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Research Article

Effects of Total Suspended Solids at Different Levels on the Eggs and Larvae of Endemic Fish, Tarek (*Alburnus tarichi* Güldenstädt, 1814) in the Karasu River (Van, Turkey)

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ABSTRACT

The Karasu River (Van, Turkey) is one of the most important sources of intensive reproduction for Tarek (*Alburnus tarichi* Güldenstädt, 1814) in the Van Lake basin. However, sand pits and sand extraction activities in the Karasu River threaten the spawning areas of *A. tarichi*. This study aimed to investigate how increased sediment concentration in the Karasu River affects the eggs and larvae of *A. tarichi* during the reproductive period. The study in the laboratory was designed by measuring total suspended solids and turbidity associated with the river over two years for five different experimental groups (corresponding to 0, 10, 50, 100, and 600 mg L^{-1}, and associated groups: control group, Group 1, Group 2, Group 3, and Group 4, respectively). The percentage of hatched out larvae was statistically different between the groups ($p<0.05$). The highest percentages of hatched out larvae were found in the control group (73.98±5.89%) and Group 1 (68.05±2.96%). The highest survival rates in larvae were determined in the control group (53.35±5.62%) and Group 1 (44.98±4.41%). These results demonstrate that *A. tarichi* is sensitive to suspended solid concentrations that are very common in the natural environment. In conclusion, sand pits and sand extraction activities must be stopped completely during the reproductive period of *A. tarichi* between May and July and strict controls should be put in place during this time. These measures will provide an important contribution to ensuring the continuity of this species.

Keywords: Destruction zones, sand extraction activities, sand pits, sediment effects, turbidity

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INTRODUCTION

Alburnus tarchi (Güldenstädt, 1814) is endemic to the Van Lake Basin (Elp et al., 2014, 2016; Sen et al., 2015). Considering the biological characteristics of this species, *A. tarichi* migrates to the rivers from Van Lake for the purpose of reproduction and shows mass distribution in the rivers between May 15 and June 15 (Cetinkaya et al., 2014). Moreover, tons of A. tarichi are caught from Van Lake every year during the fisheries season and offered for human consumption (TUIK, 2020).

Sediment transport, suspension and settlement play important roles in water quality and aquatic lives. Because of their influence on density, light penetration, and nutrient availability, total suspended solids effects eutrophication processes, biological and chemical reaction rates in the water column (Sutherland & Meyer, 2007; Jin & Ji, 2013).

The Karasu River is one of the most important sources of intensive breeding for *A. tarichi*. However, it has been reported that this density is reducing each passing year (Elp et al., 2006). In the first half of 2000s, there was over 90% reproductive success compared to today. In the Van Lake basin, sand pits and sand extraction activities in the Karasu River, threaten the reproductive areas of *A. tarichi*. Due to sand extraction activities, the silt forms a sediment that slowly settles at the river bed and then passes into the form of total suspended solids in the water. During this sedimentation process, silt accumulates on the eggs and hatching of the eggs is prevented (Atici et al., 2018).

It was seen that sand has been extracted from the river beds of Hosap, Ilıca, Karmuc and Zilan as well as the Karasu River. Also the structure of the river bed had been damaged by sand extraction and produced observable turbidity in the river. Sand washing activities in sand pits also affects the water turbidity. On the other hand, while the sand extraction activities are stopped due to snow in winter, they are reactivated with the arrival of spring and the warming of the weather. These activities take place in the spring during the breeding migration of *A. tarichi* in the Karasu River (Sen et al., 2015). Because of sand extraction from the Karasu River, mass fish mortality was observed in 2003 to 2004, especially in the months of May to June (Elp et al., 2006).

Studies on sand pits and sand extraction effects have shown that these activites are very harmful for the ecosystem. There are many sand pits in Karasu River and increasing sand extraction activities in spawning aeras threaten the amount of *A. tarichi* catching in Van Lake (Atici et al., 2018).

On the other hand, while there are many studies that report on bioecology, life history, environmental conditions, morpholgy, systematic characters, population structure, growth, condition, reproduction biology, feding, stock assessment, fisheries management and histologic studies on *A.tarichi*, there are no data about the effects of suspended solid concentrations on the population of *A. tarichi*. This study was performed to determine how increased suspended sediment concentration in the Karasu River affects the eggs and larvae of the *A. tarichi* during the reproductive period.

