemia. All dead fish were counted and their blood and body fluids were examined for the presence of trypanosomes. The second part of the study was conducted under the similar conditions as first part to measure the pathological effects induced by the infection of *T. danilewskyi* strain FCc 1. Two groups, (A & B) each containing 40 fish (5 months old) were inoculated with 50,000 live trypanosomes/fish in group A and 0.2 ml PSG/fish in group B (control). Blood was examined at 10 days intervals and used for the estimation of parasitemia, hematocrit, hemoglobin and thin blood smear preparations. In the first part of study no fish died in groups A & D, 12.5 % mortality was observed in group B and 50 % in group C. The second part revealed a significant decrease in hematocrit, hemoglobin, erythrocyte counts and significant increase in leukocytes counts was observed in infected fish (group A) when compared to the control (group B) at the same interval of time. Thus it is concluded that *Trypanosoma danilewskyi* strain F Cc 1 is a potential hemoflagellate pathogen of juvenile common carp as it causes mortality, anemia and altered blood parameters.

Key words: pathogenic effects, *T. danilewskyi* strain FCc 1, hemoglobin, leukocytes, carp, mortality.

INTRODUCTION

Fish hemoflagellate parasites like *Trypanosoma danilewskyi* (Lom and Dykova, 1992) and *Trypanoplasma borreli* (Kruse *et al.* 1989; Steinhagen *et al.* 1989 & 1990) are known pathogens of juvenile common carp, *Cyprinus carpio*. In experimental infections, trypanosomes prevail against the immune system of the host resulting in pronounced diseased conditions and ultimately death of the host (Lom, 1979; Khan, 1985; Ahmed and Ollevier, 2001, 2002). There are reports on tissue changes, with emphasis on blood parameter changes induced by some other species of fish trypanosomes (Tandon and Chandra, 1977; Pulsford, 1984) while the pathological effects caused by *T. danilewskyi* (MA strain) in gold fish, *Carassius auratus*, have been well documented by Islam and Woo (1991a,b) and Lom and Dykova (1992). *Trypanoplasma borreli* has caused severe parasitemia, mortality, altered blood parameters in laboratory-infected juvenile common carp, *Cyprinus carpio* (Kruse *et al.* 1989; Steinhagen *et al.* 1989, 1990; Wiegertjes *et al.* 1995). Other fish trypanosome specie like *Trypanosoma murmanensis*, have been reported to induce lethargy, anemia and changes in blood parameter in Atlantic cod, *Gadus morrhua* (Khan, 1977), longhorn sculpin, *Myoxocephalus octodecemspinosis* and three other marine fishes, *Pseudopleuronectes americanus*, *Myoxocephalus scorpius* and *Myoxocephalus octodecemspinosis* (Khan *et al.* 1980). Significant effects on blood glucose, cholesterol and various enzyme levels due to the infection of *Trypanosoma singhii* in freshwater fishes like *Wallago attu* (Tandon, 1986) and *W. attu* and *Heteropneustes fossilis* (Gupta and Gupta, 1986) have also been reported. Another trypanosome specie, *Trypanosoma attii* has been described as an etiological agent of anemia in *W. attu* (Gupta and Gupta, 1990). Tandon and Chandra (1977) have reported lowered cholesterol level due to trypanosome (species not mentioned) infections in 6 freshwater fish species, (*Cirrhina mrigala*, *Clarias batrachus*, *Heteropneustes fossilis*, *Mastocembelus armatus*, *Mystus seenghala* and *W. attu*). Woo (1994, 1998, 2001, 2003) conducted several studies on *Trypanoplasma salmositica* infection in rainbow trout, *Oncorhynchus mykiss* and reported severe pathological effects and immune system damage in the host. Similar evidences of pathological effects of Cryptobiosis in rainbow trout have also been documented by Lowe-Jinde (1986). *Cryptobia salmositica* is found to cause the

severe anorexia (Li and Woo, 1991) and impaired immune response during the infection in rainbow trout, *O. mykiss* (Chin, *et al*. 2004). And the same parasite has caused serious histopathological effects in rainbow trout and these effects were related with severity of parasitemia (Bahmanrokh and Woo, 2001).

