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RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

Effects of ceramic balls on trout welfare during their live transfer conditions

Canlı balık transferi süresince kullanılan seramik topların alabalık refahı üzerine etkisi

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Abstract: The effects of ceramic balls on live transfer conditions of rainbow trout (*Oncorhynchus mykiss,* (Walbaum,1792)) were determined by examining ammonia, pH, dissolved oxygen, and temperature of water and gill histology of fish. Salt addition and various pH levels of water were also studied for comparison. The study was designed two different pH levels (high 7.98±0.2 and low 6.01±0.1), salt (6g/L), and ceramic ball (10g/l) addition and fish stock density to be 62.3 kg/m^3. The trial tanks were mounted on a vehicle to represent actual transport conditions. Fish were sampled every hour for gill histology. As a results, the lowest ammonia value was determined as 1.98 mg/L in the ceramic ball. The highest ammonia value in the high pH group was 2.83 mg/l. When the gill tissues of the fish were examined, the significant differences observed were oedema and epithelial lifting in the control and high pH groups. Hyperplasia, epithelial lifting, and multiple deformations were observed in all the experimental groups except the ceramic ball group. This study showed that based on the histological results of the gills and the stability of the pH levels of the water and the effect on the reduction of the ammonia value of the water, that the ceramic balls are particularly useful for the transport of live fish.

Keywords: Trout, ceramic balls, pH, ammonia, gill histology

Öz: Seramik topların gökkuşağı alabalığının (*Oncorhynchus mykiss*, (Walbaum, 1792)) canlı transfer koşulları üzerindeki etkileri, balıkların solungaç histolojisi ve taşıma suyunun amonyak, pH, çözünmüş oksijen ve su sıcaklığı değerleri incelenerek belirlenmiştir. Seramik topların karşılaştırılması için tuz ilavesi yapılmış grup ve farklı pH seviyeleri hazırlanmış taşıma grupları oluşturulmuştur. Çalışma iki farklı pH seviyesi (yüksek 7.98±0.2 ve düşük 6.01±0.1), tuz (6g/L) ve seramik top (10g/l) ilavesi ve balık stok yoğunluğu 62.3 kg/m^3 olacak şekilde tasarlanmıştır. Deneme tankları, gerçek nakliye koşullarını temsil etmek için bir araca monte edildi. Solungaç histolojisi için balıklardan her saat başı numune alındı. Sonuç olarak seramik bilyede en düşük amonyak değeri 1,98 mg/L olarak belirlendi. Yüksek pH grubunda en yüksek amonyak değeri 2,83 mg/l idi. Balıkların solungaç dokuları incelendiğinde, kontrol ve yüksek pH gruplarında ödem ve epitel dokuda ayrılma olduğu gözlendi. Seramik top grubu dışındaki tüm deney gruplarında hiperplazi, epitel dokuda ayrılma ve çoklu deformasyonlar gözlendi. Bu çalışma, solungaçların histolojik sonuçlarına ve suyun pH seviyelerinin stabilitesine ve suyun amonyak değerinin azalmasına olan etkisine dayanarak, seramik bilyelerin özellikle canlı balıkların taşınması için yararlı olduğunu göstermiştir.

Anahtar kelimeler: Alabalık, seramik top, pH, amonyak, solungaç histolojisi

INTRODUCTION

There are several stages in aquaculture production. Many practices such as brood stock fish care, egg fertilization, grading, disease treatment, and hygiene applications are carried out in fish farms (Kayis, 2019). An important stage during these practices is the transportation of live fish to different systems. Transporting fish to other systems in different life stages has become very common and applicable in a wide geography. Today, live fish can be transported even between different countries. Systems and transportation procedures that allow the transfer of ornamental fish from the Far Eastern countries to countries in other regions of the world are widespread.

In this sense, practices such as starvation of fish before the transfer, the addition of oxygen and antibiotics in water, fish density, and different anaesthetic additions (tetracycline and nitrofurazone) help fish to be healthy during these transfers (Belema et al., 2017).

