

Taste Sensitivity of Common Carp *Cyprinus Carpio* to Free Amino Acids and Classical Taste Substances

A. O. Kasumyan, A. M. Kh. Morsi

Moscow State University, Biological Faculty, Vorob'evy Gory, Moscow, 119899 Russia

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Abstract—Taste response of the yearlings of carp *Cyprinus carpio* of 9–12 cm in length to 21 free amino acids (L = stereoisomers) and classic taste substances is ascertained by in testing behavioral methods. It is found that these compounds can be divided by their taste properties into groups of substances that are highly attractive (cysteine, proline, glutamic and aspartic acids, alanine, glutamine, citric acid, calcium chloride), neutral (histidine, lysin, leucine, tyrosine, glycine, asparagine, isoleucine, norvaline, sodium chloride, saccharose) and deterrent (tryptophane, arginine, threonine, methionine, phenylalanine, serine, valine). Threshold concentrations are determined for the most attractive substances. It is estimated that the absolute quantity of the substance in pellet which is sufficient for stimulating a significant taste response is 4.27 and 3.39 μg or 3.53×10^{-10} and 1.77×10^{-10} mole for cysteine and citric acid. Positive correlation between palatability of pellets and the duration that are retained by fish in the oral cavity is shown. The anosmiating of fish did not cause any changes in the features and intensity of fish gustatory response, nor in range of effective concentrations. The relationship between the palatability of amino acids and some of their structural features, and the physiological requirements of carp for them, is considered. A hypothesis of the zonality of the intraoral gustatory buds sensitive to different types of substances is made.

The carp *Cyprinus carpio* belongs to the traditional and special inhabitants of the world's freshwater aquaculture. Its existence spans many centuries (Balon, 1995; Hoffmann, 1995). In Russia and many other countries the carp takes first places in the volume of artificially cultivated fish. Just owing to the great economic importance of the fish many noted works, many of which are devoted to further improving carp rearing technology, are dedicated to the investigation of the different aspects of its biology. In connection with the important role of chemosensory system in the regulation of the feeding behavior of fish (Pavlov, Kasumyan, 1990) and the prospects of creating highly effective olfactory and gustatory stimulations, great attention is given to the study of the chemosensory system of carp, particularly the odor and taste stimulants.

We investigated the macromorphology and ultrastructural organization of the olfactory organ and its development in the ontogenesis of carp (Pashchenko, 1986; Yamamoto, Ueda, 1978; Appelbaum, 1981; Datta, Deb, 1989), the structure of the surface layers of the olfactory bulb (Alonso *et al.*, 1989), and the ion composition of the olfactory mucus (Gdovskii *et al.*, 1991). Using electrophysiological methods of recording the electrical activity in the olfactory tracts and bulbs, we revealed carp's sensitivity to different odorous substances of various natures and found the threshold concentration of some of them; we showed that there is an existence of the carp olfactory receptors with different sensitivity and selectivity to olfactory stimulants (Ruzhinskaya, 1976, 1978; Il'in *et al.*, 1983;

Ruzhinskaya, Gdovskii, 1988; Goh, Tamura, 1978; Satou *et al.*, 1983), and investigated special items connected with the mechanisms of olfactory reception (Potapov, 1991; Ruzhinskaya, Gdovskii, 1992; Ohno *et al.*, 1984; Fesenko, 1989). We found out the nature of behavioral responses of carp to the natural and artificial olfactory stimulants, and traced the development of olfactory sensitivity in ontogenesis (Bondarenko *et al.*, 1984, 1986; Kruzhalov, 1986; Kasumyan, Ponomarev, 1990; Kuz'min, 1991; Saglio, Blanc, 1983; Saglio *et al.*, 1990). Special attention was given to the research and identification of the nature of the olfactory stimulants in feeding behavior. The different groups of substances were discerned from the composition of natural food organisms, their attracting effect for carp was assessed, and the complex multicomponent composition of natural feeding olfactory signals in fish was found (Kasumyan, Ponomarev, 1991; Tsushima, Ina, 1978).