The present study was aimed to pursue the pathogenic effects like mortality, anemia, and changes in the blood parameters like hematocrit values, hemoglobin concentration, total blood cell counts and increase or decrease in number of differential blood cells in laboratory infected juvenile common carp, *Cyprinus carpio* when infected with *Trypanosoma danilewskyi* strain FCc 1.

MARETIALS AND METHODS

Four months (5.5 ± 0.12 cm) and 5 months (7.67 ± 0.11 cm) old single breed juvenile common carp were purchased from the Experimental Fish Facility, Department of Zodiac, Agriculture University Wageningen, Netherlands. All fish were kept in glass aquaria (1.5 x 2.0 x 1.5 ft) at 20° C for 2 weeks prior to experimental infection in flow through system (flow rate 4 lit/hr). *Trypanosoma danilewskyi* strain FCc 1 (blood stream forms isolated and maintained in single breed juvenile common carp until 12th sub-passages) was the same as used in the previous studies (Ahmed and Ollevier, 1998, 2001; Ahmed *et al*. 2001). All fish were fed with TrouVit to an equivalent of 2.5 % body weight twice daily. Blood was drawn from caudal vein puncture with 1 ml heparinized syringe, examined and the parasitemia was estimated from wet blood preparation by matching method (Herbert and Lumsden, 1976). The hematocrit centrifuge technique (HCT) (Woo, 1969) was applied where the parasitemia was very low. For hematocrit, 40 µl bloods were centrifuged in preheparinized hematocrit capillaries for 5 minutes at 1500 g. Blood hemoglobin was measured by spectrophotometer (Spectronic 1201, Milton Roy Company), according to Kampen and Zijlstra (1961) and Wedemyer and Yasutake (1977). Erythrocytes counts were taken from diluted blood (200 times in RPMI 1640 medium pH 7.2) by hemocytometer chamber (Brüker, 0.0025 mm) from 40 small chambers in triplicate and the mean value was multiplied by 10^5 and dilution level to calculate the total number of cells/ml blood (Archer, 1965). Thin blood smears were prepared on precleaned glass slides, air dried, fixed with methanol (analytical grade) and stained with May-Grunwald Giemsa stain (Möhr, 1981) for differential blood cell identification and counts. One hundred blood cells (all type) were counted (3 times) from well stained slides, averaged and percentage was calculated for each cell type.

Parasite induced mortality: Single breed juvenile common carps (4 months old) were used to observe the parasite induced mortality and inoculum size related parasitemia. Four groups (A- D) in duplicate each containing 20 fish were inoculated intraperitoneally with 1000, 25,000 and 50,000 live trypanosomes/fish in groups A - C respectively while group D (control) was injected with 0.2 ml of PSG (phosphate buffered saline with glucose, pH 7.0, filtered sterilized)/fish. Blood was examined at 10 days intervals to monitor the development of parasitemia. The dead fish were counted and their blood and body fluids were diagnosed for the presence of trypanosomes by smear preparation on precleaned glass slide and examined under phase contrast microscope.

Parasite induced pathological effects: Another experiment under similar conditions of the previous study was conducted to investigate the parasite induced pathological effects in single breed juvenile common carp. Two groups (A & B) in duplicate each containing 40 fish (5 months old) were inoculated intraperitoneally with 50,000 live trypanosomes/fish in group A and 0.2 ml PSG (phosphate buffered saline with glucose, pH 7.0, filtered sterilized)/fish in group B (control). Five fish were sacrificed at 10 days intervals and blood was used for the development and estimation of parasitemia, measurement of hematocrit, hemoglobin concentration and thin blood smear preparations for differential blood cell counts.

Two-way analysis of variance was applied to compare various blood parameters like hematocrit and hemoglobin levels and changes in erythrocytes and leukocytes counts of control and infected groups followed by Bonferoni (Dunn) t test by using SAS/ETS® Software (SAS Institute Inc. 2001).

RESULTS

Mortality: In first experiment, the mortality caused by *Trypanosoma danilewskyi* strain FCc 1 in four months old carp was zero in group A (1,000 trypanosomes/fish), 12.5 % in group B (25,000 trypanosomes/fish) and 50 % in group C (50,000 trypanosomes/fish). No fish died in control (group D) (Fig. 1). Mortality was significantly higher ($P<0.05$) in infected groups B-C when compared to group A and control (group D). A significant difference ($P<0.05$) in mortality was also observed among infected groups B & C.

