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The Effect of Water Parameters and Dissolved Minerals on the Hematological Parameters of during Breeding Period Catfish (*Silurus glanis*)

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HIGHLIGHTS

- In this research on Çelik Lake (Adıyaman, Turkey) the blood parameters of catfish (*Silurus glanis* Linnaeus, 1758) were evaluated.
- Blood sampling on before, during and after reproduction was performed by hematological analysis.
- Hematological values during all time were affected by the water quality parameters.

Abstract: In this study, the blood parameters of catfish (*Silurus glanis* Linnaeus, 1758) inhabiting Çelik Lake (Adıyaman, Turkey) were investigated. The study was conducted between April 2013 and October 2013. After obtaining a blood sample from each catfish has been hematologically analyzed. Then, the water temperature, dissolved oxygen, pH, calcium, magnesium and nitrate values were determined at before breeding (April), breeding period (June – July) and after breeding of fish (October) and the impacts of these data on hematological values were investigated. According to this study, it was observed that the

hematological values obtained at before breeding period, breeding and after the breeding period were affected by the water quality parameters.

Keywords: Çelik Lake; hematological values; reproduction; water quality parameters.

INTRODUCTION

Various studies have been conducted for many years in the field of hematology of fish in our country and the world. The studies have been mostly dealing with erythrocyte and leukocyte count, hematocrit, leucocrit and hemoglobin levels. Especially to get good yields in the fish farms, it has become a necessity to investigate the fish in terms of biological, physiological, biochemical, histological and hematological in detail. The survey conducted on fish farming, identification of diseases and parasites in fish is an important issue. Therefore, one of the methods to be used for early detection of diseases has become a subject of concern of researchers in the fish hematology in recent years. For this purpose, comparative studies have carried out on blood samples taken from live fish or anesthetized fish [1-9].

In addition to interpret the hematological findings correctly in fish, it must be taken into account the physiological and biochemical effects of changes in negative environmental factors or pollution. In this context, hematological values in fish are alter with gender, reproductive period, sexual maturity, temperature, dissolved oxygen, pH, some water quality parameters such as calcium, magnesium and nitrate, diseases and other environmental factors[10-14].

In this study, the effects of some water quality parameters such as water temperature, dissolved oxygen, pH, calcium, and magnesium and nitrate values on the hematological values of the *Silurus glanis* caught from Çelik Lake during breeding periods were determined. Thus, in terms of the correct diagnosis of the factors causing the disease, minimize the disease and taking the necessary precautions, hematological findings can be used as prior knowledge in fisheries.

MATERIAL AND METHODS

Research was conducted between April 2013 and October 2013. Live catfish has been supplying from Çelik Lake in Gölbaşı Region of Adıyaman. Çelik Lake (37° 42' 08.5" F, 37° 30' 08.9" D) is located 63 km away from Adıyaman and 17 km west of the Gölbaşı (Figure 1) which used for irrigation and fishing[15]. The maximum depth of the lake, which is 2 km long, is 13.6 m. Its surface area is 1.09 km². Çelik Lake, which has a rich ecosystem, lives important fish including catfish (*Silurus glanis*) and carp (*Cyprinus carpio*) with high economic value [15]. Pinter nets are used when fishing. The measurements of these nets were measured with a steel meter with a length of 5 meters and the material thickness with a digital caliper. In order to determine hematological values of the fish at before breeding, breeding and after the breeding period, some previous references are used [15-19]. For this purpose a total of 51 adult fish were caught. Males (20 individuals) had a weight of 48.5-99 cm and weight of 710-6560 g, while females (31 individuals) were 49.7-96.5 cm and 704-5158 g.



Figure 1. Geographical location of Çelik Lake [20]

Fish were anesthetized with benzocaine (50 mg / L) to obtain blood collection [21,22]. Fish were set up with a towel after anesthesia and taking the blood from the caudal vein with a sharp and fine-tipped syringe, then blood was filled into ethylene diamine tetra-acetic acid (EDTA) tubes [3]. In the counting of blood, thoma slides was used in erythrocyte and leukocyte counts. After determination of erythrocyte and leukocyte counts in 1 mm³ blood, they were examined in immersion lens in light microscope. Hematocrit and leucocrit values were detected with microhaematocrit method using centrifuge. For this, blood was taken into heparinized hematocrit capillary tubes. After centrifugation (12500 rpm 5 minutes), percentages calculated. Also, hemoglobin levels were determined by cyanomethemoglobin method using spectrophotometer [4, 23-26].

