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Research Article

The effects of Potassium Chloride and Calcium Sulphate on Egg Production and Heart Rate in *Daphnia magna* (Crustacea: Cladocera)

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Article Info

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ABSTRACT

Daphnia magna is capable of rapid reproduction in suitable environmental conditions and temperature (15-22 °C). Additionally, reproduction of water fleas under laboratory conditions is quite simple and cheap. Moreover, daphnids are commonly used in laboratory experiments. Therefore, the effects of potassium chloride (KCl) and calcium sulphate ($CaSO_4$) on heart rate and egg production in *D. magna* were assessed in this study. Daphnids of treatment groups were exposed to distinct concentrations of KCl and $CaSO_4$ (37.5, 75, 150 mg/L). Ten individuals were used in each group. All experiments were made in triplicate. Heart rate variability was examined for 96 h and the number of heartbeats was calculated by using a video camera. The number of eggs per individual in each experimental group was recorded for 21 days. An increase in both heart rate and egg production due to exposure to $CaSO_4$ was generally observed, depending on the concentration gradients. However, KCl caused a decrease in the egg production of medium and high-dose groups. Furthermore, although an increase in heart rate was examined for two successive days after exposure to KCl, a decrease in the heart rate of medium and high-dose groups was observed during the last 48 h. When $CaSO_4$ treatment groups compared with the control group, it can be concluded that $CaSO_4$ contributes positively to egg production. When it comes to KCl, it can be concluded that KCl negatively affects egg production in the medium and high dose groups.

Potasyum Klorür ve Kalsiyum Sülfatın *Daphnia magna* (Crustacea: Cladocera) Türünde Yumurta Üretimi ve Kalp Hızı Üzerine Etkileri

ÖZET

Daphnia magna uygun çevre koşullarında ve sıcaklıkta (15-22 °C) hızlı üreme yeteneğine sahiptir. Ayrıca su pirelerinin laboratuvar koşullarında üremesi oldukça basit ve ucuzdur. Dahası su pireleri laboratuvar deneylerinde yaygın olarak kullanılmaktadır. Bu nedenle bu çalışmada potasyum klorür (KCl) ve kalsiyum sülfatın ($CaSO_4$) *D. magna* bireylerinde kalp hızı ve yumurta üretimi üzerine etkileri değerlendirildi. Uygulama gruplarındaki su pireleri KCl ve $CaSO_4$'ün farklı konsantrasyonlarına (37.5, 75, 150 mg/L) maruz bırakıldı. Her grupta on birey kullanıldı. Tüm deneyler üç tekrar halinde yapıldı. 96 saat boyunca kalp hızı değişkenliği incelendi ve video kamera kullanılarak kalp atım sayısı hesaplandı. Her deney grubundaki birey başına düşen yumurta sayısı 21 gün boyunca kaydedildi. $CaSO_4$'e maruz kalma nedeniyle hem kalp hızında hem de yumurta üretiminde konsantrasyon derecelerine bağlı olarak genellikle bir artış gözlendi. Ancak KCl orta ve yüksek doz gruplarında yumurta veriminde azalmaya neden oldu. Ayrıca KCl'ye maruz kaldıktan sonra art arda iki gün boyunca kalp hızında bir artış incelenmiş olmasına rağmen, orta ve yüksek doz gruplarında kalp hızında son 48 saat içinde bir azalma gözlendi. $CaSO_4$ uygulanan gruplar kontrol grubu ile karşılaştırıldığında $CaSO_4$'ün yumurta üretimine olumlu katkı sağladığı sonucuna varılabilir. KCl'ye gelince, KCl'nin orta ve yüksek doz gruplarında yumurta üretimini olumsuz etkilediği sonucuna varılabilir.

1. INTRODUCTION

Daphnia is a genus of small planktonic crustaceans which is characterized by flattened leaf legs used as the filtering apparatus. Daphnids feed on small, suspended particles which is usually made up of planktonic algae in water. They are the most widely used live food items particularly during the development of some fish species (perch, freshwater bream, catfish, carp, sturgeon etc.) in fish farming [1, 2]. Therefore, water fleas are important members of the food chain in freshwater. *Daphnia magna* is an extensively used model organism in ecotoxicological research because of its common distribution, short life cycle, rapid maturation, and reproduction [3, 4].