The gustatory reception of carp is investigated not in every detail. The information on morphology and topography of olfactory buds is very scanty (Edwards, 1930; Hirata, 1966; Sibbing, 1986). Data on the sensitivity of taste receptors in carp, sensitive to substances such as salts, inorganic acids, sugars, and amino acids, are obtained. The threshold concentrations for a number of substances are determined. The specificity of taste receptors in carp is investigated by a method of cross-adaptation (Vasilevskaya, 1974; Vasilevskaya, Polyakova, 1977, 1980; Konishi, Zotterman, 1961; Konishi, Niwa, 1964; Konishi, 1966; Konishi *et al.*, 1966;

Hidaka, 1970b, 1970c; Marui *et al.*, 1980b; 1989). Possibility to develop the instrumental and conditioned reflexes in carp, with taste receptor stimulation by salts and acids, is shown (Vasilevskaya, Nikitina, 1975, 1978). The effects of toxicants (salts of heavy metals) on the functional characteristics of fish gustatory system is traced, with reference to carp (Hidaka, Yokota, 1967; Hidaka, 1970a). Carp's response to the taste of substances has been poorly studied until now (Bondarenko, 1985; Appelbaum, 1980).

The goal of this work was to investigate the features of carp's gustatory behavioral responses to free amino acids and to traditional tasting substances. The results of this work will expand the limited list of fish species for which the gustatory spectra of behavior-effective substances are known (Kasumyan, Sidorov, 1992, 1993, 1994a, 1994b; Hidaka *et al.* 1978; Adams *et al.*, 1988; Jones, 1989, 1990; Kasumyan, 1995; Kasumyan, Sidorov, 1995). The comparative interspecific analysis of the composition of these spectra will allow the elucidation of the role of gustatory reception in ensuring the feeding specificity of fish, and their selection and consumption of appropriate food organisms. The possibility of finding the extent of the effectiveness of gustatory stimulants when recording the responses at the systemic (chemosensory) (Marui *et al.*, 1983b) and organismic (behavioral) levels is also obtained. The data on the specificity and intensity of the carp's behavioral gustatory responses is of significant practical interest in view of creating highly effective chemical feeding stimulants and using them to increase the attractiveness of artificial foods.

MATERIALS AND METHODS

We conducted experiments in carp yearlings with lengths (1) of 9–12 cm and masses of 12–15 g, which, after they were received from the fish farm (All-Russian scientific and production association for fish rearing, Moscow oblast) they were retained for 3–4 months in a common aquarium (450 l) at water a temperature of 16–17°C and fed with live or frozen fresh Chironomidae larvae. We placed the fish, one by one, in aquaria of organic glass (12 l) 1–3 days before the experiments and trained them to catch the feed introduced into the aquarium—at first the larvae of chironomids, and then the experimental pellets. The trained, experimental fish caught the agar-agar pellets 100% of the time. Air was supplied to each of the aquaria by the microcompressors AN-4, through the fine-porous ceramic sprayers. The produced upwards water flows suspended a pellet in a water column during the whole experiment. The individual aquaria had the upper covers with a hole in the center, through which feed or the agar-agar pellets were supplied.

The experiment began with the adding of a single pellet, containing one of the substances to be tested, to an aquarium. From the moment the experimental fish caught the pellet we recorded for one minute the

following parameters: 1) number of times the added pellet was caught for length of experiment; 2) the duration the first pellet was retained in mouth after the fish caught it (seconds); 3) total duration the pellet was retained in the mouth of the fish for the entire experiment (seconds); 4) rate at which the pellets were eaten, i.e. whether the pellet was swallowed or rejected. Subsequently we determined the percentage of eaten pellets from the total number of those presented and from the number of pellets containing one of the tested substances. We also calculated the index of palatability of the substances by the formula:

$$Ind_{pal} = \frac{R - C}{R + C} 100,$$

where Ind_{pal} —index of palatability of substance; R—consumption of pellets with the substance, %; C—consumption of control pellets, %.