MATERIALS AND METHODS

Migration area

Van Lake (pH 9.81, 22‰ total salinity), is the largest soda-lake on Earth, present an extreme living environment to which comparatively few orgasims have adapted. The only commercial fish species known to occur in Van Lake is the anadromus cyprinid *A. tarichi* (Danulat & Sekcuk, 1992). Since the freshwater environment is needed for the eggs to hatch, they must migrate to the Karasu River (pH 8.36, 0.31‰ total salinity). Karasu River, which sources from the Turkey - Iran border, is poured into Van Lake from the Citoren Reeds (38°34′51.54″N - 43°13′21.42″E) to the north of the Van center (Figure 1). There are Sarımehmet Dam and Satibey Regulator built in 1991 for irrigation purposes on the river (Cetinkaya et al., 1994).

Experimental design

This study was carried out during the reproductive period of the *A. tarichi* between May 2016 and August 2016. During the experimental period, temperature, oxygen and pH parameters were measured with a multimeter (HACH model DR-5000, Loveland, USA). The total suspended solids were analyzed using a spectrophotometer (HACH model HQ-40d, Loveland, USA) and turbidity was measured in nephelometric turbidity unit (NTU) with a turbidy meter (HACH model 2100-Q, Loveland, USA).

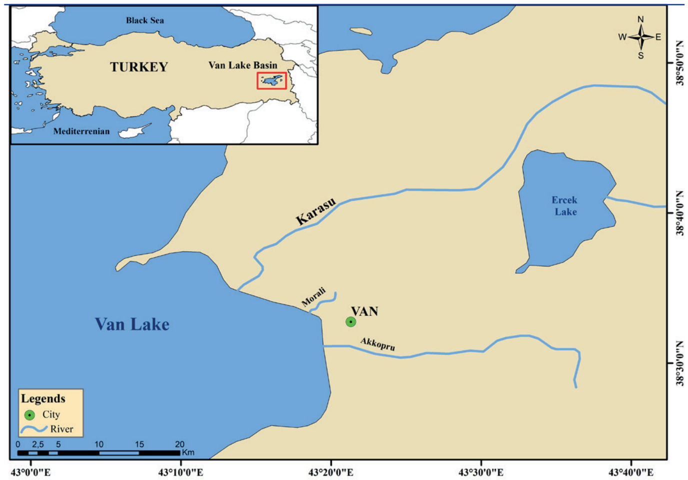

Figure 1. Map of the Karasu River in the Van Lake basin.

Sediment suspension apparatus were modified from Sutherland (2005, 2006) and Sutherland and Meyer (2007). The all experiments were carried out in 10 experimental units, each composed of a 30 liter glass aquarium (50 × 20 × 30 cm) and a motor-driven paddle which moves slowly (~3 – 5 mm sec^{-1}) back and forth along the bottom of the aquarium (Figure 2). The paddle was traveled along the bottom, sediment that has settled, was resuspended. To aid in resuspension, air lines have been attached to each paddle allowing it to function as a slow moving air diffuser.

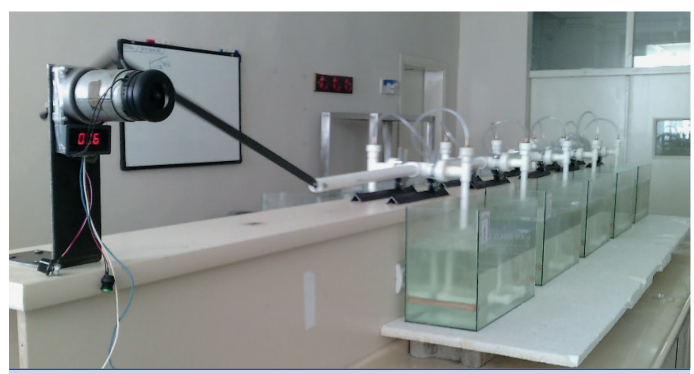

Figure 2. Sediment suspension apparatus used in the experiments: drive mechanism, drive shaft cross coupling, and arm (Original).