The gonads were macroscopically examined to confirm gender of the fish according to the terminology of [4].

The temperature, dissolved oxygen and pH levels of the lake water were measured before, during and after the breeding period of fish. According to this, oxygen meter with 0.01 sensitivity was used to determine water temperature and dissolved oxygen levels, and a pH meter of 0.003 sensitivity was used for determination of pH. Calcium, magnesium and nitrate values were measured by ion exchange chromatography using the method specified by [27].

In this study, ANOVA multivariate Duncan's test was applied using the SPSS 17.0 package program for statistical evaluation of blood parameters between the reproductive periods. Student t-test was applied to statistical comparisons between blood parameters of female, male and all individuals at the same reproductive period. The results are expressed in "A, B, C". The relationship between hematological parameters in the blood samples of the catfish and the data obtained from some water quality values of the lake water depending on reproduction and gender was determined by Pearson's correlation coefficient [27].

RESULTS

Some blood parameter values (hematocrit and leucocrit values, erythrocyte and leukocyte counts, hemoglobin level) of the fish that are examined depending on breeding periods and gender are given in Table 1.

The level of hematocrit between consecutive reproductive periods for male, female and for all was significant ($p < 0.05$) (Table 1). It was determined that the difference in hematocrit level between male and female individuals in the same reproductive period was statistically insignificant ($p > 0.05$) (Table 1). It was determined that the leucocrit ratios of the female, male and all individuals in the same reproductive period were not statistically significant ($p > 0.05$) but for pre- and reproductive period and post-reproductive period were significant ($p < 0.05$).

Erythrocyte counts between consecutive reproductive periods; between the reproductive period and the other periods for female, male and all individuals it was found to be insignificant ($p > 0.05$) but between pre-reproduction period and post-reproductive

period were insignificant ($p > 0.05$) (Table 1). The differences between erythrocyte counts of male, female and all individuals was found to be statistically insignificant ($p > 0.05$) for pre- and reproductive period, but were insignificant for post reproductive period ($p < 0.05$) (Table 1).

Leucocyte counts among consecutive reproductive periods for female were not significant ($p > 0.05$) but was significant between breeding and other periods for male fish ($p < 0.05$). But insignificant between pre- and post-breeding period ($p > 0.05$); between pre- and post-breeding period it was insignificant ($p < 0.05$) for all individuals (Table 1). For all reproductive period, all individuals were statistically insignificant ($p > 0.05$).

Hemoglobin levels for female individuals at pre-reproductive and reproductive period were significant ($p < 0.05$), but no significant at post-reproductive period ($p > 0.05$); for male fish between the all periods were no significant ($p > 0.05$) (Table 1). The difference in hemoglobin amounts of female, male and all individuals at the same reproductive period was statistically insignificant ($p > 0.05$) (Table 1).

Table 1. Comparison of some blood parameter values of pre-breeding (PreB), breeding (B) and post-breeding (PostB) catfish species (mean \pm standard deviation)