The end of the retaining of the pellet in the oral cavity and the swallowing were recorded on the basis of completion of the characteristic masticatory movements by the jaws, and the resumption of the rhythmic movements of the opercula. We recorded the time the pellet was retained in oral cavity using a hand stopwatch "Agat" of the summing type.

The experiments in which the pellet was not caught by the fish over one minute's time were not taken into account (the number of such occurrences was extremely insignificant). The pellets containing different test substances were supplied in random sequence and alternated with those containing an extract of chironomids. Uneaten pellets were removed from the aquarium after the end of the experiment. The interval between trials performed with the same specimen was at least 10–15 min.

The pellets of 2.5 mm in length and 1.5 mm in diameter were cut out from agar-agar gel (2%) using stainless steel pipes just before the trial. In order to prepare the gel, we added agar-agar powder (Reanal) to water and heated it on a water bath at 70–80° until it was completely dissolved. Then we added the Ponceau 4R stain (0.033) giving a bright red color to the gel, and also to one of the tested substances or water extract of chironomids (75 g/l). We used citric acid, sodium chloride, calcium chloride, and saccharose (all REAKHIM, pro analysis grade) as the substances inducing the basic types of gustatory sensations in human. A list of the used, free amino acids (L-stereoisomeres, Fluka, NBC, Calbiochem) is given in Table 1. The agar-agar pellets containing no substances other than the dye, were used in the control group. The gel containing amino acids and the classical tasting substances were kept at +5° for not more than seven days. The gel containing the extract of chironomids was kept under the same conditions for not more than 3–4 days.

In order to elucidate the chemosensory channel responsible for the observed behavioral responses of the carp to the pellets we conducted experiments in the

Table 1. Taste responses of carp to free amino acids

Stimulant	Concentration, M	Consumption of pellets, %	Index of palatability, %	Number of catching acts	Duration of retaining pellet, sec.		Number of tests
					After the first catching	During all test	
Extract of chironomids	75.0	100***	74.4	1.0***	9.8 ± 0.3***	9.8 ± 0.3**	109
Cysteine	0.1	99.0 ± 1.0***	74.1	1.0 ± 0.02***	16.5 ± 0.5***	16.7 ± 0.5***	100
Proline	0.1	51.6 ± 5.2***	55.7	4.9 ± 0.4***	5.3 ± 0.7***	18.1 ± 1.1***	93
Glutamic acid	0.01	36.3 ± 5.1***	42.4	2.5 ± 0.2	6.5 ± 0.9***	11.6 ± 1.1***	91
Aspartic acid	0.01	34.9 ± 5.2**	40.7	2.3 ± 0.2	7.1 ± 1.0***	12.5 ± 1.2***	86
Alanine	0.1	34.0 ± 4.9**	39.6	3.3 ± 0.3***	4.1 ± 0.7	11.0 ± 1.0**	94
Glutamine	0.1	33.7 ± 5.0**	39.3	3.9 ± 0.3***	3.5 ± 0.5	12.4 ± 1.1***	92
Histidine	0.1	11.1 ± 3.3	-14.0	1.9 ± 0.2	2.9 ± 0.5	5.4 ± 0.8	90
Lysin	0.1	9.6 ± 3.3	-21.0	1.9 ± 0.2	2.8 ± 0.4	5.0 ± 0.6	83
Leucine	0.01	9.2 ± 3.1	-23.0	1.8 ± 0.1	2.3 ± 0.5	4.8 ± 0.8	87
Tyrosine	0.001	8.9 ± 3.0	-24.6	1.7 ± 0.1*	2.5 ± 0.5	4.4 ± 0.7	90
Glycine	0.1	8.5 ± 2.9	-26.7	2.6 ± 0.2	6.0 ± 0.6***	9.4 ± 0.6**	94
Asparagine	0.1	7.4 ± 2.7	-33.0	3.6 ± 0.2	2.4 ± 0.3	6.7 ± 0.6	95
Isoleucine	0.01	6.9 ± 2.7	-36.1	1.9 ± 0.2	1.8 ± 0.2	4.3 ± 0.6*	87
Norvaline	0.1	6.5 ± 2.6	-38.7	2.2 ± 0.2	2.2 ± 0.2	5.2 ± 0.7	93
Tryptophane	0.01	4.5 ± 2.2*	-53.1	3.3 ± 0.3***	1.5 ± 0.1*	3.8 ± 0.5**	89
Arginine	0.1	3.7 ± 2.1*	-59.8	1.7 ± 0.2	1.6 ± 0.1*	3.6 ± 0.6***	81
Threonine	0.1	3.5 ± 2.0**	-61.5	2.1 ± 0.2	2.2 ± 0.5	4.1 ± 0.5*	86
Methionine	0.1	1.2 ± 1.2**	-84.9	1.7 ± 0.1*	1.6 ± 0.1*	2.9 ± 0.5***	83
Phenylalanine	0.1	1.0 ± 1.0***	-87.3	2.7 ± 0.2*	1.6 ± 0.1*	4.2 ± 0.4**	96
Serine	0.1	1.0 ± 1.0***	-87.3	2.4 ± 0.2	1.8 ± 0.3	3.9 ± 0.4**	97
Valine	0.1	0***	-100.0	2.6 ± 0.2	1.5 ± 0.1*	4.0 ± 0.3**	95
Control	-	14.7 ± 3.7	0	2.1 ± 0.2	2.6 ± 0.4	6.6 ± 0.8	95