The study in the laboratory was designed by measuring total suspended solids and turbidity associated with the river over two years (Atici et al., 2018) for five different experimental groups (corresponding to 0, 10, 50, 100, and 600 mg L^{-1}, and associated groups: C group, G1, G2, G3, and G4, respectively). Suspended sediment treatments were set up with 2 replicates of 5 inorganic sediment concentrations (Figure 3). Natural sediment used in trials was collected from Karasu River.

Egg trials

The mature female and male *A. tarichi* were taken from the Karasu River in May 2016. The eggs and sperms were stripped from the spawner artifically and fertilized by the dry method under labora-

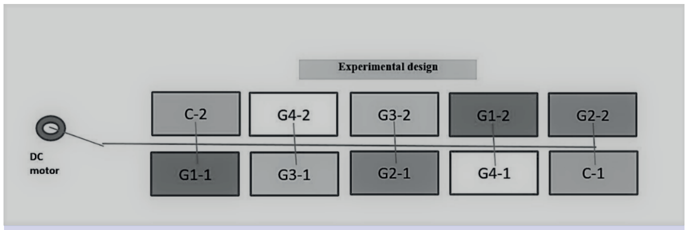

Figure 3. The experimental design in the egg and larval experiments (Original).

tory conditions. Fertilized eggs ranged from 329-344 were placed in the incubator of trial aquariums (i.e. 5 treatments × 2 tanks per treatment level × 3 trials × 329-344 egg / hatchery). For each egg experiment, a total of 10 incubators (20 × 14.3 × 4.5 cm) were used. The incubators were placed at 10 cm above the bottom of trial aquariums. Egg experiments lasted 7 days and the average temperature, dissolved oxygen, and pH were found to be: 22.1 ± 0.04 ˚C, 8.43 ± 0.00 mg L^{-1} and 8.41 ± 0.00, respectively.

Sediment accumulation at hatchery were measured to the nearest 0.01 mm using a digital caliper. The percentage values of hatched out eggs (HE), dead larvae (DL) and live larvae (LL) were calculated using the equations 1, 2, and 3, respectively.

HE (%) = the number of dead larvae (%) + the number of live larvae (%) (1)

DL (%) = [the number of dead larvae ÷ the total values of fertilized eggs]×100 (2)

LL (%) = [the number of live larvae ÷ the total values of fertilized eggs]×100 (3)

Larvae trials

The larval experiments were carried out with larvae hatched from eggs of *A. tarichi* in the laboratory. The fertilized eggs were incubated in at 18 ± 1 °C incubators for approximately 4 days. On the 5th day after hatching, larvae were fed with commercial feed (45% crude protein, 4% crude fat and 3% crude fiber) twice a day and the water in the tanks was changed daily. After acclimation, the larvae were exposed to sediment treatments for 14 days. The same procedures used in the first larval experiment were also performed for the second larval trial.Two 14-day trials were conducted using 1-1.5 month old post larvae. In each of two trials, 300 lab-reared *A. tarichi* were randomly chosen from holding tanks and placed in the experimental aquariums (i.e., 30 fish per tank × 2 tanks per treatment level × 5 treatments × 2 trials = 600 fish). Before each trial began, the fish were allowed to acclimate to the apparatus for 48 hours. The mean temperature, dissolved oxygen, and pH were found as 23.0 ± 0.01 °C, 8.43 ± 0.00 mg L^{-1} and 8.40 ± 0.00, respectively.

The initial weight and final weight of the larvae were measured on scales to a precision of 0.0001 g. The number of dead larvae (DL) was determined by equation 4. The survival rate (SR) was calculated using the equation 5 (Pechsiri & Yakupitiyage, 2005).

The number of DL= the total number of larvae -the number of live larvae (4)

SR (%)= [number of live fish÷initial number of fish]×100 (5)

Statistical analysis

A statistical software package, SPSS 21.0, was used to statistically evaluate the data obtained at the end of the experiment. A one-way ANOVA analysis was applied and the difference between the averages was assessed by the Duncan Multiple Comparison Test at a significance level of 0.05. The results are expressed as the mean ± standard deviations (SD). Pearson's rank correlation was used to establish relationships between parameters (Uzgoren, 2012).

RESULTS AND DISCUSSION

The total suspended solids (TSS) in water are known to have an effect on the reproduction, growth and nutrition of fish such as salmon, trout, carp, bass, herring, anchovy, and killifish (Newcombe & Jensen, 1996; Barton, 2002). Sand pits and sand extraction activities in the water column can result in increased TSS levels. However, although the source and the effect of TSS are known, these activities need to be expressed numerically. In order to sustain the Karasu River as a breeding environment for *A. tarichi*, there is a need for these studies.