		Hematocrit level (%)	Leukocrit level (%)	Eritrocyte count ($\times 10^6/\text{mm}^3$)	Leukocrit count ($\times 10^4/\text{mm}^3$)	Hemoglobin value (g/100ml)
Female+ Male (N=51)	PreB	33.47 \pm 2.55 ^{aA}	2.06 \pm 0.66 ^{aA}	1.35 \pm 0.05 ^{aA}	4.64 \pm 0.48 ^{aA}	6.34 \pm 0.68 ^{aA}
	B	24.40 \pm 7.13 ^{bA}	2.60 \pm 0.91 ^{a,bA}	1.23 \pm 0.12 ^{bA}	4.03 \pm 0.36 ^{bA}	5.97 \pm 0.84 ^{aA}
	Post B	16.95 \pm 4.08 ^{cA}	2.95 \pm 0.97 ^{bA}	1.33 \pm 0.04 ^{aA}	4.38 \pm 0.27 ^{cA}	6.11 \pm 0.95 ^{aA}
Female (N=20)	PreB	33.86 \pm 2.85 ^{aA}	2.00 \pm 0.58 ^{aA}	1.36 \pm 0.05 ^{aA}	4.59 \pm 0.46 ^{aA}	6.43 \pm 0.40 ^{aA}
	B	24.83 \pm 9.45 ^{bA}	2.83 \pm 0.75 ^{bA}	1.16 \pm 0.13 ^{bA}	4.17 \pm 0.31 ^{aA}	5.63 \pm 0.79 ^{bA}
	Post B	16.29 \pm 3.59 ^{cA}	2.43 \pm 0.53 ^{a,bB}	1.29 \pm 0.03 ^{aB}	4.27 \pm 0.22 ^{aA}	5.77 \pm 0.59 ^{a,bA}
Male (N=31)	PreB	33.20 \pm 2.44 ^{aA}	2.10 \pm 0.74 ^{aA}	1.34 \pm 0.04 ^{aA}	4.68 \pm 0.51 ^{aA}	6.27 \pm 0.83 ^{aA}
	B	24.11 \pm 5.73 ^{bA}	2.44 \pm 1.01 ^{a,bA}	1.28 \pm 0.09 ^{bA}	3.94 \pm 0.37 ^{bA}	6.19 \pm 0.84 ^{aA}
	Post B	17.33 \pm 4.44 ^{cA}	3.25 \pm 1.06 ^{bC}	1.35 \pm 0.03 ^{aC}	4.44 \pm 0.28 ^{aA}	6.31 \pm 1.09 ^{aA}

a, b, c: The same group in the column is no significant with the same letter ($P > 0.05$); A, B: Between consecutive gender groups are no significant with the same letter ($P > 0.05$).

Some water quality values of Çelik Lake are shown in Table 2.

Table 2. Some water quality values of the lake water are cited.

Parameters	Pre-reproductive period (April)	Reproduction period (June)	Post-reproductive period (October)
Temperature (°C)	17.2	25	18.8
Oxygen (mg/L)	9.76	7.40	8.63
pH	7.65	8.32	7.95
Calcium (mg/L)	41.55	30.71	47.81
Magnesium (mg/L)	18.48	15.22	19.37
Nitrate (mg/L)	0.566	0.125	0.310

The correlation coefficients (r) determined in the correlation analysis between the data obtained from these water quality values and the hematological parameters of the cat fish are also given in Table 3.

According to the statistical analysis between the water temperature and the hematological parameters of the catfish (*S. glanis*), there was no significant difference between temperature and hematocrit value, leukocyte count and hemoglobin amount for female fish ($p > 0.05$). On the other hand, the difference between temperature and leucocrit value ($p < 0.05$) and erythrocyte count was significant ($p < 0.01$). For male fish, there was no significant difference between temperature and haematocrit value, leucocrit value and hemoglobin level ($p > 0.05$). On the other hand, the difference between the temperature and the number of erythrocytes ($p < 0.05$) and leukocyte count was significant ($p < 0.01$). Statistical analysis showed that there was no significant difference ($p > 0.05$) between oxygen levels and the haematocrit value for female catfish.

There was no significant difference between the oxygen level and leucocrit value and hemoglobin amount for male fish ($p > 0.05$). In contrast, the differences between the oxygen level and the haematocrit value ($p < 0.01$), the number of erythrocytes ($p < 0.05$) and the number of leucocytes were significant ($p < 0.01$).

Statistical analysis revealed that there was no significant difference between pH and hematocrit value for female fish ($p > 0.05$). In contrast, the difference between pH and leucocrit value ($p < 0.05$), erythrocyte count ($p < 0.01$), leukocyte count and hemoglobin amount was significant ($p < 0.05$). For male fish, there was no significant difference between pH and leucocrit value and hemoglobin level ($p > 0.05$). In contrast, the difference between pH and haematocrit value ($p < 0.05$), erythrocyte count ($p < 0.05$) and leukocyte count was significant ($p < 0.01$).

Table 3. Some water quality values of the lake water are cited.

