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RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

A study on nutrition of medicinal leech (*Hirudo verbana* Carena, 1820): Cannibalism?

Tıbbi sülük (*Hirudo verbana* Carena, 1820)'ün beslenmesi üzerine bir araştırma: Kanibalizm?

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Özet: Bu çalışma ile tıbbi sülük *Hirudo verbana*'nın yapay koşullarda beslenme esnası ve sonrası davranışlarının araştırılması amaçlanmıştır. Sülükler taze sığır kanı ile beslenmişlerdir ve toplu tartım yöntemiyle beslenme sonrasında %97.74 oranında ağırlık artışı olmuştur. Yeteri kadar beslenememiş bazı sülüklerin, hareket kabiliyetlerini kısmen kaybetmiş olan bazı tok sülüklere saldırdıkları, kanibalistik eğilim sergiledikleri, ancak kanibalizmin net olarak şekillenmediği görülmüştür. Bazı sülüklerin beslenmeden 66. gün sonrasında dahi tükettikleri kanın bir kısmını kustukları görülmüştür. Kusulan kanın bazı bireylerin uyarımına neden olduğu, ancak kanın bulunduğu bölgeye gelen sülüklerin kısa süreli bir incelemenin ardından kana ilgisiz davrandıkları ve bölgeden uzaklaştıkları gözlenmiştir. Yetiştiricilik şartlarında periyodik besleme gruplarının oluşturulması, tüm sülüklerin uyarılarak beslenmeye hazır hale gelmeleri ve beslenme sonrasında tok ile yeterince beslenmemiş bireylerin ayrı ortamlara alınmaları ile tıbbi sülük *H. verbana*'da potansiyel kanibalizm eğiliminden kaynaklı problemlerin önüne geçilebileceği düşünülmektedir.

Anahtar kelimeler: Tıbbi sülük, *Hirudo verbana*, Besleme Davranışı, Kanibalizm Eğilimi, Kan Kusma.

Abstract: It was aimed to investigate the behavior of the medicinal leech *Hirudo verbana* during and after feeding in the artificial conditions with this study. The leeches were fed with fresh cattle blood, and weight gain occurred at the rate of 97.74% using by bulk weighing after the feeding. Some leeches which were not fed enough, attacked to the some satiated leeches which have lost their ability to move partially, and they demonstrated cannibalistic tendency, but the cannibalism wasn't occurred clearly. It was observed that some leeches vomited the certain portion of the blood even 66 days after the feeding. This vomited blood caused to stimulate the some leeches, but the stimulated leeches acted uninterested and moved away from the region after the short-term investigation of vomited blood. It was thought that the problems which originated from the potential cannibalism tendency of the medicinal leech *H. verbana* can be avoided by the creation of periodic feeding groups, stimulating of the all leeches to make them ready for feeding and placing the satiated and insufficient fed individuals in different environments after the feeding in the breeding conditions.

Keywords: Medicinal Leech, *Hirudo verbana*, Feeding Behavior, Cannibalism Tendency, Vomiting Blood.

INTRODUCTION

The most important factor affecting the distribution and density of the leeches is the presence of food (host) in the habitats (Sawyer, 1986). Medicinal leeches feed by sucking blood of the vertebrates such as mammals, fish, amphibians, birds and reptiles (Dickinson and Lent, 1984; Sawyer, 1986; Merilä and Sterner; 2002; Kutschera and Roth, 2005), and their sucking capability is up to 8.9±0.2 times of their initial weight (Dickinson and Lent, 1984). Water temperature affects the feeding frequency of medicinal leeches; feeding frequency is once every 8-12 months at 10-20^{o}C and 3-6 months at 20-35^{o}C (Spencer and Jones, 2007).

The medicinal leech populations have been decreased in Europe. The most important factor of this situation is especially the destruction of ponds and marshes (Elliott and Tullett, 1984; Merilä and Sterner, 2002; Elliott and Kutschera, 2011). This dramatic situation is also derived from the fact that the horses and cattles have under control in last 200 years and their water requirement has met in the farms (Mann, 1955), and consequently the nutrition of medicinal leeches are mainly covered by frog blood with lower energy comparing with the mammalian blood (Davies and McLoughlin, 1996).

Although the most observations based on laboratory conditions, the cannibalism, defined as the intra-species predation, is widely available in many species. The dynamics of cannibalism in the natural populations are largely neglected (Fox, 1975; Persson and Elliott, 2012). Cannibalism was reported in the some species such as *Erpobdella octoculata* (Mann, 1953), *Erpobdella punctata*, *Dina lineata*, *Trocheta subviridis*, *Haemopis sanguisuga* (Elliott and Mann, 1979), *Trocheta haskonis* (Grosser and Kutschera, 2004) and *Hirudo medicinalis* (Kutschera and Roth, 2005) with the studies on the Hirudinea class.