Parasite induced pathological effects: In second experiment, where 5 months old carps were used, all inoculated fish developed parasitemia (Fig. 2 a) and were found anemic during the course of infection. Anemia increased with the increase in parasitemia and was highly pronounced in heavily infected fish. A significant decrease ($P<0.001$) in hematocrit (Fig. 2 b) was observed from day 10-60 post infection (p.i) and the lowest value

(25.71 %) was obtained at the peak of infection (day 30 p.i). The total number of erythrocytes (Fig 2 c) decreased ($P<0.05$) from day 30 – 60 p.i while the total number of leukocytes (Fig 2 d) significantly increased in infected fish ($P<0.05$ on day 20 & 60 p.i and $P<0.01$ on day 30, 40 & 50 p.i). The relative percentage of erythrocytes (RBC) (Fig 3 a) decreased with an increase in parasitemia and was lower at the peak and post infection. The relative percentage of differential leukocytes was altered (Fig 3 b) and a significant increase ($P<0.05$) in differential leukocytes (macrophages, granulocytes, neutrophils and monocytes) was observed from day 20 – 60 p.i. Thrombocyte counts were changed but not significantly different from control. The lymphocytes (smaller & bigger) lowered among other leukocytes and a decrease ($P<0.05$) was observed on day 30, 40 and 60 p.i as compared to the control at the same sampling time. A significant decrease in hemoglobin/ml of blood was observed on day 20 p.i ($P<0.05$) and from day 30 – 60 p.i ($P<0.01$) as compared to the control (Fig. 3 c). The lowest hemoglobin level (5.21 g/100 ml) was observed on day 30 p.i. The concentration of hemoglobin per erythrocyte was $3.9 \pm 0.27 \times 10^{-5}$ µg and $3.7 \pm 0.24 \times 10^{-5}$ µg in the blood of control and infected fish respectively. The lowest value per cell in infected blood was $3.6 \pm 0.14 \times 10^{-5}$ µg on day 30 p.i. However, the concentration of hemoglobin per cell was not significantly different from the control group. A negative correlation was observed between parasite number and erythrocyte counts ($r = -0.821$, $P<0.023$), hemoglobin ($r = -0.8928$, $P< 0.006$) and hematocrit ($r = -0.928$, $P<0.002$). The ratio between erythrocytes and white blood cells (RBC/WBC) decreased on day 10, 20 & 60 ($P<0.05$) and on day 30, 40 & 50 p.i ($P<0.01$) as compared to the control at the same sampling time interval (Fig. 3 d).

DISCUSSION

The mortality was 50 % in 4 months old juvenile common carp when inoculated with a large size of inoculum. The fish died due to pathogenic effects induced by *Trypanosoma danilewskyi* strain FCc 1 in juvenile common carp, (*Cyprinus carpio*). Lom (1979) found similar pathogenic effects in goldfish due to another trypanosome strain (MA strain) of *Trypanosoma danilewskyi* which has caused high mortalities depending on the size of inoculum and age of the fish. Other species of piscine trypanosomes were also found pathogenic causing severe damage and death of the hosts; i.e. *Trypanosoma murmanensis* caused 65 % mortality in less than one year old Atlantic cod, *Gadus morrhua* (Khan, 1985).

Anemia, manifested as lowering of hematocrit and hemoglobin levels, is one of the most common feature of the infection in fishes alongwith other clinical signs. During the infection of *T. danilewskyi* strain FCc 1 in juveniles common carp, the fish were lethargic and anemic with a significant decrease ($P<0.001$) in hematocrit and hemoglobin levels. This study has confirmed the previous findings that anemia is directly related with the increase in parasitemia during the trypanosome infection in fishes (Thomas and Woo, 1990; Li and Woo, 1991; Islam and Woo, 1991 b, Chin *et al* 2004; Ahmed and Ollevier, 2001, 2002; Ahmed *et al*. 2001). Significant decrease in hematocrit values was observed during the infection of *Trypanoplasma borreli* in common carp, *C. carpio* (Steinhagen *et al*. 1990), *T. salmositica* in rainbow trout, *Oncorhynchus mykiss* and *Cryptobia salmositica* in rainbow trout, *O. mykiss* (Lowe-Jinde 1986; Woo, 1994, 2001, 2003). The hematocrit values also decreased during the infection of *Trypanosoma attii* in *Wallago attu* (Gupta and Gupta, 1990) and *Trypanosoma murmanensis* in Atlantic cod, *G. morrhua* (Khan, 1985).