The average sediment accumulations in the egg experiments were recorded as 0.00 ± 0.00 cm (control group), 0.31 ± 0.07 cm (G1), 0.55 ± 0.12 cm (G2), 1.35 ± 0.12 cm (G3) and 1.70±0.17 cm (G4) at the end of the 7th day (Table 1). The percentages of hatched eggs (HE) were highest in the control group (73.98 ± 14.43%) and in G1 (68.05 ± 7.25%), while the rate of hatched eggs in G2 (44.62 ± 21.85%), G3 (8.14 ± 5.09%) and G4 (2.87 ± 2.50%) decreased with sediment accumulation. A negative correlation was found between TSS and the number of hatched eggs, and there was a positive relationship with the sediment accumulation (Table 2). These results show that the rate of hatching decreases with an increasing amount of TSS. The duration and degree of exposure to TSS are important factors to consider in determining effects on aquatic organisms (Berry et al., 2003). In another study, salmon (*Oncorhynchus keta*) eggs were exposed to 97 mg L^{-1} of TSS for 117 days resulting in a mortality rate of 77% (Langer, 1980), while rainbow trout (*Oncorhynchus mykiss*) eggs were exposed to 57 mg L^{-1} of TSS for 62 days, resulting in a mortality rate of 47% (Slaney et al., 1977). In addition, it was observed that egg development was slowed and hatching was delayed in striped bass (*Morone saxutilis*) and white perch (*Morone americana*) exposed to 800 mg L^{-1} TSS for 24 hours (Morgan et al., 1983). Sutherland (2005) reported whitetail shiner (*Cyprinella galactura*) eggs in the experimental group exposed to 500 mg L^{-1} TSS could not be opened. In our study, hatching percentages were found to be very low in G2, G3 and G4. It was determined that this situation was related with the amount of sedimentation on eggs at the high TSS levels (100-600 mg L^{-1}) for 7 days.

In other studies, mean TSS were reported as 212.5 mg L^{-1} at Karasu River (Atici et al., 2018), 11.8 mg L^{-1} at Catakdibi (Aydın, 2018), 18.5 mg L^{-1} at Donerdere, 6.5 mg L^{-1} at Yumruklu, 110 mg L^{-1} at Dolutas, and 44 mg L^{-1} at Degirmigol Ponds (Atici, 2020) in Van Lake basin. In the measurements made using sediment trays to determine sediment accumulation in the Karasu River has accu-

Table 1. The total number of fertilized eggs (FE), the percentage values of hatched out eggs (HE), dead larvae (DL) and live larvae (LL) and the values of sediment accumulation (SA) in three trials (Mean ± SD).

	Groups				
	C	G1	G2	G3	G4
FE	344.67±10.51	329.17±17.51	329.83±12.15	331.83±10.61	331.00±13.64
HE (%)	73.98±14.43^{a}	68.05±7.25^{a}	44.62±21.85^{b}	8.14±5.09^{c}	2.87±2.50^{c}
DL (%)	12.38±7.25^{b}	16.51±6.81^{b}	17.94±5.32^{b}	3.42±2.06^{a}	0.76±0.51^{a}
LL (%)	61.61±18.44^{c}	51.54±13.01^{c}	26.68±23.47^{b}	4.72±6.00^{a}	2.11±2.65^{a}
SA (cm)	0.00±0.00^{a}	0.31±0.07^{b}	0.55±0.12^{c}	1.35±0.12^{d}	1.70±0.17^{e}

In the same column, the differences between the values indicated by the same letters are statistically insignificant (p>0.05) and the differences between the different letters are significant (p<0.05).

mulated an average of 3.23±0.35 cm sediment at the end of the 3rd day (Atici et al., 2018). The present study, the highest sediment accumulations in the egg experiments were recorded as 1.35 ± 0.12 cm in G3 and 1.70±0.17 cm in G4 at the end of the 7th day. Due to the excess amount of sediment carried in the study conducted in Karasu River, approximately twice as much sediment was accumulated in the half time of the current study.

It has been observed that increased sedimentation was effective in reduced the success of hatching (Figure 4).