It was determined that *Hirudo verbana* was confused with *H. medicinalis* by the some recent genetic studies (Trontelj and Utevsky, 2005; Siddall et al., 2007). It was aimed to investigate the feeding behavior of *H. verbana* whose

ecological data is insufficient (Neubert and Nesemann, 1999; Elliott and Kutschera, 2011) in the artificial conditions with this study.

MATERIALS AND METHOD

In total, 348 specimens of *H. verbana* Carena, 1820, collected from Gelendost (2010/10/15) and Yenice (2010/10/21) locations around Lake Eğirdir, were transferred to Eğirdir Fisheries Research Institute production unit. They were placed in a tank, converted in accordance with the purpose, with the dimensions of 1.20x1.20x0.60 m equipped with soil, stones and macrophyte materials, and the tank was filled with water up to half in the manner similar to natural environment. Then, they were acclimated the environment until the beginning of the experiment.

The dry bowel for making sausage and fresh cattle blood obtained from slaughterhouse of city of Eğirdir were used for the feeding of leeches. Dry bowel was immersed in warm water for 10 minutes in order to soften and then was cut in 10 pieces each of 25 cm length. The one ends of the each bowel were knotted and then were filled with fresh cattle blood through a funnel. The other ends were knotted also after the blood filling, and these feeding materials were placed on the soil in the tank. The water was disturbed by hands strongly in order to stimulate the all leeches for feeding. The leeches were fed ad libitum from 09:30 a.m. until 16:00 p.m. (2010/11/12).

In order to determine weight gain, the average weight of leeches was determined by bulk weighing method before and after feeding on the electronic scales with 0.01 g weighing sensitivity removing the water and blood droplets on them. Food materials were removed from the tank by controlling in terms of the presence of leeches, the water in the tank was completely changed after feeding and the leeches were kept in the plastic pail during these operations lasting approximately half-hour. The study was continued for 66 days after feeding and during this period the leeches weren't fed. All the water in the tank was changed twice a week for the first month and in the rest of the experiment period once a week. The water temperature, pH and dissolved oxygen concentration were measured in different days. The behaviors of leeches were observed throughout the study.

RESULTS

The water temperature, concentration of dissolved oxygen and pH values were measured as 13.60±0.20 °C, 3.57±1.45 mg/L and 7.44±0.21, respectively during the study.

It was observed that the most leeches were stimulated due to the mobilization of water and swam dispersedly around the area of water vibration during the preparation prior to the feeding. Although the water was vibrated by hands strongly in order to stimulate the all leeches for feeding, it was observed that some leeches didn't leave the area where they were and they didn't participate in feeding. The satiated leeches, which were apparently distinguished by their plump body structures, left the feeding material suddenly and dived deep into the water due to couldn't carry their own weights or the sense of satiation, and they were become vulnerable against the other leeches not enough fed.

Figure 1. The attack of the poorly fed individuals to the satiated leech that has partly lost its motion capability

Figure 2. The attack of the poorly fed individuals to the satiated leech that has partly lost its motion capability

The bowels were removed from the tank after feeding, and it was observed that many leeches were positioned in the bowels as a result of an investigation on the inner walls of the bowels. It was identified that some of the poorly fed or unfed leeches apparently distinguished by their loose structure and comfort in motions, attached with their posterior suckers to the body wall of the satiated leeches, which were in difficulty to move, and made an attempt for feeding with their anterior suckers in the plastic pail during the relocation of the them into the tank containing refreshed water (Figure 1 and Figure 2). But, it was not observed any cut (bite) trace or blood leakage on the body wall of the individuals who were exposed to attack. These extraordinary behaviors were never seen again during the study.

The average weight of leeches before feeding was determined by bulk weighing as 2.21 g, after feeding as 4.37 g with an increase of 97.74% and 3.60 g in the 66 days following the nutrition.

It was observed that the water in the tank was blurred due to the wastes of metabolism especially the first three weeks after nutrition. Therefore, the water in the tank was renewed twice a week in the first month and once in the following

period. It was observed that the satiated leeches were located under the layer formed by leaves, stones and soil, and their active mobility was dropped the minimum level. Furthermore, it was also observed that the insufficient fed or hungry leeches were located closely to the water surface or attached to the side walls of the tank, and when the water was vibrated they swam disorderly towards the source of vibration in a mass with gradually increasing number. It was attention grabbing that the some satiated leeches were grouped together without motion in the center of water discharged tank (in the area where the substrate slope is ended)

Figure 3. The blood vomiting individual

Figure 4. The stimulated individual due to the blood vomited by other

It was detected that the some leeches vomited some portion of the consumed blood even in the last day of the period of 66 days (Figure 3), and some leeches were stimulated with the vomited blood (Figure 4). However, it was observed that the leeches acted uninterested and moved away from the region after the short-term investigation of vomited blood. It was noted permanent morphological changes and deaths in the some blood vomiting individuals.