Note: Concentration of chironomids extract is expressed in g/l. *, **, *** – significance of the difference, $P > 0.95, 0.99, 0.999$, respectively, with respect to control.

anosmiated specimens. Anosmiation was made by cauterization of the olfactory rosettes.

During the experiments we fed the fish with chironomids ad libitum once a day after the ends of the trials.

For the statistical analysis of the obtained results we used the Statgraphics software package, version 5.0. In total we performed 2902 experiments on 20 normal and five anosmiated specimens of carp.

RESULTS

Gustatory Responses to Free Amino Acids

It is found that the carp gustatory responses to the pellets with different amino acids differ in nature and intensity. In general, all amino acids could be divided into three roughly equal groups: group 1—the amino acids having the stimulating properties, i.e. increasing

a consumption of pellets; group 2—the amino acids having the deterrent, i.e. repellent taste properties and group 3—the amino acids having indifferent taste properties for the carp (Table 1).

The first group includes six amino acids, one of which cysteine, shows an extremely high palatability. The pellets with amino acid were eaten up by carps in almost 100% of the experiments and swallowed after the first catching of the pellet, i.e. the pellets containing cysteine were practically never rejected by the fish in course of each particular test. The average duration of retaining the pellets containing cysteine in oral cavity amounted to 16 sec., and was sometimes longer, compared to the pellets containing other amino acids, especially, the deterrent ones. Palatability of other stimulating amino acids—proline, glutamic acid, aspartic acid, alanine, and glutamine, was essentially less than that of cysteine. The duration these pellets were retained by

Table 2. Coefficients of correlation between parameters of taste responses of carp to free amino acids

Parameters of taste response	Number of catching acts	Duration of retaining pellet, sec.	
		After the first catching	During all test
Consumption of pellets, %	0.06	0.92***	0.89***
Number of catching acts	–	–0.17	0.41
Duration of retaining pellet after the first catching	–	–	0.78***

@ $P > 0.999$.

the fish during the first catching was shorter too. However the total duration of the gustatory testing of the pellets was close for all stimulating amino acids and significantly longer than for the pellets with all other amino acids.

The deterrent properties were shown by seven amino acids, most of them—methionine, phenylalanine, serine, and valine—resulted in almost complete refusal of carps to consume the caught pellets. In the course of the experiment the pellets containing the deterrent amino acids were more than once rejected and then were repeatedly caught by the experimental fish. The duration the pellet was kept in the oral cavity, in particular after the first catching, was extremely low, ten times lower than, for example, the duration of retaining the pellets with cysteine (highly attractive for carp).

