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Effect of Different Concentrations of Classic Taste Substances on Parameters of Feeding Behavior of *Rutilus frisii kutum*

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Abstract: Feeding behavior studies of fish are useful to improve of aquaculture and make a proper diet. Parameters of feeding behavior of *Rutilus frisii kutum* were determined to different concentrations of classic taste substances (citric acid, calcium chloride, sodium chloride and sucrose) by testing behavioral methods. Behavioral parameters that were recorded include: 1) the number of pellet grasps by fish, 2) retention time of the pellet in mouth after the first grasp, 3) retention time of the pellet in mouth during all grasps and 4) number of pellets consumed. It was found that these compounds created different behavioral responses in kutum. Pellets containing citric acid increased consumption of pellets. Consumption of pellets in concentration of 0.026 M of citric acid had significant difference with other concentrations. In citric acid and sucrose, the effect of concentration change on two parameters, consumption of pellets and proportion of the number of pellets consumed to the number of grasps was significant, but in calcium chloride and sodium chloride wasn't significant. The effect of concentration change on retention time of pellets after of the first grasp(s) in all substance was significant. Concentrations of palatability index and consumption of pellets in sodium chloride hadn't significant difference together.

Key words: Behavioral Responses · Classic Taste Substances · *Rutilus frisii kutum*

INTRODUCTION

Feeding is an elective process with the animal selecting specific food items from a variety of nutrient sources within the environment [1]. Feeding behavior is a complex behavior where a series of consequent phases and stages are discerned from the moment the animal receives the signal on the presence of a food object to the final realization by the individual of a decision to swallow or reject the caught food [2]. Fish exposed to food-related chemical stimuli initiate feeding behavior [3]. Food object are rejected only when they enter the oral cavity and presumably contact the walls of the pharynx. Pellets with highest level of palatability are immediately trapped in the pharynx and transported back toward the esophagus for subsequent ingestion [4].

The behavioral taste response in fish is relatively simple and includes several basic components: snap, retention of the pellet in the oral cavity, rejection of the pellet and its repeated snaps. The frequency and duration of these components differ and depend on the taste attractiveness of the pellet and the fish species [5]. Taste is primarily a close-range sense and its function is the identification of nutrients and the avoidance of noxious substances.

Taste receptors in vertebrates monitor material taken into the mouth and verify its palatability. They respond to a wide range of concentrations of a large number of chemical substances in the outside milieu (chemical substances are included: free amino acids, classic taste substances, nucleotides, nucleosides, organic acids and other types of substances) [6]. In the vertebrate taste system, environmental chemical compounds are received by taste buds, cell groups of epithelial origin [7]. There are taste buds on the surface of the lips, mouth cavity, gill cavity and the beginning of the esophagus [8] and the resulting signals are transmitted to the central nervous system through taste nerves innervating the taste bud cells [9]. Taste reception is responsible for the determination of the quality of food items and the

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assessment of their properties to the food requirements of the organism [10]. Fish may detect food by a variety of sensory mechanisms, in particular vision and olfaction, but gustatory system plays the key role in determining whether a particular food item is swallowed or rejected [11]. Chemical substances present in the diet of an animal may act as feeding stimulants, feeding enhancers (that is, they induce the animal to eat more than it normally would), or as feeding deterrents. The presence or absence of these compounds in the diet will determine whether a food item is eaten or rejected and, to some extent, the quantity consumed [12, 13]. It has been shown that these compounds evoke different types of behavioral taste responses, thus they are perceived as different by fish. Each of these substances creates different feeding behavior in fish. According to this feeding behavior can determine the suitability diet. Also different concentrations of these substances may be creates different feeding behavior in fish. So we can also determine the best concentration of these substances.

The aim of this study is to scrutiny feeding behavior of *Rutilus frisii kutum* juveniles to different concentrations of classic taste substances (citric acid, calcium chloride, sodium chloride and sucrose) and study effect of concentration change of these substances on parameters of feeding behavior of *Rutilus frisii kutum*.