DISCUSSION

It was observed 0.98-fold weight gain after the feeding. It is thought that the difference between our findings and the results reported for 8.9-fold weight increase in *H. medicinalis* by Dickinson and Lent (1984) is derived from the fact that the studies have been made on different leech species, and our method based on the batch weighing, and some of the leeches were not fed at all, and the difference between the initial weight of the leeches in our study (2.21 g) and the initial weight of the *H. medicinalis* (1-1.5 g) used by mentioned researchers, and the satiety level of the leeches, and the water temperature difference regarding the research environments.

It was calculated that the daily average weight loss for each leech was 11.67 mg, and the initial weight will be attained in 185 days (~6 months). This value appears to be compatible with the frequency of feeding requirement reported by Dickinson and Lent (1984) and Spencer and Jones (2007). However, it should be considered that the frequency of needing for re-feeding is changed inversely proportional with water temperature.

Nutrition materials, such as bowels etc. ensuring housing/hiding place for the leeches, should be removed from environment after controlling in the breeding conditions. Otherwise, the individuals who perforated the wall of nutrition material with their teeth and penetrated into the inner area maybe be also removed with waste material from the environment. This undesirable situation will cause to economic losses in the medicinal leech sector in which an individual leech may have customer demand up to $15.70 (Leeches USA, 2013) depending on its size and the amount of demand.

Because of the feeding frequency of the medicinal leeches maybe as long as one year (Spencer and Jones, 2007), it is possible that some leeches acted uninterested to the bowels filled with blood during the feeding experiment. Therefore, it was thought that the some leeches collected from wild for this study were fed a short time ago in their habitats, and they weren't fed during this feeding experiment, in spite of stimulate them for feeding. Kipling and Frost (1970) and Leonardsson (1991) reported that the extreme density and then the occurrence stress might cause the cannibalism in the laboratory conditions in the different animal species. We concluded compatible with the previous authors that the leeches stressed when they were kept in the plastic pail, and the insufficient fed leeches attacked to the satiated conspecifics. Considering that the attacks weren't observed again during the study, this extreme behavior may also be a result of the attempt to continue of the feeding that couldn't be completed during the feeding process, with the stimulation of the blood consumed by the over-satiated leeches in the stress conditions. However, the cannibalism was not occurred, since it was not found any trace of cuts (bite) and blood leakage on the body wall of the individuals who were exposed to attack, so it was concluded that the threshold of predation couldn't be exceeded. Due to the fact that the feeding and growth of the medicinal leeches are closely related with the diversity and abundance of the nutrients in their habitats (Davies and McLoughlin, 1996; Merilä and Sterner, 2002; Elliott, 2008), the cannibalistic tendency and the predation threshold should be researched in the *H. verbana* populations by ecological and experimental studies.

The attack behavior of the *H. verbana*, observed in this study, may be considered in the context of parasitism, not as cannibalism tendency, from a different perspective. But,

although the cannibalism, defined as intraspecific predation (Fox, 1975), was described a process including killing and eating an individual on the conspecifics (Polis, 1981), the feeding characteristic such as sucking blood should be considered for the evaluation. Therefore, we agree with Kutschera and Roth (2005) who classified *H. medicinalis* in the cannibalistic annelid, and we conclude that, although the sucking blood from the satiated conspecifics and killing were not occurred, *H. verbana* maybe categorize as cannibalistic tendency, at this stage. This situation should be clarified in the future detailed studies which include the many environmental and biological factors.

It was noted that some of the individuals vomited the certain portion of the sucked blood even 66 days after feeding. The blood vomiting phenomenon is considered as a manifestation of the most sensitive physiological response to the chemical changes in environment. For the prevention of the aforementioned reaction, the mouths of the individuals were tied with string before the treatment with ethanol in order to keep them still prior to taking consumed blood samples (Vaughan, 2009). It is concluded that the vomiting of the digested blood during the study may be due to the physicochemical properties of the environment, the quantity and composition of the consumed blood and the physiological characteristics of the leeches. The lack of interest of the individuals who came to the area where the blood vomiting occurred and their departure from the area after a short investigation was derived from the quality of blood that has lost its charm of consumption and the starvation level of the individuals. The future studies will be useful to clarify the situation in which physicochemical and/or physiological reasons causing blood vomiting in the different stages of after feeding.

It was revealed that post-feeding process is a critical stage for *H. verbana* in the artificial environments with this study. It is concluded that the insufficient fed leeches are potential danger for the satiated leeches with restricted mobility. As a solution to this undesirable situation, it is recommended to create periodic feeding groups, stimulate the all leeches to make them ready before feeding and place the insufficient fed and satiated leeches in different environments after the feeding in the breeding conditions.

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