As most of the other species in the genus *Daphnia*, *D. magna* reproduces by parthenogenesis. In a typical growth season, sexual reproduction in life cycle of *Daphnia* is also observed. Dormant encapsulated eggs (ephippia) produced by sexual reproduction are usually strongly melanized and contain two large eggs. Ephippium with only one or no egg is not uncommon. Ephippium is triggered by conditions announcing or associated with an unfavorable environment [5, 6]. *Daphnia* is a keystone genus in freshwater and environmental conditions effect offspring sex and whether they reproduce asexually or sexually [7]. Chemical exposure effects not only hatching of dormant eggs, but also *D. magna* hatchling survival and movements [6].

There are two types of circulatory system in animals which are the open circulatory system and the closed circulatory system. Heart is an important component of circulatory system because its muscular contraction provides to move fluid (hemolymph or blood) for distribution of respiratory gases and nutrients. There are two types of animal hearts which are neurogenic and myogenic hearts [8, 9]. Initiation of heartbeat in neurogenic heart is under nervous control. However, initiation of heartbeat in myogenic heart is under muscular control [10, 11].

There is diversity in heart types of Crustacea. This taxon includes species with neurogenic hearts or myogenic hearts. *D. magna* has myogenic heart. Therefore, the heart of *D. magna* and other ancestral crustaceans such as *Triops longicaudatus* bear structural resemblance to the hearts of vertebrate animals. Additionally, *Daphnia* may represent a model system for understanding the myogenic hearts of vertebrates [9]. Moreover, changes in *D. magna* heartbeat have been reported to be an indicator for toxicity evaluation [12, 13]. Therefore, heart rate may be a sensitive physiological indicator that can reflect differential concentration effects of chemical compounds to *D. magna* circulatory system.

Calcium sulphate ($CaSO_4$) is a naturally occurring mineral used in various industrial applications. It has the longest history not only in industrial usage but also in clinical usage. Moreover, to date, many clinical *in vivo* and *in vitro* studies have been conducted. Potassium is one of the three most important minerals which is used to produce the electrical charge to function properly of the body cell. Because the cells produce electrical charges through electrolytes (eg, sodium and potassium). Additionally, KCl helps regulation of heartbeat and blood pressure, contributes nerve and muscle activities, water balance. However, high dose potassium causes a decrease in blood pressure and cardiac arrhythmia [14, 15].

In the present study, the effects of mineral salts constituting water hardness on egg production and heart rate of *D. magna* were analyzed. We evaluated the changes of these features after exposure to different concentrations of KCl and $CaSO_4$. This species was chosen because of its size and the heart can be easily observed by optical methodologies. Additionally, a 21-day standard reproductive test was carried out to evaluate the effects of KCl and $CaSO_4$ on egg production of *D. magna*.

2. MATERIAL and METHODS

The experiment was carried out with *D. magna* which we have cultured in the laboratory for more than three years. Daphnids were maintained in aerated fresh water at temperature of 20-22 °C with a 14 h light and 10 h dark cycle. Stock solutions were prepared by dissolving potassium chloride (KCl) and calcium sulfate ($CaSO_4$) in fresh water. Three different concentrations of KCl and $CaSO_4$(37.5, 75, 150 mg/L) were diluted from the stock solution. These concentrations were determined based on literature data [16, 17]. The culture medium was renewed twice a week. Daphnids were fed with a mixture of *Spirulina* microalgae and TetraPond fish food once a day. Each beaker contained 60 mL solution and ten daphnids. All experimental samples were made in triplicate. Ten adults (older than 14 d) were used for heart rate analysis. Each daphnid was transferred on a slide with a drop of the culture medium and examined by light microscopy. Heartbeats of each daphnid were recorded in slow motion by a video camera for 10 seconds. Changes in heart rate of *D. magna* were examined for 96 h. In the second part, a 21-day reproductive test was performed with neonates (<24 h). The number of eggs per individual in each experimental group was noted. Data were presented as mean with standard deviation. The differences were compared for statistical significance by one-way ANOVA with post hoc analysis using Tukey test. Statistical evaluations were carried out via PASW statistics (SPSS) 18 software. We set the significance level at $p<0.05$.