The group of the gustatory indifferent amino acids was the most numerous. It includes eight amino acids. The parameters of fish gustatory response to the pellets

with indifferent amino acids were characterized by the intermediate values.

The correlation analysis of the individual parameters of the carp gustatory response revealed a high positive correlation between the share of the eaten pellets and the duration of their gustatory testing of the fish (total and after the first catching) and the absence of a significant correlation between the share of the eaten pellets and the number of catching acts. The duration the pellet was retained in the oral cavity after the first catching by the fish and the duration it was retained in the oral cavity during the whole of the test, i.e. from moment of the first catching up to the moment it was swallowing, or finally rejected, by the fish (Table 2).

Gustatory Responses of Carp to Classical Taste Substances

The strongest preference was for citric acid; the consumption of the pellets containing citric acid approached 100%. Stimulating the effectiveness of calcium chloride was significant, but was 1.78 times lower than that of citric acid. Sodium chloride and saccharose had no significant effect on the consumption of the pellets. The most palatable pellets with citric acid were swallowed by the fish after the first catching in most tests, while the less attractive pellets and the pellets with saccharose were more than once rejected by fish and then repeatedly caught again. With that, the carps retained the pellets with citric acid in the oral cavity for a considerably longer time after the first catching, while the gustatory testing of the less attractive pellets did not last as long (Table 3).

Determination of the Threshold Concentrations of Some Substances

The threshold concentrations are determined for the most effective amino acids, cysteine, as well as for citric acid and sodium chloride. It was revealed that consumption of pellets abruptly reduces with increasing the concentration of the substances (Table 4). Use of palatability index for assessing the intensity of fish's

Table 3. Gustatory responses of carp to classical taste substances

Stimulant	Concentration, %	Consumption of pellets, %	Index of palatability, %	Number of acts of catching	Duration of retaining pellets, sec.		Number of trials
					After the first catching	During the experiment	
Citric acid	0.26 (5.0)	92.1 ± 3.4***	53.9	1.1 ± 0.03***	12.3 ± 0.7**	12.7 ± 0.6	63
Calcium chloride	0.9 (10.0)	51.7 ± 6.5**	30.4	1.8 ± 0.2**	9.3 ± 0.9	11.5 ± 0.8	60
Sodium chloride	1.73 (10.0)	43.5 ± 6.3	22.4	1.9 ± 0.2**	8.3 ± 0.8	10.6 ± 0.8	62
Saccharose	0.29 (10.0)	20.9 ± 5.2	–13.8	2.4 ± 0.2	7.0 ± 0.7	10.0 ± 0.7	62
Control	–	27.6 ± 5.6	0	2.6 ± 0.2	8.3 ± 1	11.4 ± 1.2	65

Note: See note to Table 1

Table 4. Gustatory responses of carp to low concentrations of highly palatable substances

Stimulant	Concentration, M	Consumption of pellets, %	Index of palatability, %	Number of acts of catching	Time of retaining pellet, sec.		Number of trials
					After the first catching	During all trial	
Cysteine	0.1	100***	59.0	1.0***	15.3 ± 0.4***	15.3 ± 0.4**	48
Cysteine	0.01	49.9 ± 6.2*	31.8	1.4 ± 0.2	9.3 ± 1.2**	12.4 ± 1.4	48
Cysteine	0.001	28.3 ± 9.3	4.6	1.5 ± 0.2	7.6 ± 1.0	9.8 ± 1.3	48
Control	–	25.8 ± 8.0	0	1.8 ± 0.2	4.4 ± 1.1	7.8 ± 1.5	48
Citric acid	0.05	59.4 ± 8.8**	74.7	1.2 ± 0.1***	11.1 ± 1.3***	11.8 ± 1.4**	32
Calcium chloride	0.09	16.7 ± 6.9*	32.0	1.7 ± 0.2	3.2 ± 1.0	5.0 ± 1.1	30
Control	–	8.6 ± 4.8	0	1.9 ± 0.2	4.4 ± 0.9	6.9 ± 1.0	35
Citric acid	0.005	30.6 ± 7.8**	73.4	1.9 ± 0.2	5.3 ± 1.2	9.8 ± 1.4	36
Citric acid	0.0005	2.0 ± 2.2	40.3	2.8 ± 0.3	3.3 ± 0.4	6.1 ± 0.6	36
Control	–	4.7 ± 3.2	0	2.1 ± 0.3	4.2 ± 0.7	6.2 ± 0.7	43