Rainbow trout (*Oncorhynchus mykiss*) has an important place among the fish used for human consumption. Therefore, it is one of the most intensively produced species. During the production process, trout are frequently subjected to live transfer. In live trout transfer, water temperature values are required to be maintained at 8–10 °C, and dissolved oxygen value not to fall below 5–6 mg/L. Similarly, it is stated that the pH value should be around 7–8. Water conditions, fish size and weight, and transportation time determine the stocking density of trout transfer. An average stocking density of 100 kg/m^3 is acceptable (Shabani et al., 2016). At this stage, the addition of certain substances to the transport water has been suggested in the literature. Zeolite and ceramic materials that act as ammonia retainers and pH regulators are good examples. In recent years, the addition of ammonia retainers such as zeolite has come to the fore to prevent the accumulation of ammonia and reduce ammonia toxicity during transportation. In addition, natural ceramic balls or rings with ammonia retainers and pH regulators with various effects on bacteria have become useful for fish welfare, especially in the aquarium industry. These products are the subject of studies; especially for the improvement of wastewater, values related to pH, ammonia excretion, and temperature have been investigated (Sajuni et al., 2010). In addition, it has been reported that ceramic products are used as a suitable attachment material for bacteria and contribute to bacterial growth. Contrarily, it has also been reported that ceramic balls are used to inhibit the growth of pathogenic *E. coli* bacteria (Ni et al., 2007).

A few indicators determine the sustainability of ecological balance or the welfare of living organisms in aquatic ecosystems. These reagents can be biotic or abiotic. For example, heavy metals, antibiotics, and nitrogenous compounds are considered to be abiotic markers of pollution in aquatic ecosystems (Barlas et al., 2005; Topal et al., 2012). Biotic markers are used for some bacteria and many aquatic organisms. Gills are the leading tissues that can be used as markers and reveal the environmental effects of fish diseases and welfare (Strzyżewska-Worotyńska et al., 2017). Since gills are the centres of respiration and excretion of many metabolic wastes that can be toxic (Abrahamson et al., 2008), gills can develop symptoms in adverse conditions earlier than other tissues (Sorour, 2001). Because, gills play an important role on the uptake of dissolved substances (suspended solids, heavy metals, nitrogenous compounds, pesticides and other pollutants) from the water. Therefore, gills are the most used bioindicator organs in the determination of water pollution (Birungi et al., 2007).

In the present study, we aim to determine changes in water during the live transfer of rainbow trout by examining water quality parameters and gill histology.

MATERIAL AND METHODS

In this study, the effects of two different pH levels, salt addition, and ceramic ball addition to water for live transport of rainbow trout (18.7±0.8 g) were tested. For this purpose, five different groups were designed, namely, control (C), low pH (LpH), high pH (HpH), salt (S), and ceramic balls (CB). Low pH was named for the value lower than neutral pH, and high pH was named as the value higher than neutral pH. The contents of the ceramic balls are given in Table 1. 0.1 N NaOH was used to increase the pH value of the water for the HpH group (pH 7.98), and 0.1 N HCl was used to decrease for the LpH (pH 6.01) group. The amounts and properties of the substances used in the experimental groups are given in Table 2. The pH value of the ceramic ball group was initially recorded as 7.91 due to the addition of the ceramic ball. The experimental fish were observed on a fish farm located in the Eastern Black Sea Region of Turkey. In the study, the fish were transported at a density of 62,3 kg/m^3.

Table 1. The contents of the ceramics ball

Content	(%)
Al_2O_3	>99
Fe_2O_3	<0.1
Na_2O	<1.0
SiO_2	<0.2
MgO	~0.11
CaO	<1.0
Max. temperature resistance	>1500°C
Water absorption	<1.0
Specific gravity	3.50-3.75 (g/cm^3)

Table 2. The amount and properties of the substances used in the experimental groups, and fish weight and some water quality parameters. Control (C), High pH (HpH), Low (pH), Salt (S), ceramic ball (CB)

Parameters and Properties	Experimental Groups				
	C	H (pH)	L (pH)	S	CB
Temperature (ºC)	11.1±0.2	11.5±0.4	11.4±0.2	11.2±0.1	11.1±0.3
pH	7.10±0.3	7.98±0.2	6.01±0.1	6.71±0.2	7.91±0.4
Dissolved oxygen (mg/l)	14.1±0.1	14.2±0.2	13.9±0.2	14.0±0.3	14.2±0.1
Fish weight (g)(total)	187±2.1	187±2.3	186±2.1	186±2.2	188±2.4
*Ceramic ball (g/l)	-	-	-	-	10
Salt (NaCl) (g/l)	-	-	-	6	-
Initial Ammonia (mg/l)	0.043±0.008	0.034±0.008	0.034±0.122	0.033±0.011	0.037±0.004