MATERIALS AND METHODS

Fish Maintenance: The study was carried out on 4-5 months old kutum juveniles with 58.8±7.5 mm body length (TL) and 1.5±0.5g body weight) reared on the Fish Farm Sijaval (Turkmen Seaport, Golestan Province, Iran). Fish were transported to the Shahid Fazli Aquaculture Research Station of the Gorgan University and were maintained in common 100 L aquaria with water temperature 22-23°C and natural photoperiod during one month. Fish were fed manually once per day, until apparent satiation with on a commercial formulated diet (Biomar 0.8 mm) containing 56% of protein, 18% lipid and 10.4% ash.

A 20 days before trials, 20 fish were placed each in separate organic glass aquaria (5 L) equipped with air pump and do not contain gravel or other material on the bottom surface. The back and side walls of aquaria were opaque, for visual isolation of the fish. Observations were made through the transparent frontal wall of the aquarium. The fish were fed daily with thaw out frozen chironomid larvae. Chironomid larvae were offered the fish one by one in the front part of the aquarium. A part of water in aquaria was daily replaced with fresh water. After several days that the fish were adapted to the observer, new conditions and feeding procedure, the fish were trained to take artificial agar pellets flavored with chironomid larvae extract, 75 g L^{-1}. The fish training was lasted 5-7 days when fish were catching a dropped pellet after several seconds. During the experimental work the fishes were fed ad libitum with chironomid larvae once daily after the completion of trial session in order to ensure that they have the same feeding motivation in each trial.

Preparation of Pellets: Pellets were prepared from 2% agar gel. After dissolving agar in boiling distilled water, a dye (Cr_2O_3, 0.35%) and solution one of the four classical substances was added into hot agar solution. Homogenate of chironomid larvae was filtrated through a cotton cloth after extraction during 5 minutes at 18°C and obtained extract was added to cooled (50°C) agar solution. Then agar solution was mixed and poured into Petri dish. The concentrations of substances in the agar matrix are indicated in the Tables 1, 2, 3 and 4. Blank pellets with dye only were used as control. The cylindrical pellets with 1.1 mm in diameter and 1.5 mm in length pellets were cut from cold gel with a stainless steel tube just ahead of each trial. Agar gel contained classical taste substances and chironomid larvae extract were kept at +5°C up to 7 and 3-4 days respectively. In total, 23 types of agar gels distinguished by substances and their concentrations were prepared [14].

Experimental Design: Twenty fish were used for experiments. Each trial was lasted during 1 minute and fish behavior was recorded by the video camera Panasonic SDR-H250GS-S, Japan. During 1 minute we introduced a few plates to fish when fish swallowed the pellet, we introduced next plate.

Several behaviors were registered in each trial: 1) the number of pellet grasps made by fish during the trial, 2) retention time of the pellet in mouth after the first grasp, 3) retention time of the pellet in mouth during all grasps and 4) pellet consumption (was pellet swallowed or rejected in the end of trial). The retention time was recorded with a hand stop watch. The moment of the pellet consumption was determined by the termination of characteristic chewing movements of the fish jaws and the restoration of normal movements of gill covers. Pellet which was not grasped or not swallowed 1 minute after introduction was removed from the aquarium.

Table 1: Means comparison of behavioral responses in citric acid

Stimulus	Concentration, % (M)	Consumption of pellets, %	Index of palatability, %	Proportion of the number of pellets consumed to the number of grasps,%	Number of grasps	Retention time of pellets in the mouth after of the first grasp (s)	Average retention time of pellets in the mouth
Citric acid	10 (0.52)	25.8^{bc}	27.37^{bc}	17.92^{bc}	4.32^{c}	6.82^{a}	4.3^{ab}
Citric acid	5 (0.26)	29.2^{bc}	45.29^{ab}	25.65^{b}	4.96^{bc}	5.45^{ab}	8.56^{a}
Citric acid	3.5 (0.182)	29.4^{bc}	35.42^{abc}	24.39^{bc}	4.68^{bc}	6.07^{ab}	4.99^{ab}
Citric acid	2.5 (0.13)	24.4^{bc}	28.27^{bc}	21.02^{bc}	4.60^{bc}	2.96^{bc}	4.42^{ab}
Citric acid	0.5 (0.026)	48.49^{a}	59.87^{a}	42.40^{a}	5.77^{b}	3.66^{bc}	3.4^{b}
Citric acid	0.2 (0.01)	29.26^{bc}	36.5^{abc}	27.79^{bc}	5.8^{b}	3.1^{bc}	2.8^{b}
Citric acid	0.1 (0.0052)	12.91^{cd}	14.66^{cd}	12.18^{cd}	7^{a}	2^{c}	2.39^{b}
Citric acid	0.05 (0.0026)	10.25^{cd}	2.56^{d}	8.87^{cd}	6.54^{ab}	1.7^{c}	1.97^{b}
Control	0	5.88^{d}	0	4.52^{d}	5.8^{b}	0.6^{d}	0.95^{c}