Note: See note to Table 1

response enabled us to find out that the correlation between the palatability of the pellets and the content of the actual substances in them has a more complicated nature with using citric acid as compared with using cysteine (Fig. 1). With reducing the content of the substance the time when the pellet is being retained in oral cavity both after the first catching and during the trial a number of the repeated acts of catching of pellet increases. For cysteine the threshold concentration at which the consumption of pellets still significantly exceeded the level of the control pellet consumption, which was 0.01 M. For citric acid the threshold concentration was 0.005 M (0.1%). Decreasing the concentration of calcium chloride by one order of – to 0.9 M (1.0%) resulted in the consumption of the pellets with this substance not differing from the consumption of the control pellets (See Table 4).

Influence of Anosmiating on Gustatory Responses

The experiments were conducted in the carps ten days after bilateral cauterization of the olfactory rosettes. As a gustatory stimulant we used the amino acid, cysteine, having a high stimulating effect (See Table 1). The anosmiated carps swallowed the pellets with cysteine (0.1 M) in 100% of the cases and the swallowing of the pellets occurred after the first catching, just like in the intact specimens. The time the pellet was retained in the oral cavity was long—more than 14 sec. The pellets in which a concentration of cysteine was reduced to 0.01 M became less palatable for the fish; the number of acts of catching a pellet increased and time of its gustatory testing decreased. The gustatory responses of the anosmiated fish to the pellets with a cysteine concentration of 0.001 M did not differ

significantly from the fish's responses to the control pellets in all response parameters recorded (Table 5).

DISCUSSION

The results of this work give evidence of the carp'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 free amino acids, can have quite different taste properties for the carp, ranging from highly attractive tastes to deterrent ones. With that, the intensity of carp's responses to substances of the same taste group, for example, the stimulating or deterrent ones, also varies significantly. The experiments performed on anosmiated specimens showed that anosmiating the fish did not result in a change in the intensity and direction of the response or the range of the effectiveness of the concentrations. These data allow us to conclude that the olfactory system does not participate in sensory ensuring of the observed differences in the behavioral responses of the experimental fish to pellets with different substances, and also that the nature and intensity of responses appear to be completely controlled by intraoral gustatory reception. A comparison of our data with the results of other authors (Bondarenko, 1985; Appelbaum, 1980) shows that taste responses to a number of substances are similar or agree, and that the observed differences may be attributed to differences in the used methods of investigation and in the active concentrations of substances.

The presence of those free amino acids that cause an enhancement of pellet consumption, i.e. those that may be classified as feeding stimulants (Mackie, 1982; Sakata, 1989), is not unexpected and is in agreement with the results of previous investigations (Kasumyan, 1995; Hidaka *et al.*, 1978; Mackie, 1982; Adams *et al.*,