In order to carry out the experiment, the fish were placed in plastic containers (each of 5 L capacity) with a black surface to prevent light transmission and minimize stress. HACH DR 3900 model device and HACH kits were used to measure the ammonia content of the water. Temperature, dissolved oxygen, and pH values were measured with the HACH LANGE HQ40d portable multiprop. The experiment was conducted in two sets. Ten fish were placed in each group containing three liters of water. Thus, a total of 100 rainbow trout were used. The study was initiated by moving the vehicle on which the tanks were mounted. At the end of the one-hour movement period, ammonia, pH, and temperature values of the water were recorded for each group. In addition, samples were taken from each group every hour for gill histology that were fixed in neutral buffered formalin (10%). The trial was terminated at the end of the fourth hour. This time has been determined by considering the average time spent in fish transfer in the region.

The live fish were anesthetized with benzocaine, and transferred as a whole to neutral formalin. One day later, the fish were transferred to 50% ethyl alcohol. The gills of the fish were removed, passed through alcohol series and xylose, and kept in paraffin at 65°C for 12 h, after, which they were embedded with paraffin. Tissues were sectioned in 0.5 µm thickness with a microtome device (Leica RM2125 RTS), kept at 65°C overnight, then passed through xylose and alcohol series, and stained with haematoxylin and eosin. Stained sections covered with rapid mounting medium (Entellan, Merck 107961) and were inspected under a light microscope (Leica DMi1) and photographed (Luna, 1968). Histopathological changes were graded as follows; none (-), low (+), middle (++) and extreme (+++).

Data were statistically analyzed using the SPSS 25 Software program for Windows. Statistical significance was determined at p=0.05. Prior to statistical analysis, the normality of data was tested. All the data were analyzed using one way analysis of variance (ANOVA). Significant differences between groups were determined by Tukey Post Hoc test.

The study above has been approved by the Local Ethics Commitee of Recep Tayyip Erdogan University (Decision no:2016/31).

RESULTS

As an important detail, there were no deaths in any group during the trial. It was observed that the initial ammonia content of the experimental water was 0.037 mg/l. At the end of the experiment, the lowest ammonia value was in the CB group at 1.98 mg/l. The highest ammonia value was measured in the HpH group at 2.83 mg/l. At the end of the 4th hour, CB group had a lower ammonia value than the control group, while ammonia values in the HpH and S group were higher than the control group ($p<0.05$). There was no statistical difference between the LpH group and the control ($p>0.05$) (Figure 1).

Although the pH value of the CB group was 7.98 at the beginning of the experiment, it remained at a constant value of 6.4 in the subsequent measurements. This standard value measurement was not observed in the other groups. In the HpH group, the suddenly falling pH value tended to increase during the study. Similarly, in the salt group, the pH value was in an up and down course. In the control group, a result was obtained in continuous descent. Although no significant difference was observed in the low pH group, a continuously increasing trend was noted (Figure 1).

When the gill tissue samples of each group were examined histologically, the most observed histopathological finding was hyperplasia. This pathology was mostly detected in the salt, HpH, and LpH groups. Histological changes occurring in the gills are given in Table 3.