3. RESULTS

The effects of KCl and $CaSO_4$ on heart rate and egg production of *D. magna* were analyzed in the current study. Heart contractions were visually counted by slowing down the speed of video playback. The number of eggs per individual in each experimental group (including control, low, medium, and high-dose groups) was also calculated for 21 days. The heart of *D. magna* is located dorsally and anterior from the brood chamber (Figure 1). As a result of the exposure to $CaSO_4$, an increase in heart rate depend on concentration gradients (37.5, 75, 150 mg/L) was generally examined. Heart rate changes were statistically significant in all treatments groups compared to control except for medium-dose group data at 72 and 96 h. Although exposure to different concentration of KCl (37.5, 75, 150 mg/L), caused increasing of heart rate for two consecutive days, a decrease in the heart rate of medium and high-dose groups was observed during the last 48 h. Although a decrease in the heart rate of medium and high-dose groups was observed during the last 48 h, the heart rate of medium group at 96 h was not lower than the control group. When the control group was compared to the treatment groups, it was statistically significant except for the data related to the heart rate of the medium-dose group at 96 h. The mean of heart rate (mean ± s.d.) for each group was presented in Tables 1 and 2.

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Figure 1. Adult *Daphnia magna* of control group. The spherical heart is located on the dorsum of animal and in front of the brood chamber where the eggs are carried. There are two eggs in the brood chamber.

Table 1. The mean and standard deviation (mean ± s.d.) of *Dapnia magna* heart rate in the control group and treatment groups with different concentrations of calcium sulfate ($CaSO_4$) were presented.

Experimental Groups	24 h	48 h	72 h	96 h
Control	392.4±28.49	403.2±12.20	394.2±22.04	399±50.52
37.5 mg/L	401.4±54.26	393±38.06	400.8±72.43	406.2±14.96
75 mg/L	432±32.01	424.2±18.18	418.2±16.08	420±20.07
150 mg/L	441±35.44	437.4±15.74	435±23.43	445.2±43.13

Table 2. The mean and standard deviation (mean ± s.d.) of *Dapnia magna* heart rate in the control group and treatment groups with different concentrations of potassium chloride (KCl) were presented.

Experimental Groups	24 h	48 h	72 h	96 h
Control	370.8±13.36	374.4±21.66	391.2±18.74	387.6±36.51
37.5 mg/L	414.6±9.79	422±15.36	423.6±17.23	424.2±27.42
75 mg/L	444±7.74	444.6±23.12	420.6±28.22	384.6±44.32
150 mg/L	471.6±22.41	480.6±25.53	451.2±17.46	364.2±22.77

Figure 2. Adult *Daphnia magna* in the medium-dose group of KCl bearing melanized eggs known as Ephippium. A closer microscopic photograph of the ephippium with two eggs on the left side.

The eggs were observed in the brood chamber located dorsally beneath the carapace of *D. magna*. The embryos hatched from the eggs after about 1 day at 20-22 °C but they remained in the brood chamber for further development. After 2-3 days, the young individuals were released by the mother daphnid from the brood chamber. Although the newborn looked more or less like the adult daphnid, the brood chamber was not developed in newborn individuals. The first eggs were examined into the brood chamber of nearly 9-10-day-old female water fleas and daphnids lived for 35-40 days in laboratory conditions. *Daphnia* produces a different type of egg for resting. The resting eggs were enveloped with a chitinous melanized structure called ephippium (Figure 2). Resting eggs were uncommon in our experimental groups and they were rarely observed in only one or two individuals in medium-dose concentration of KCl. After exposure to $CaSO_4$, an increase in egg production depend on concentration gradients was observed. However, exposure to KCl gave rise to a decrease in egg production of the medium-and high-dose groups. Our values were presented by graphs (Figures 3, 4).