In the present study it was observed that the decrease in hemoglobin concentration was accompanied with decrease in the number erythrocytes in the blood of infected fish which has confirmed that significant destruction of erythrocytes has occurred during trypanosome, trypanoplasm or cryptobia infections in fishes (Khan, 1985; Lowe-Jinde, 1986; Woo, 1994, 2001, 2003; Ahmed and Ollevier, 2001, 2002; Ahmed *et al*. 2001). This destruction of erythrocytes was positively correlated with the severity of parasitemia during the infection of *T. danilewskyi* in goldfish (Islam and Woo, 1991a). Because of this, the number of mature erythrocytes in the blood decreased, which ultimately resulted in less hemoglobin per ml of blood. Another factor that is an increase in immature erythrocytes (smaller in size, rounded in shape, grayish-blue in color and contain less hemoglobin) was observed in the infected blood during the infection of *T. danilewskyi* strain FCc 1 in juvenile common carp. That was because of rapid proliferation of erythrocytes from erythrocyte stem cells. The significant decrease in hemoglobin concentration in the blood of infected fish was not only due to an increase in immature erythrocytes but also relative decrease in the number of mature erythrocytes in relation to the number of leukocytes, which eventually lowered the overall relative percentage of mature erythrocytes in the infected blood. Thus the changes in the final composition of infected blood resulted in lowered level of hemoglobin. These results are also corroborating with the finding by Lowe-Jinde (1986). She further found a significant decrease in hemoglobin level due to the destruction of erythrocytes during the infection of *Cryptobia salmositica* in rainbow trout, *O. mykiss*.

Biochemical studies also indicated changes in the blood parameters during trypanosome infections in fishes. Blood glucose level decreased during the infections of *T. singhii* and *T. attii* in *Heteropneustes fossilis* and *Wallago attu* (Gupta and Gupta, 1986). In *W.*

attu, hemoglobin, serum cholesterol, serum iron, serum cholinesterase, aldolase, acid phosphatase and ascorbic acid were decreased while alkaline phosphatase, 5´nucleotidase and lactate dehydrogenase were increased during experimental infection of *T. attii* (Tandon, 1986).

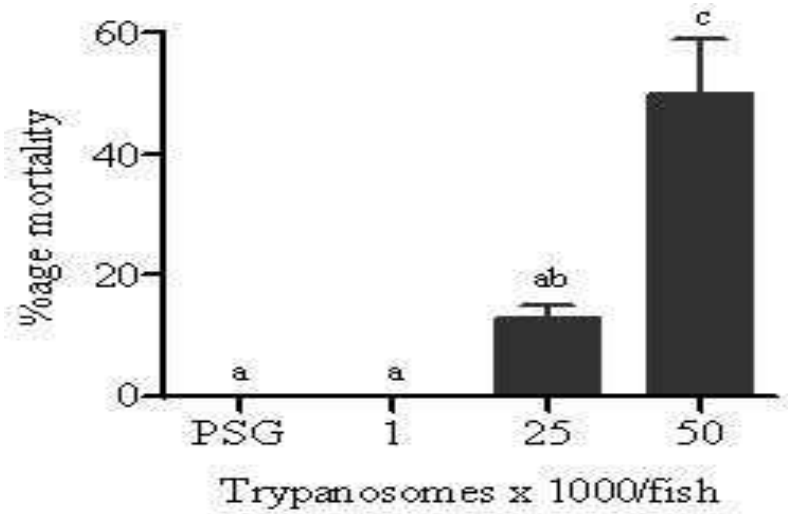

Figure 1. Mortality caused by blood stream forms of *Trypanosoma danilewskyi* strain FCc 1 during experimental infection in single breed juvenile common carp at different inoculum size. The superscripts at the bar show the levels of significance (a = not significant, b = P<0.05 and c = P<0.01).