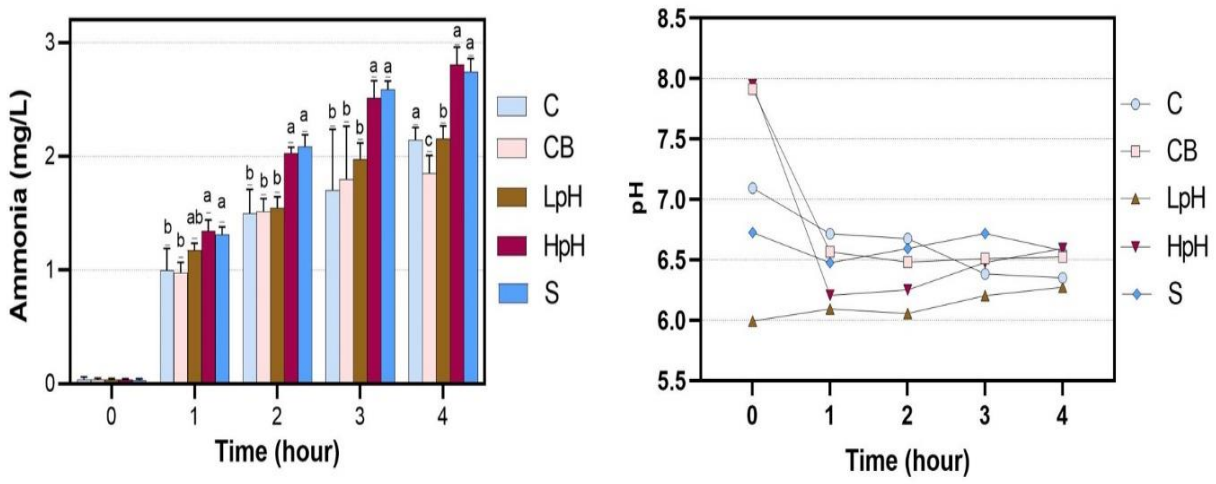

Figure 1. Ammonia and pH values of the water during the experiment. Control (C), High pH (HpH), Low (pH), Ceramic ball (CB)

Table 3. Histological changes occurred at the end of the experiment. control (initial FC), control (end of the trial LC), High pH (HpH), Low (pH), ceramic ball (CB)

Histopathological change	FC	LC	CB	H (pH)	L (pH)	S
Hyperplasia	-	+	+	+++	+	++
Hypertrophy	-	-	-	+	-	-
Epithelial lifting	-	++	-	+++	-	+
Lamellar fusion	-	-	+	+	-	-
Deformation	-	-	-	++	+	+
Oedema	-	++	-	-	-	-

none (-), low (+), middle (++) and extreme (+++)

Another important pathology of the gills was epithelial lifting in the HpH and control groups. In addition, a few deformations of the gill structure in the control group were observed. However, the most distinctive feature observed was oedema; this pathology was observed only in the control group (Figure 2, 3).

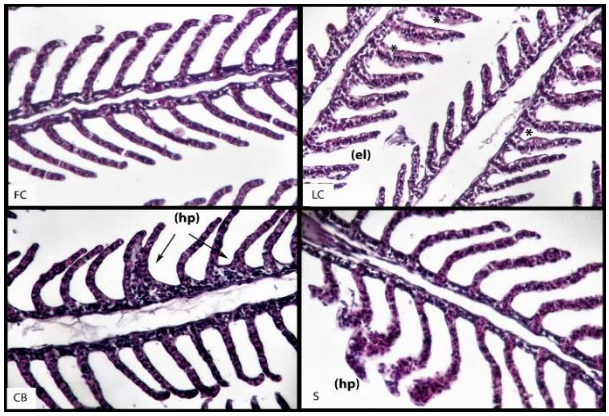

Figure 2. Histopathologic changes on gills. (el) epithelial lifting, (hp) hyperplasia, (•) oedema. First control (FC), last control (LC), Salt (S), ceramic ball (CB)

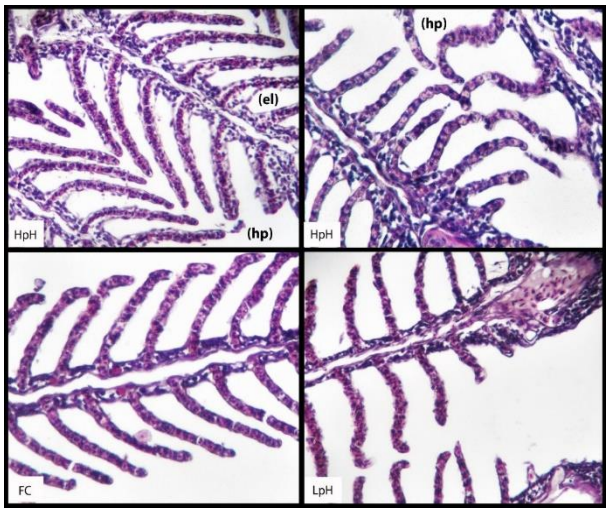

Figure 3. Histopathologic changes on gills. (el) epithelial lifting, (hp) hyperplasia. First control (FC), high pH (HpH), low (LpH)