Water quality parameters	Gender	Number of Sample	Hematological parameters				
			Hematocrit value (%)	Leucocrit value (%)	Eritrocyte number ($\times 10^6/\text{mm}^3$)	Leukocyte number ($\times 10^4/\text{mm}^3$)	Hemoglobin amount (g/100ml)
Temperature (°C)	F	20	-0.177	0.474*	-0.751**	-0.392	-0.405
	M	31	-0.202	-0.024	-0.439*	-0.610**	-0.047
Oxygen (mg/L)	F	20	0.413	-0.505*	0.751**	0.461*	0.491*
	M	31	0.457**	-0.133	0.371*	0.604**	0.035
pH	F	20	-0.387	0.504*	-0.755**	-0.455*	-0.484*
	M	31	-0.430*	0.115	-0.381*	-0.608**	-0.037
Calcium (mg/L)	F	20	-0.295	-0.294	0.555*	0.167	0.143
	M	31	-0.296	0.291	0.440*	0.457**	0.055
Magnesium (mg/L)	F	20	-0.161	-0.360	0.636**	0.242	0.229
	M	31	-0.157	0.221	0.456**	0.520**	0.054
Nitrate (mg/L)	F	20	0.493*	-0.504*	0.731**	0.475*	0.512*
	M	31	0.544**	-0.191	0.334	0.585**	0.029

Insignificant ($p > 0,05$), *Significant ($p < 0,05$), ** Significant ($p < 0,01$), negative Correlation (-), Female (F), Male (M)

The statistical analysis of the relationship between the calcium values of Çelik Lake and the haematological parameters of the catfish revealed that there was not significant difference between the amount of calcium and haematocrit value, leucocrit value, leucocyte count and hemoglobin amount for female fish ($p > 0.05$). In contrast, the difference between the amount of calcium and the number of erythrocytes was significant ($p < 0.05$). For male fish, there was no significant difference between the amount of calcium and haematocrit value, leucocrit level and hemoglobin level ($p > 0.05$). On the other hand, the difference between the amount of calcium and the number of erythrocytes ($p < 0.05$) and the number of leukocytes was significant ($p < 0.01$).

There was no significant difference between magnesium amount and hematocrit value, leucocrit value, leucocyte count and hemoglobin amount for female fish ($p > 0.05$) when the relationship between magnesium levels in water of Çelik Lake and haematological parameters of catfish were statistically analyzed. However, the difference between the amount of magnesium and the number of erythrocytes was significant ($p < 0.01$). For male fish, there was no significant difference ($p > 0.05$) between magnesium amount and hematocrit value, leucocrit value and hemoglobin amount. In contrast, the difference between the amount of magnesium and the number of erythrocytes and leukocytes was significant ($p < 0.01$).

The difference between the nitrate amount and the number of erythrocytes haematocrit value, leucocrit value, leucocyte count and hemoglobin amount was found to be significant ($p < 0.01$). There was no significant difference between nitrate amount and leucocrit value, erythrocyte count and hemoglobin amount for male fish ($p < 0.01$). In contrast, the difference between nitrate amount and hematocrit value and leucocyte count was significant ($p < 0.01$).

DISCUSSION

In this study, pre-reproductive hematocrit values were determined as 33.47% for all individuals of (*S. glanis*), and this value was determined as 24.40% and 16.95% in reproductive period and post-reproductive period, respectively. Hematocrit values were

found to be 29.75 %, 19.3% and 22.3% 3.4 and 20% 8. It is believed that the differences in haematocrit values in all three periods examined were due to differences in fish blood sampling periods. In this study, leucocrit levels were determined as 2.06%, 2.60% and 2.95% in reproductive period, pre-reproduction and post-reproductive period, respectively. Different blood parameters were previously investigated in studies on catfish, but no evidence of leucocrit was found. For this reason, leucocrit values in this study are thought to contribute to the work to be done later.

Kocabatmaz and Ekingen [3,4] determined the number of erythrocytes in $1.00 \times 10^6 / \text{mm}^3$ to $1.38 \times 10^6 / \text{mm}^3$ and these values were $1.40 \times 10^6 / \text{mm}^3$ in Docan [8]. In this study, it was determined that erythrocyte counts were $1.35 \times 10^6 / \text{mm}^3$ for male and $1.33 \times 10^6 / \text{mm}^3$ for female fish before and after reproductive period respectively, and these values were similar to the studies of the mentioned above. However, the number of erythrocytes in the reproductive period was reported to be $1.23 \times 10^6 / \text{mm}^3$. In this study, leucocyte counts of the pre-breeding, breeding and post-breeding period were determined as $4.64 \times 10^4 / \text{mm}^3$, $4.03 \times 10^4 / \text{mm}^3$, and $4.38 \times 10^4 / \text{mm}^3$, respectively. Leucocyte counts were determined as $4.15 \times 10^4 / \text{mm}^3$ [3], $4.60 \times 10^4 / \text{mm}^3$ [4] and $17.0 \times 10^4 / \text{mm}^3$ [6]. The leucocyte counts obtained in this study were in agreement with some of the above mentioned studies, and they were in contrast to some others. It is believed that the cause of these differences may be due to factors such as age and size of the fish, environmental conditions and physiological status of the fish.