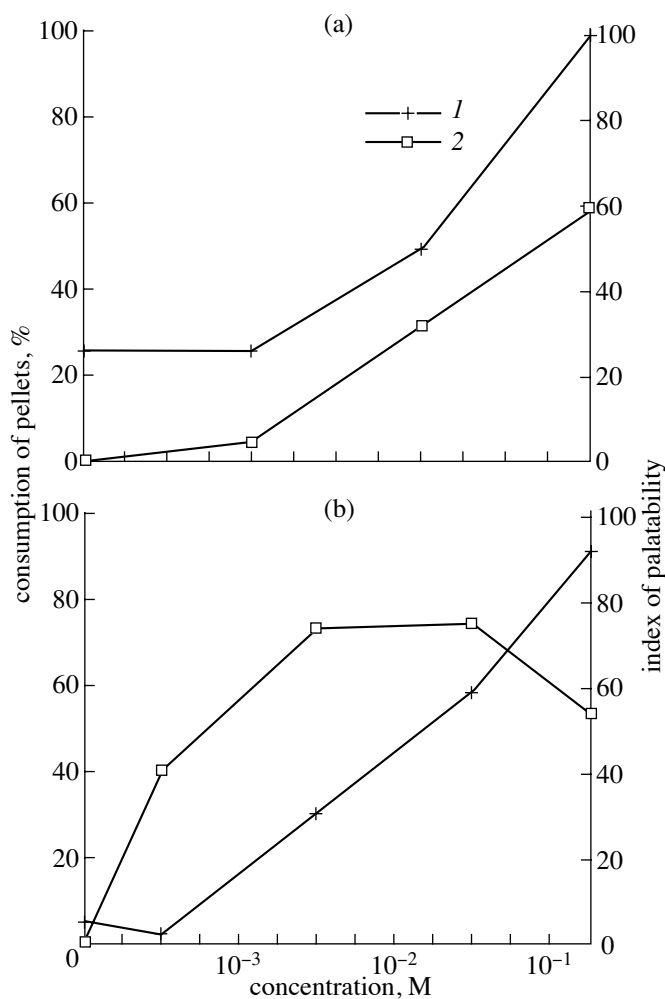


Fig. 1. Changing in pellet consumption and index of palatability of cysteine (a) and citric acid (b) depending on their content in pellets, %.

1988; Jones, 1989). A share of the stimulating amino acids in carp amounts to about 29% of the total number of amino acids tested. In previously investigated *Salvelinus alpinus erythrinus*, Caspian *Salmo trutta caspius*, keta *Oncorhynchus keta*, Russian sturgeon *Acipenser gueldenstaedti*, Siberian sturgeon *A. baeri*,

and starred sturgeon *A. stellatus* the share of the stimulating amino acids was 19, 29, 67, 29, 37 and 14% respectively (Kasumyan, Sidorov, 1992, 1994a; Kasumyan, Sidorov, 1995; Kasumyan, 1995). In *Tilapia zillii* (Johnsen, Adams, 1986) the palatable amino acids amounted to 37%. Thus, for only a single species, keta, most of the tested free amino acids being, known to be present in many food organisms (Dabrowski, Rusiecki, 1983; De la Noue, Choubert, 1985; Holm, Walther, 1988; Kumai *et al.*, 1989), had an attractive taste. In Japanese horse mackerel *Trachurus japonicus* only one of 20 tested amino acids, tryptophane, showed a stimulating effect (Ikeda *et al.*, 1988b). On the whole in Japanese horse mackerel the amino acid fraction made up an insignificant share of high palatability of food organism extract (Ikeda *et al.*, 1988a, 1988b). In other species—*T. zillii* (Johnsen, Adams, 1986), rainbow trout *Salmo gairdneri* (Mearns *et al.*, 1987), Japanese eel *Anguilla japonica* (Takeda *et al.*, 1984), marbled rockfish *Sebasticus marmoratus* (Takaoka *et al.*, 1990)—one can observe the opposite, the palatability of the food organism extract or its imitating mixture of substances was in full or to a large measure ensured by the presence of free amino acids.