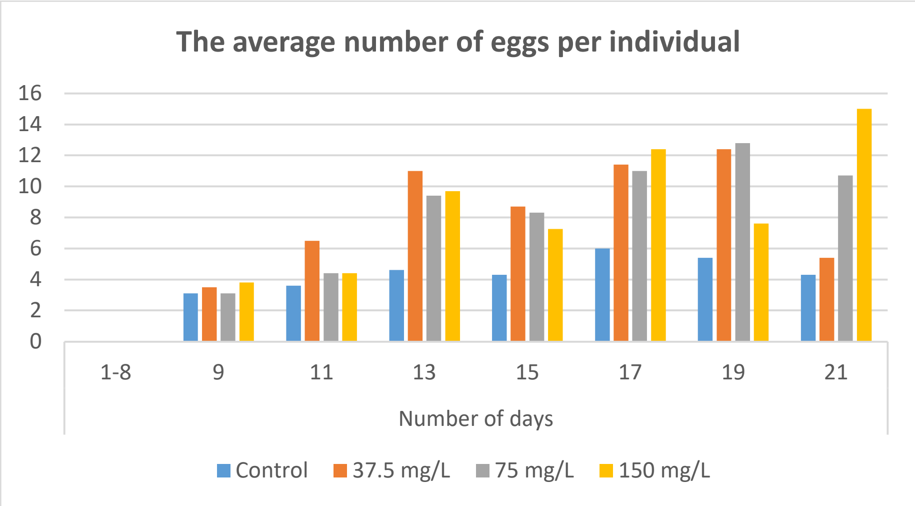

Figure 3. The graph shows the average number of eggs per individual in the control group and the treated groups with different concentrations of calcium sulfate ($CaSO_4$).

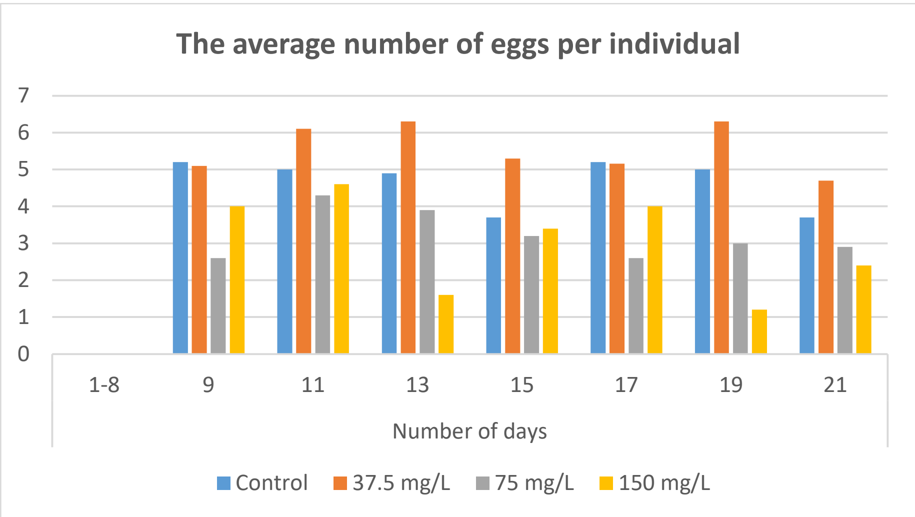

Figure 4. The graph shows the average number of eggs per individual in the control group and the treated groups with different concentrations of potassium chloride (KCl).

4. DISCUSSION

The effects of different concentrations of $CaSO_4$ and KCl on *D. magna*, which is an important part of the food chain in the aquatic ecosystem, were evaluated. $CaSO_4$ is the compound with the longest history not only in industrial usage but also in clinical usage. KCl is a naturally occurring form of potassium salt, typically extracted from land or sea. KCl is found in many foods due to fact that it is considered safe and has high antimicrobial activity compared to sodium chloride. Potassium participates in various functions in the body, such as regulating blood pressure levels, neurotransmission, cardiovascular, skeletal, and muscular system activities [15, 18]. Therefore, the present study is important to evaluate the positive or negative effects of these two compounds on *D. magna* which is an invertebrate model organism, frequently used in laboratory studies and ecotoxicology studies. Moreover, *D. magna* is used in model in the toxicity assessment of pharmaceuticals. Ketoprofen is a nonsteroidal anti-inflammatory and analgesic drug commonly used in human and veterinary medicine. Bownik et al. [19] reported that the results showed ketoprofen induced a time- and concentration-dependent decrease of daphnid heart rate. Additionally, *Daphnia* heart activity was analyzed after exposed to cardioactive drugs: ouabain, metaproterenol, metoprolol, and verapamil. Results indicated that ouabain, metaproterenol and metoprolol caused similar effects on heart of daphnids to those observed on humans due to positive systolic inotropic effects [20].