Hemoglobin levels of pre-breeding, reproductive and post-breeding period were determined as 6.34 g / 100 ml, 5.97 g / 100 ml and 6.11 g / 100 ml, respectively. In a study carried out on catfish [4], the amount of hemoglobin was found to be 6.06 g / 100 ml, and this value correlated with the amount of hemoglobin in the breeding period in this study. In other studies, these values were determined as 7.0 g / 100 ml [8] with 4.4 g / 100 ml 3, 9.02 g / 100 ml [6].

According to this study, when the water temperature is increased; hematocrit value, erythrocyte and leucocyte counts and hemoglobin levels decreased and leucocrit levels increased in pre-breeding and breeding periods. Leucocyte counts and hemoglobin levels increased and hematocrit and leucocrit values and erythrocyte counts decrease with decreasing water temperature after reproductive period. A decrease in hematocrit values (31.8% and 43.5%), erythrocyte counts (0.964 and $1.318 \times 10^6 / \text{mm}^3$) and hemoglobin amounts (1.36 g / 100 ml) were found to be related to the increase in water temperature before and during reproductive period. Hemoglobin values ($1.391 \times 10^6 / \text{mm}^3$) and hematocrit values (39.3%) and erythrocyte counts ($1.191 \times 10^6 / \text{mm}^3$) decreased after the reproductive period [11]. Şahan and Cengizler [13] found that the amount of hemoglobin decreased (6.39 g / 100 ml) due to the increase in water temperature and increased (7.33 g / 100 ml) depending on the drop of the water. In another study, erythrocyte counts ($0.89 \times 10^6 / \text{mm}^3$) and hematocrit values (42.60%) decreased according to the water temperature decrease [14]. The findings obtained by all the researchers mentioned above comply with our findings. However, it has been emphasized in other researches that there is an increase in leukocyte counts with the increase of water temperature and there is no significant effect of water temperature on hematocrit values, hemoglobin amounts and erythrocyte counts [29, 30]. In a study carried out by Joshi [5], hemoglobin levels (8.18 – 10.95 g / 100 ml), erythrocyte counts ($2.75 - 4.45 \times 10^6 / \text{mm}^3$) 100 ml) and hematocrit values (40.85% - 44.27%) increased with increasing water temperature in pre-breeding period (March-June), but observed no increase in erythrocyte counts and hematocrit values in breeding period (July-September). According to this result, there are differences between the research findings obtained by [5] and our findings. It is thought that these differences may be due to the size of the fish, habitat, fish species, breeding periods, seasons and other environmental conditions.

In this study, when the pH values increased; hematocrit values, erythrocyte and leucocyte counts and hemoglobin levels are decreased and leucocrit levels were increased in breeding and pre reproductive period. But after reproductive period leucocyte counts and hemoglobin levels increased with decreasing pH values and hematocrit and leucocrit values and erythrocyte counts decreased. In a study, hematocrit values (31.8 - 43.5%), erythrocyte

counts ($0.964 - 1.318 \times 10^6 / \text{mm}^3$) and hemoglobin levels ($1.36 \text{ g} / 100 \text{ ml}$) decreased with increasing pH values in pre-reproductive and reproduction period. However hemoglobin amounts increased ($1.48 \text{ g} / 100 \text{ ml}$) [11]. It was determined that hematocrit values and erythrocyte counts in our study are opposite to other studies, depending on the increase or decrease of the pH values, and other findings are similar.