At the beginning of the experiment, there were 30 larvae in all groups (Table 3), but at the end of the experiment there was a statistically significant difference between the dead larva groups ($p<0.05$). The highest numbers of dead larvae were in G2 (26.0 ± 2.0), G3 (28.0 ± 2.0) and G4 (29.0 ± 2.0), while the lowest values were in the control group (14.0 ± 4.0) and G1 (17.0 ± 4.0).

The survival rates in the groups showed statistically significant differences ($p<0.05$) (Table 3). The survival rate was the highest in the control group (53.35 ± 11.24%) followed by G1 (44.98 ± 8.82%). The survival rate was comparatively less in the G2 (13.33 ± 2.74%), G3 (7.50 ± 6.34%) and G4 (2.50 ± 3.20%) groups. The larval survival rate was reported as 94.3% at 25 mg L^{-1} TSS in Arctic grayling (*Thymallus arcticus*), 58% at 500 mg L^{-1} TSS in striped sea bass (*Morone saxatilis*), and 82% and 64% at 100 and 500 mg L^{-1} TSS respectively, in American shad (*Alosa sapidissima*) (Auld & Schubel, 1978; Berry et al., 2003). In increasing amounts of sediment, mucus and sediment accumulates in the gills of fish, the gill epithelium thickens, respiratory function is reduced and the fish epidermis is affected by mechanical damage (Goldes, 1998). The highest larval survival rate for *A. tarichi* was determined to be in the control group and G1, which had the lowest level of TSS. Our study is consistent with other studies, which show that as the amount of TSS increases, the survival rate of the larvae decreases (Table 3).

There was a statistical difference between the final live weights of the larvae in the groups ($p<0.05$). The final live weights were measured as 0.50 g (control group), 0.37 g (G1), 0.16 g (G2), 0.13 g (G3), and 0.06 g (G4), respectively.

A negative correlation was found between the increase in the amount of TSS and the survival rate. However, there was a positive relationship between TSS and the number of dead larvae (Table 4).

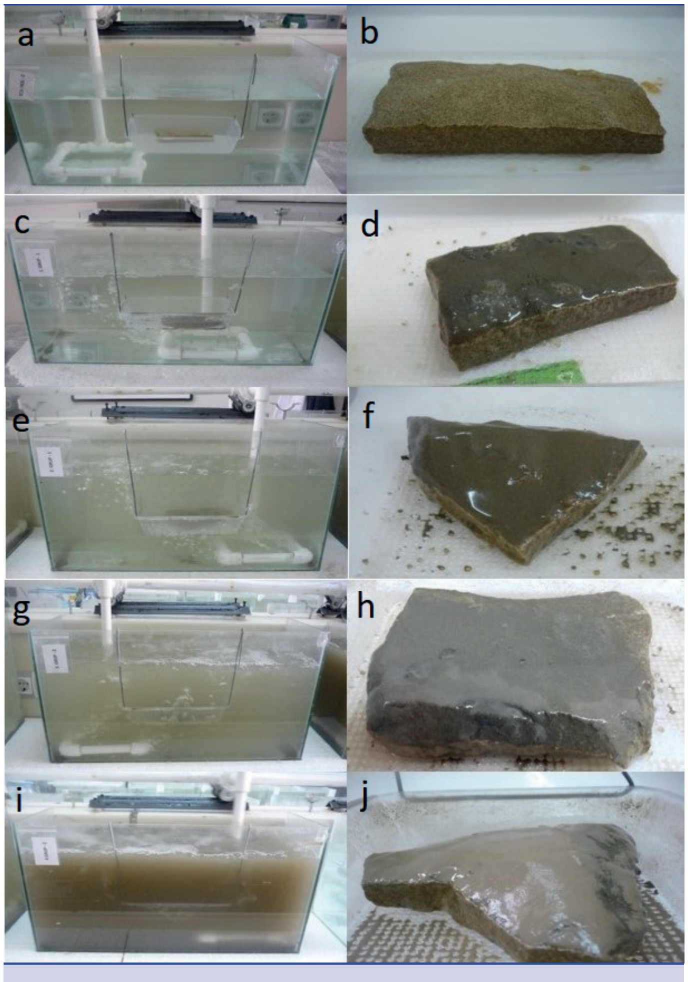

Figure 4. Sediment accumulation in the egg experiments: a. experiment of control group, b. sediment accumulation in control group, c. experiment of G1, d. sediment accumulation in G1, e. experiment of G2, f. sediment accumulation in G2, g. experiment of G3, h. sediment accumulation in G3, i. experiment of G4, j. sediment accumulation in G4 (Original).