Different superscript letters designations indicate a significant difference at $P \leq 0.05$.

Table 2: Means comparison of behavioral responses in calcium chloride

Stimulus	Concentration, % (M)	Consumption of pellets, %	Index of palatability, %	Proportion of the	Number of grasps	Retention time of pellets in the mouth after of the first grasp (s)	Average
Calcium chloride	10 (0.9)	5.25^{b}	-5^{b}	4.61^{b}	8.20^{a}	1.44^{b}	2.51^{a}
Calcium chloride	5 (0.45)	14.94^{ab}	9.66^{ab}	14.08^{ab}	5.45^{b}	1.71^{b}	1.75^{b}
Calcium chloride	1 (0.09)	18.43^{a}	32.22^{a}	14.12^{a}	6.05^{b}	2.21^{a}	2.23^{a}
Calcium chloride	0.5 (0.045)	10.05^{ab}	3.7^{ab}	8.26^{ab}	6.94^{ab}	1.6^{b}	1.64^{b}
Control	0	5.88^{b}	0	4.52^{b}	5.8^{b}	0.6^{c}	0.95^{c}

Different superscript letters designations indicate a significant difference at $P \leq 0.05$.

Table 3: Means comparison of behavioral responses in sodium chloride

Stimulus	Concentration, % (M)	Consumption of pellets, %	Index of palatability, %	Proportion of the number of pellets consumed to the number of grasps,%	Number of grasps	Retention time of pellets in the mouth after of the first grasp (s)	Average retention time of pellets in the mouth
Sodium chloride	10 (1.73)	5.60^{a}	-2.7^{a}	6.74^{a}	7.27^{a}	1.8^{a}	2.8^{a}
Sodium chloride	1 (0.173)	14.30^{a}	12.96^{a}	12.68^{a}	5.33^{b}	1.91^{a}	1.64^{b}
Sodium chloride	0.5 (0.0865)	9.31^{a}	13.33^{a}	7.79^{a}	6.05^{ab}	2.08^{a}	1.37^{bc}
Sodium chloride	0.1 (0.0173)	11.55^{a}	6.11^{a}	10.30^{a}	4.8^{b}	1.03^{b}	0.91^{cd}
control	0	5.88^{a}	0	4.52^{a}	5.8^{b}	0.6^{c}	0.95^{d}

Different superscript letters designations indicate a significant difference at $P \leq 0.05$.

Table 4: Means comparison of behavioral responses in sucrose

Stimulus	Concentration, % (M)	Consumption of pellets, %	Index of palatability, %	Proportion of the number of pellets consumed to the number of grasps,%	Number of grasps	Retention time of pellets in the mouth after of the first grasp (s)	Average retention time of pellets in the mouth
Sucrose	10 (0.29)	1.88^{b}	-12.97^{b}	1.51^{b}	8.81^{a}	1.63^{b}	1.7^{a}
Sucrose	5 (0.145)	14.47^{a}	16.50^{a}	13.24^{a}	5.85^{b}	1.07^{c}	0.98^{b}
Sucrose	1 (0.029)	26.07^{a}	33.51^{a}	21.43^{a}	7.05^{ab}	2.39^{a}	1.67^{a}
Sucrose	0.5 (0.0145)	18.33^{a}	29.33^{a}	18.03^{a}	6.9^{ab}	1.84^{ab}	1.86^{a}
Sucrose	0.1 (0.0029)	9.62^{ab}	5.26^{ab}	9.4^{ab}	6.21^{ab}	1.1^{c}	1.13b^{b}
Control	0	5.88^{b}	0	4.52^{b}	5.8^{b}	0.6^{c}	0.95^{b}

Different superscript letters designations indicate a significant difference at $P \leq 0.05$.