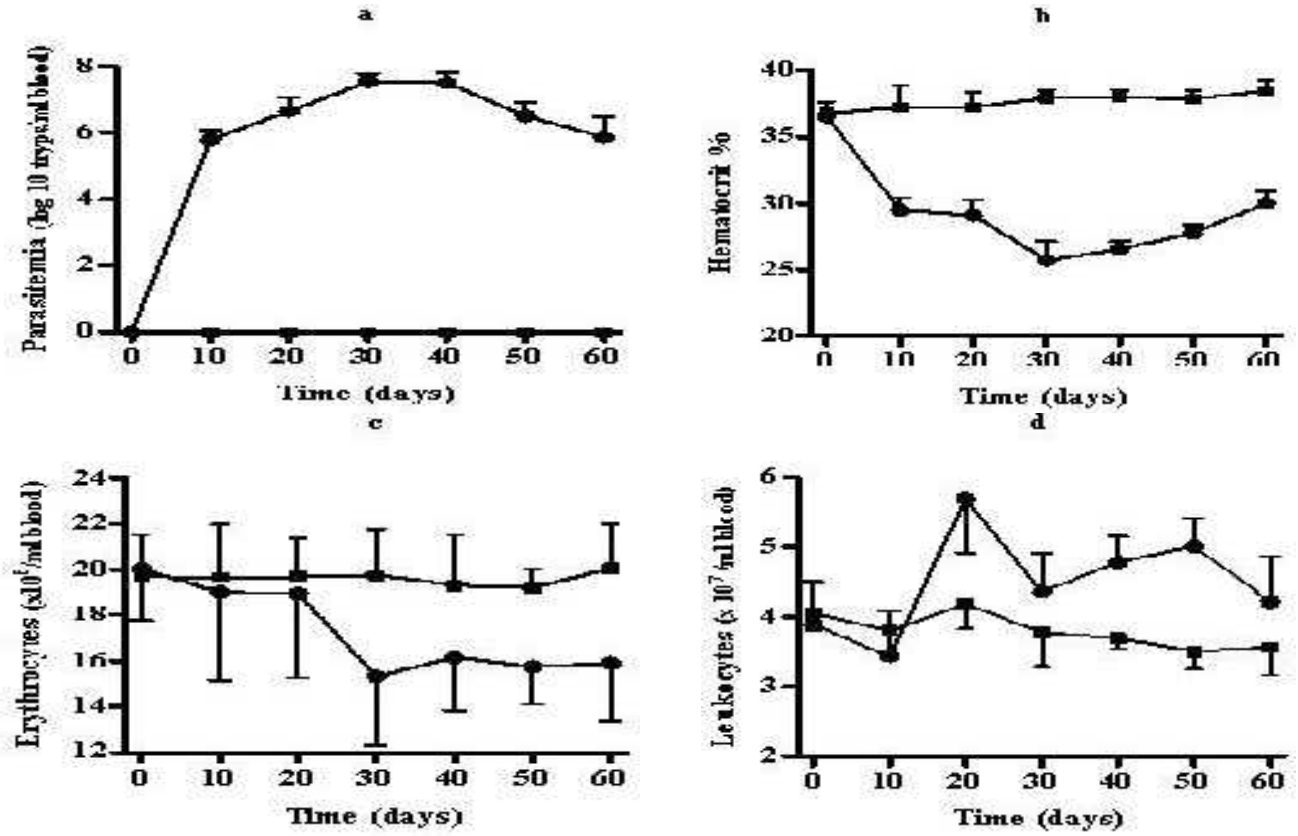

Figure 2: Experimental infection: (a) development of parasitemia, (b) change in hematocrit %, (c) total number of erythrocytes, and (d) leukocytes counts during the infection of blood stream forms of *T. danilewskyi* strain FCc 1 in juvenile common carp. (Control fish —■—, infected fish —●—, Leu; Leukocytes, Thr; Thrombocytes, Lym; Lymphocytes; n = 5).