After exposure to KCl, an increase in heart rate was observed for two consecutive days however a decrease in heart rate was observed in the medium and high-dose groups during the last 48 hours. Cohlmia et al. [21] investigated the effects of KCl and monophosphate glutamate (MSG) on the heart of *D. magna*. According to the results of this study, while there was an increase in heart rate after exposure to MSG, there was a decrease in heart rate after exposure to KCl. Although potassium is the prominent cation in many physiological functions such as regulation of osmotic pressure, conduction of nerve impulse, muscle contraction particularly the cardiac muscle etc. [22], Mattu et al. [23] have reported that high concentrations of potassium give rise to abnormal heart and skeletal muscle function by lowering cell-resting action potential and preventing repolarization, leading to muscle paralysis. Soetan et al. [22] also reported that an increased level in serum potassium caused dilatation of the heart and cardiac arrest.

After exposure to $CaSO_4$, an increase in heart rate of treatment groups (except for second-day data of 37.5 mg/L) was observed compared to the control group, depending on the concentration levels of this compound. Wilson et al. [24] investigated the effects of caffeine and ethanol on the heart rate of *D. magna*. It was noted that while heart rate increased because of caffeine exposure, it decreased because of ethanol exposure. Crustacean species need to periodically shed their old exoskeleton to grow, and the exoskeleton contains $CaCO_3$. Due to continual replacement of exoskeleton at frequent intervals, crustacean species come across massive calcium fluctuations in the internal concentration. To minimize the fluctuation, they have highly advanced calcium regulatory mechanisms occur in epithelial cell layers of crustacean gills, gut, antennal glands, and integument [25].

An increase in egg production was observed depending on the $CaSO_4$ concentration levels. Similarly, Leblanc and Surprenant [16] reported that different concentrations of $CaSO_4$ (200, 290, 460, 910, 2100 mg/L) had a positive impact on the number of offspring produced per daphnid. Romero et al. [26] who studied with birds, reported that after the animals were fed with a mixture of $CaSO_4$ and zeolite (6.94% of the diet), egg yolk, egg weight and egg quality were increased. When it comes to KCl, it caused a decrease in egg production in the medium- and high-dose groups, although there was an overall increase at the low dose group. Bhattacharya and Kaliwal [27] reported that haemolymph trehalose was significantly increased after supplementing the feed with KCl to the silkworm. Trehalose is the main circulating sugar which is used for oocyte growth [28]. Based on literature data, the increase in egg production of the low-dose group may be related to trehalose level due to KCl. However, a high potassium concentration gives rise to various complex dysfunctions and abnormal cell-membrane-permeability for ion exchanging or molecule transportation [29]. Omana and Wu [30] also reported that a high concentration of KCl was effective on egg proteins due to minimized complex formation between ovomucin and other egg white proteins.

Overall, our results revealed that $CaSO_4$ and KCl induced time- and concentration-dependent changes on heartbeat and egg production of *D. magna*. Due to the fact that the heartbeat mechanism in *Daphnia* is similar in many respects to that of vertebrates, heart rate is a prominent physiological indicator that reflects differential concentration effects of chemical compounds to *D. magna* circulatory system. Given that *D. magna* is a key species in aquatic ecosystems and a significant component of the freshwater food web, our results added information about the effects of $CaSO_4$ and KCl on heartbeat and egg production of this species compared with the studies published to date.

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The Declaration of Conflict of Interest/Common Interest

No conflict of interest or common interest has been declared by the authors.

Authors' Contribution

The first author contributed 40%, the second author 30%, the third author 30%.

The Declaration of Ethics Committee Approval

This study does not require ethics committee permission or any special permission.

The Declaration of Research and Publication Ethics

The authors of the paper declare that they comply with the scientific, ethical and quotation rules of ETOXEC in all processes of the paper and that they do not make any falsification on the data collected. In addition, they declare that Environmental Toxicology and Ecology and its editorial board have no responsibility for any ethical violations that may be encountered, and that this study has not been evaluated in any academic publication environment other than Environmental Toxicology and Ecology.

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