Table 5: The effect of concentration change on behavioral responses in classic taste substances

Stimulus	Consumption of pellets	Index of palatability	Proportion of the number of pellets consumed to the number of grasps	Number of grasps	Retention time of pellets after of the first grasp (s)	Average retention time of pellets
Citric acid	0.008	0.85	0.009	0.002	0.041	0.086
Calcium chloride	0.162	0.185	0.178	0.036	0.007	0.06
Sodium chloride	0.625	0.613	0.671	0.027	0.004	0.000
Sucrose	0.003	0.004	0.003	0.128	0.000	0.000

Data Analysis: The percentage of pellets swallowed to pellets grasped and the percentage of pellets swallowed to the number of grasps were calculated for each type of pellet. The index of palatability was calculated following the ratio: $Ind_{pal} = [(R - C)/(R + C)] \times 100$, where R is the number of swallowed pellets with the substance, C, number of swallowed control pellets [14]. Statistical analyses were performed using nonparametric test.

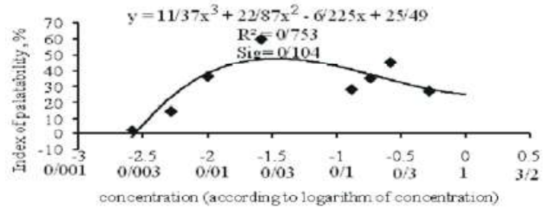

Fig. 1: Changing in index of palatability of citric acid

RESULTS

Trained fish show a high feeding activity and grasp the offered pellets almost immediately, in most cases during the first 1-2 s of a trial. Usually fish perform several repeated grasps, up to 4-9 in the same trial before to swallow or to finally refuse the pellet.

Citric Acid: Consumption of pellets, palatability index and proportion of the number of pellets consumed to the number of grasps in concentration of 0.026 M had significant difference with other concentrations. With reducing the content of citric acid up to 0.026 M these three parameters increased but afterwards decreased (Table 1 and Fig. 1). In citric acid palatability index of 0.26 to 0.01M was highest value (Fig. 1). Retention times of pellets in the mouth with reducing the content of citric acid decreased (Fig. 1). The effect of concentration changes on consumption of pellets and proportion of the number of pellets consumed to the number of grasps was significant, also in number of grasps and retention time of pellets after of the first grasp (s) (Table 5).

Calcium Chloride: Consumption of pellets, palatability index and proportion of the number of pellets consumed to the number of grasps only in concentration of 0.09 M were highest value and other concentrations hadn’t significant difference together (Table 2). The effect of concentration changes on index of palatability, consumption of pellets and proportion of the number of pellets consumed to the number of grasps wasn’t significant (Table 5). With reducing concentration of calcium chloride up to 0.09 M these three parameters increased but afterwards decreased (Table 2). Also with reducing concentration number of grasps decreased. Retention times of pellets in the mouth showed different values in different concentration (Table 2).

Sodium Chloride: The effect of concentration changes on index of palatability, consumption of pellets and proportion of the number of pellets consumed to the number of grasps wasn’t significant (Table 5). Concentrations of these three parameters hadn’t significant difference together. Sodium chloride was an indifferent taste substance for kutum, because it did not lead significant increase in the consumption plates. Decreasing the concentration of sodium chloride resulted in the consumption of pellets with this substance not different from the consumption of the control pellets. Number of grasps and average retention time of pellets in the mouth with reducing concentration was decreased but not found dramatically differences between concentrations (Table 3).

Sucrose: The effect of concentration changes on behavioral responses except in number of grasps in other parameters was significant (Table 5). Highest level of palatability and consumption of pellets was observed in concentration of 0.029 M that except with concentration of 0.29 M hadn’t significant difference with other concentrations. Concentration of 0.29 M was deterrent and reduced consumption of pellets Number of grasps with reducing concentration decreased but not found dramatically differences between concentrations (Table 4).