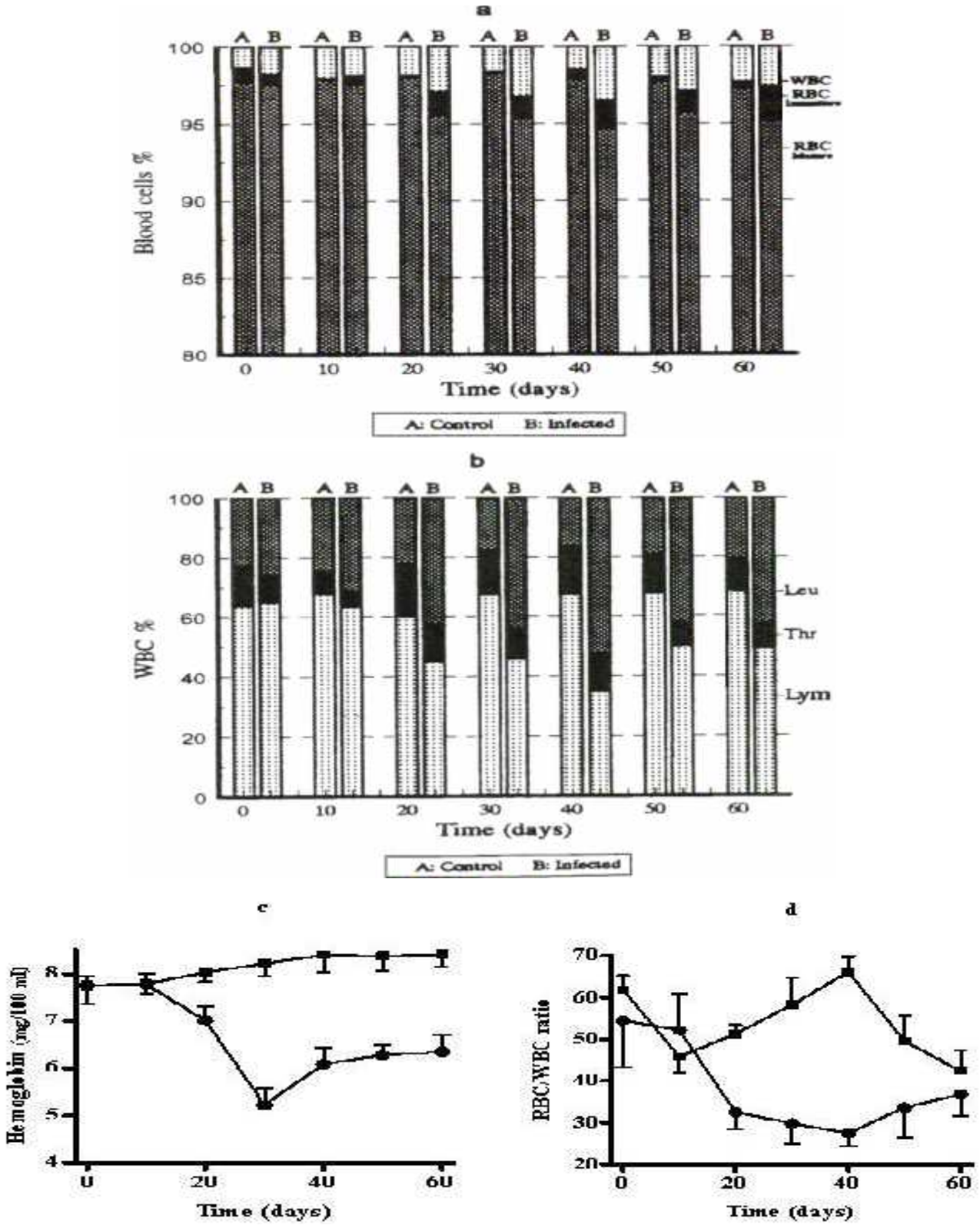

Figure 3: Experimental infection: Changes in the relative percentage of: (a) erythrocytes (mature & immature) and total leukocytes in the blood, (b) differential leukocytes, (c) hemoglobin concentration (mg/100 ml), and (d) RBC/WBC ratio during the infection of blood stream forms of *T. danilewskyi* strain FCc 1 in juvenile common carp. (Control fish —■—, infected fish —●—, Leu; Leukocytes, Thr; Thrombocytes, Lym; Lymphocytes; n = 5).

Conclusion: The present study has revealed that *Trypanosoma danilewskyi* strain FCc 1 can be considered as a pathogen of juvenile common carp when inoculated with high doses of flagellates causing 50 % mortality. It is also confirmed that it causes severe anemia, significant changes in blood parameters like erythrocytes destruction resulting in altered erythrocyte-differential leukocyte ratios and per ml hemoglobin concentration during experimental infection.

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