Table 2. The correlation results in egg experiments.

Egg Experiment	TSS	Turbidity	LL	DL	HE	SA
TSS	1	0.999**	-0.564**	-0.660**	-0.654**	0.800**
Turbidity	0.999**	1	-0.561**	-0.660**	-0.651**	0.797**

*: p<0.05 significant, **: p<0.01 significant.

Table 3. The total number of larvae (TNL), the number of dead larvae (DL), the initial weight (IW), the final live weight (FW) and the survival rate (SR) in the two larval experiments (Mean ± SD).

	Groups				
	C	G1	G2	G3	G4
TNL	30.0	30.0	30.0	30.0	30.0
DL	14.0 ± 4.0^a	17.0 ± 4.0^a	26.0 ± 2.0^b	28.0 ± 2.0^b	29.0 ± 2.0^b
IW (g)	0.31 ± 0.06	0.30 ± 0.04	0.29 ± 0.08	0.28 ± 0.08	0.26 ± 0.04
FW (g)	0.50 ± 0.10^c	0.37± 0.06^b	0.16 ± 0.06^a	0.13 ± 0.08^a	0.06 ± 0.02^a
SR (%)	53.35 ± 11.24^b	44.98 ± 8.82^b	13.33 ± 2.74^a	7.50 ± 6.34^a	2.50 ± 3.20^a

In the same column, the differences between the values indicated by the same letters are statistically insignificant (p>0.05) and the differences between the different letters are significant (p<0.05).

Table 4. The correlation results in larvae experiments.

	TSS	Turbidity	DL	SR
TSS	1	1.000**	0.580*	-0.580*
Turbidity	1.000**	1	0.582*	-0.582*

*: p<0.05 significant, **: p<0.01 significant.

CONCLUSION

The results obtained in this study generally emphasized the effects of TSS concentrations on *A. tarichi* eggs and larvae. Studies on sand pits and sand extraction effects have shown that these activities are very harmful for the ecosystem. While there are many studies that report TSS concentrations effects for aquatic organisms, there are no data on *A. tarichi*. *A. tarichi* migrates to Karasu River lays 8500 - 9000 sticky eggs in shallow and vegetative areas (Elp, 1996) (Figure 5a). However, there are many sand pits in Karasu River and increasing sand extraction activities in spawning aeras threaten to *A. tarichi* (Figure 5b). So, this study has demonstrated under laboratory conditions how increased TSS may directly affect *A. tarichi*.

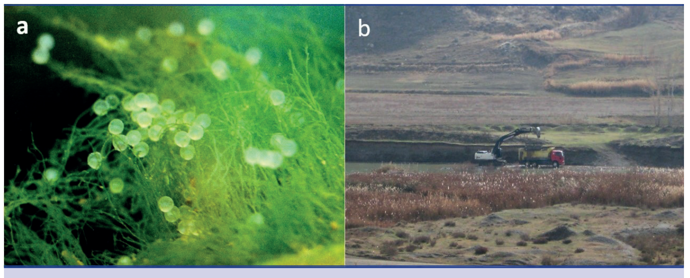

Figure 5. a. sticky eggs on vegetative structure, b. sand extraction process in Karasu River (Original).

In our study, TSS concentrations ranged from 100 to 600 mg L^{-1} were determined to have a damaging effect on the eggs and the larval survival rates were significantly lower at the these high treatment levels. These results demonstrate that *A. tarichi* is sensitive to suspended solid concentrations that are very common in the natural environment. It is necessary to take various measures to sand extraction and the sand pits activities. Sand extraction activities in the reproductive regions should be prohibited during the reproductive period of *A. tarichi* between May and July and sand pits should be consciously planned when selecting their locations.

Conflict of interest: The authors declare no conflict of interest.

Ethics committee approval: Final report of the research project detailed above was approved by Van Yuzuncu Yil University Animal and Research Local Ethics Committee in the session held on 25.05.2017 (decision number 2017/05).

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