DISCUSSION

Fish remove most of their metabolic waste as ammonia from the gills. The high amount of ammonia in water causes toxicity in fish (Randall and Tsui, 2002). For trout, the highest ammonia value in water is 0.02 mg/l (Vosyliene and Kazlauskiene, 2004). One of the important factors causing stress and toxicity during the transfer of fish is the presence of nitrogenous compounds that can show high accumulation (Kurtoğlu et al., 2021). In this study, ammonia values were calculated for the different groups and matched with gill histological results. According to the results obtained at the end of the trial, the highest ammonia content was recorded in the HpH group at 2.83 mg/l. The lowest value was observed at 1.98 mg/l in the CB group. In the control group, this value was 2.13 mg/l. These results may be due to the ammonia-binding and ion-exchange-enabled properties of the ceramic balls (Bhakta and Munekage, 2009). The gill histological equivalents of these values were observed as diffuse oedema and epithelial lifting in the control group, epithelial lifting and hyperplasia in the HpH group, and hyperplasia in the salt and LpH groups. It cannot be stated that these pathologies were only related to ammonia values. It should also be noted that high pH levels are an important factor affecting ammonia toxicity. As the fish transport time increases, the CO_2 in the water increases and causes to decrease the pH of the water towards acidity. The pH levels in the water around 7-9 are considered optimum for nitrification bacteria and fish. pH can cause rapid changes in fish stress levels, but buffers can be used to stabilize the pH of the water during fish transport (Berka, 1986).

The presence of nitrification bacteria is very important in recirculating aquaculture system. Because these bacteria are affected by sudden pH changes (Allison, and Prosser, 1993). The results show that ceramic balls positively affect the survival of nitrification bacteria because they keep the pH of the water in balance. In this study, the fact that the ammonia values in the CB group were lower than the other groups explains this situation. In this sense, in the experimental CB group, the presence of the lowest ammonia value and the fact that the pH level did not undergo a sudden change and remained constant at 6.5 revealed a healthier histological structure for the gills in this group.

Salt is a common chemical used in the treatment of parasitic, mycotic and bacterial diseases of fish and for the prevention of nitrite toxicity in static aquatic systems. (Schelke et al. 2011; Williams and Eddy, 1986). In addition, the use of salt (NaCl at the rate of 5 g/l) in the live transfer of fish is a recommended practice to balance osmoregulation and reduce stress (Tacchi et al., 2015). It has even been stated that salt application in the form of salt addition to transport water has the effect of suppressing pathogenic bacterial load. However, Amend et al., (1982) reported that the addition of salt during fish transportation had no effect on increasing fish welfare. In this study, adding salt at the rate of 6 g/l to transport water did not suppress the ammonia value in the salt group more than in the other groups. It was also determined that salt application

was not very effective in keeping the pH values constant and eliminating pathologies on the gills.

The usage of ceramic balls has widely increased in recent years. In addition to creating an attachment surface for beneficial bacteria and inhibiting some harmful bacteria, it has also been used to improve water quality in ensuring fish welfare (Ni et al., 2007; Sajuni et al., 2010). In order to increase the effectiveness of ceramic balls in improving water quality, the use of porous form has been studied. In the study, it was determined that porous ceramic balls were 10 times more effective than normal (Di et al., 2013). In another study, in which the effect of ceramic balls on the hardness of water was studied, it was also stated that these balls were effective on the water hardness (Kawarada and Pironneau, 2019). Although there are many similar studies on water quality and microbial load, our study plays a leading role as a research between fish welfare and ceramic ball. In this study, ceramic balls with a large amount of aluminium oxide (Al_2O_3) were used. In addition, these ceramic materials containing Fe, Na, Si, Mg, and Ca have been shown to reduce ammonia accumulation in water compared to that of other groups. In addition, the ability of this substance to buffer the pH value of water has drawn much attention. This feature can be recommended as a very useful application in ensuring fish welfare, especially for live transport. The data obtained at the end of this study and the gill histology findings revealed that the use of ceramic products can be particularly useful for live fish transport.

CONCLUSION

During the transportation of live trout, the most important challenge is that the ammonia value of the transport water gets much higher than the optimum level. The practices that will keep this value constant or at lower levels are very important for live fish transport. The fact that the addition of ceramic ball (B type) to trout transport water can keep the ammonia value of the water at a lower level than that of the other groups emerged as an important result. Fish welfare in the live transfer can be achieved by generalizing this practice. High pH level in fish farming has a significant effect on ammonia toxicity. Therefore, it is desirable to keep the pH levels stable, especially below 7. In this study, it has been observed that ceramic balls can keep the pH level close to 7 and provide stability. In this respect, this application can also be applied in other growth conditions (especially in closed circuit systems). In addition, important results were obtained in this study during gill histological studies. It has been determined that ceramic ball application ensures that the gill structure, which is especially important for fish, is not damaged as much as possible during transportation. In this study, fish transportation was carried out for 4 hours, the effects of longer transportation should be investigated. In addition, histological changes in the gills may be related to enzyme activity and stress hormones in fish. Accordingly, different materials that will increase fish welfare in fish transfer should be researched.

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