In another study, hematocrit values ($26.50 - 31.50$), erythrocyte counts ($1.540 - 1.820 \times 10^6 / \text{mm}^3$), leucocyte counts ($4.04 - 4.66 \times 10^4 / \text{mm}^3$) and hemoglobin amounts ($8.20 - 9.60 \text{ g} / 100 \text{ ml}$) were found to be increased [7]. In addition, Cengizler and Şahan Azizoğlu [12] investigated some blood parameters in mirror carp living in Seyhan Dam Lake and reported that there was a decrease in erythrocyte ($1.322 - 2.997 \times 10^6 / \text{mm}^3$) and leukocyte ($13.385 - 17.045 \times 10^6 / \text{mm}^3$) counts with decreasing pH. According to these results, there are differences between Örün et al. [7] and Cengizler and Şahan Azizoğlu [12] findings and our findings. All these differences are thought to be due to fish species, fish size, age difference and life of fish.

When the amount of oxygen decreased, haematological parameters decreased before and after reproductive period. After reproductive period decrease in hematocrit and leucocrit levels and increase in erythrocyte and leucocyte counts and hemoglobin levels were seen with increasing oxygen level. Docan et al. [8] showed a decrease in the amount of hemoglobin ($7.17 \text{ g} / 100 \text{ ml}$) due to decreasing dissolved oxygen level and increase in hemoglobin amounts ($7.80 \text{ g} / 100 \text{ ml}$) with increasing the amount of dissolved oxygen. Cengizler and Şahan Azizoğlu [12] reported a decrease in erythrocyte ($1.322 - 2.997 \times 10^6 / \text{mm}^3$) and leucocyte ($13.385 - 17.045 \times 10^6 / \text{mm}^3$) numbers with decreasing dissolved oxygen in Seyhan Dam Lake. Another study conducted by Şahan and Cengizler [13], both of hemoglobin ($6.39 \text{ g} / 100 \text{ ml}$) and leucocyte counts ($2.54 \times 10^4 / \text{mm}^3$) decreased due to the decrease of the dissolved oxygen amount. In addition to, the authors showed that there were an increase in erythrocyte counts ($2.61 \times 10^6 / \text{mm}^3$) and hemoglobin amounts ($7.13 \text{ g} / 100 \text{ ml}$) and a decrease in hematocrit values (47.42%) due to the increase in the amount of dissolved oxygen. The findings obtained by all of the above researchers are in accordance with our findings.

In this study, leucocrit levels increased but erythrocyte counts, hematocrit values, leukocyte counts and hemoglobin levels decreased when calcium, magnesium and nitrate values decreased before and during reproductive period. Decrease in hematocrit and leucocrit with increasing the calcium, magnesium and nitrate levels after the reproductive period; and increase in erythrocyte, leucocyte counts and hemoglobin levels were observed [28]. In another study, a decrease in hematocrit values ($31.8 - 43.5\%$), erythrocyte counts ($0.964 - 1.318 \times 10^6 / \text{mm}^3$) and hemoglobin amounts ($1.36 \text{ g} / 100 \text{ ml}$) were determined according to the decrease in calcium levels before and during the reproductive period and an increase in hemoglobin levels ($1.48 \text{ g} / 100 \text{ ml}$) and hematocrit values (39.3%) and erythrocyte counts ($1.191 \times 10^6 / \text{mm}^3$) with increasing calcium levels were reported after the reproductive period [11]. Örün et al. [7] and Jerônimo et al. [29] reported that an increase in hemoglobin levels ($8.20 - 9.60 \text{ g} / 100 \text{ ml}$), erythrocyte ($1.540 - 1.820 \times 10^6 / \text{mm}^3$) and leucocyte counts ($4.04 - 4.66 \times 10^4 / 100 \text{ ml}$) and hematocrit values ($26.50\% - 31.50\%$) occurred with increasing calcium values. The findings of the researchers mentioned above are parallel to our findings.

CONCLUSION

As a result; it was determined that blood parameter values of *S. glanis* were influenced by some water quality parameters such as water temperature, dissolved oxygen amount, pH, calcium, magnesium and nitrate values. In evaluating the blood parameters, it is also necessary to consider the age and size of the fish, breeding periods, gender, seasons, physiology of the fish, habitat and environmental conditions. Catfish is an economically important species in inland waters and haematological studies on this species are expected to shed light on biological, ecological and parasitological studies to be carried out later on for other species to be cultivated.

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