Citric acid and sucrose compared to calcium chloride and sodium chloride had high consumption. The effect of concentration change on consumption of pellets and proportion of the number of pellets consumed to the number of grasps in calcium chloride and sodium chloride (with the least attractive) wasn’t significant. Therefore can be stated consumption of substance with the least attractive were influenced by concentration change a much lesser extent. Because these substance always have consumption by fish less. Pellets containing citric acid had compared to other materials three the highest level of palatability; also retention time of pellets in the mouth for this material was high.

DISCUSSION

Almost all information relating to the functional development of the taste system in fish was obtained by methods of behavioral testing. These methods allow us to determine not only the ability of juveniles to sense the presence of various substances in food objects (the artificial feed pellets with controlled chemical composition) but also to determine the behavior of juveniles to the taste of these substances [6].

All sensory systems are involved in regulation of feeding behavior in fish [15]. The gustatory reception plays a special role in feeding behavior in fish compared with other groups of animals; the gustatory reception in fish is very highly developed [2]. There is no information about feeding behavior of kutum. The results of this study showed kutum can identifies the presence of various substances in the diet; this ability may be due to the gustatory reception (taste buds).

When studying the traits of *kutum*'s behavioral taste response an attention is attracted by such outstanding traits as the highly prolonged retaining of the pellets in the oral cavity, in particular, those which contain palatable substances. The time of retention of the pellets with citric acid during the trial reached 8 or more seconds, which distinguishes the *kutum* from such fish as the Caspian *Salmo trutta* or keta whose time of retention of the pellets in the oral cavity was sometimes shorter [16]. Benthivorous fishes keep the pellets in the mouth cavity much longer than other fishes, which can be explained by the mode of feeding of these fishes, by the necessity of the separation of food items from abundant detritus material taken in with them [17]. The retention time of pellets in the mouth by the fish is especially interesting. This time is required for a whole complex of processes: reception of the taste substance, transmission of the information to the taste centers, its analysis, development of the appropriate behavioral response, ingestion of the grasped pellet or its rejection for subsequent grasps, or for final rejection [10]. Most of this time, which in fish varies more widely than in humans, is required for information analysis in brain centers and development of the appropriate behavioral response [18]. The duration of behavioral taste response in fish depends on many factors. As in humans, one such factor is the concentration of the substance. Increasing the concentration increases the retention time and therefore the duration of the behavioral taste response in fish like tench and common carp [10, 14]. In this study, similar results were obtained with tench and *Cyprinus carpio*, an increase in the concentration of citric acid the increase retention time of pellets in the mouth. In humans, however, this relationship is inversely, an increase in the concentration of the active substance reduces the duration of the taste response [18].

With decreasing concentration of a substance its stimulating efficiency decreases. The character of the dose-response relationship may be close to a linear or parabolic [14]. We conducted about palatability index of citric acid, showed that this relationship was parabolic (Fig. 1). This result is similar to results obtained on carp by Kasumyan and Morsi [14]. A clearly pronounced decrease in the substance effectiveness with decline in its concentration was observed.

Many regularities of the gustatory sensitivity in fish, their specific traits of reacting to various types of gustatory substances are important in the applied aspect and may be used for the solution of various problems of fish capture and culture. The results of investigations showed that the screening and elaboration of highly efficient stimulants and deterrents for fish are promising and make a biological basis for designing of control methods for regulation of feeding behavior in fish using gustatory stimuli [7].

CONCLUSIONS

Many patterns of taste in fish and specific characteristics of their response to various taste substances are very important in the applied aspect and could be used in fish culture and fisheries. Management of feeding behavior in fish could be developed with introduction of certain stimulators and deterrent substances. Results of the present study provide evidence that certain feeding behaviors of *Rutilus frisii kutum* can be elicited or modified by different taste stimuli, also evidence of the *kutum*'s ability to distinguish the subtleties of taste properties in the caught prey. It is shown that the substances close in chemical structure and belonging to the same class of compounds like classic taste substances, can have quite different taste properties for the *kutum*, ranging from highly attractive tastes to deterrent ones. Kutum can recognition different concentrations of one substance and it showed different behavioral response to this substance. According to this ability, substance of attractive and deterrent can be determined and then be added to the diet of kutum substance of attractive. In fact can provided suitable diet by using food behavior of animal.

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