In the carp the share of the amino acids indifferent by their taste properties is 38%. In other species that we investigated previously this group of amino acids is also the most numerous and amounts to 76, 32, 24, 71, 58 and 86% in *Salvelinus alpinus erythrinus*, *Salmo trutta*, Russian and Siberian sturgeons, and starred sturgeon, respectively (Kasumyan, Sidorov, 1992, 1994a; Kasumyan, Sidorov, 1995; Kasumyan, 1995). The share of deterrent amino acids in carp was 33% and exceeded the group of stimulating amino acids in number. In *Salvelinus alpinus erythrinus*, *Salmo trutta*, keta and Siberian sturgeon the deterrent amino acids amounted to 5, 29, 9 and 5%, respectively. However in all these fish the number of deterrent amino acids was less than that of stimulating and indifferent ones. The results of this work support once more the ability of some free amino acids to ensure the deterrent effect in the fish, which we revealed previously. The presence of not only those free amino acids which stimulate food consumption, but also the indifferent amino acids and

Table 5. Gustatory responses of anosmiated specimens of carp to cysteine

Stimulant	Concentration, M	Consumption of pellets, %	Index of palatability, %	Number of acts of catching	Time of retaining a pellet, s.		Number of trials
					After the first catching	During all experiment	
Cysteine	0.1	100***	48.1	1.0	14.4 ± 0.2***	14.4 ± 0.2**	20
Cysteine	0.01	60.0 ± 7.0*	26.3	1.8 ± 0.2	7.9 ± 1.4	11.7 ± 1.3	20
Cysteine	0.001	35.0 ± 10.9	0	1.4 ± 0.1	8.2 ± 1.5	9.0 ± 1.4	20
Control	—	35.0 ± 8.9	0	1.9 ± 0.2	4.3 ± 1.3	9.0 ± 1.7	20

Note: See note to Table 1

amino acids having a strong deterrent taste, makes a more intelligible role of this class compounds in regulating the final phase of feeding behavior of the fish, which is connected with determining the taste properties of the prey, its rejection and its acceptance.

A comparison of the amino acid taste spectra, determined by electrophysiological methods for almost 20 species of fish, reveals significant differences in the relative effectiveness of amino acids, and the different order of their arrangement in the series ranked by this parameter (Marui *et al.*, 1983b; Caprio, 1984, 1988; Ishida, Hidaka, 1987; Marui, Caprio, 1992; Hara, 1994). The information on the palatability of amino acids and some other substances obtained by testing behavioral methods is known for a smaller number of species. However a comparative analysis of these scanty data supports a conclusion on the specific nature of the taste spectra for fish. The specific differences are easy to reveal when comparing not only the spectra of the substances belonging to the group of stimulating substances, but also those which show neutral or deterrent properties. Among the stimulating and deterrent amino acids there was none that elicited a common response in carp, *Salvelinus alpinus erythrinus*, *Salmo trutta*, keta, Russian and Siberian sturgeons and starred sturgeons at the same time (Kasumyan, Sidorov, 1992, 1994a, 1994b; Kasumyan *et al.*, 1992; Kasumyan, Sidorov, 1995; Kasumyan, 1995). Taste responses of different fish to individual substances can be diametrically opposite. So, for example, cysteine and citric acid, being the most attractive for carp, show the strongest deterrent properties for keta. At the same time another substance, alanine, was the most palatable for both carp and keta, however alanine caused a deterrent response in the Siberian sturgeon. Calcium chloride enhancing the pellet consumption in carp and Caspian *Salmo trutta* resulted in the opposite effects in the Siberian sturgeon and starred sturgeon, but for *Salvelinus alpinus erythrinus* and keta this substance was neutral in taste. Undoubtedly, a peculiarity of taste spectra of the different fish indicated an important role of taste reception in ensuring feeding selectivity and feeding specialization in fish.

The role of taste and olfaction in fish behavior, including feeding behavior too, is different (Pavlov, Kasumyan, 1990; Atema, 1980). According to available data there are differences also in the substance spectra in terms of effectiveness in stimulation for the gustatory and olfactory systems of fish of the same species (Caprio, 1988; Marui, Caprio, 1992; Hara, 1994). The comparison of our results on the palatability of amino acids with previously obtained data on the behavioral effectiveness of amino acid solutions for the carp (Kruzhalov, 1986; Saglio, Blanc, 1983) shows that the responses to some of the amino acids are very different, thus supporting the existence of sensory specialization of the olfactory and gustatory systems.

In spite of intensively developed investigations in the field of taste reception of fish and other groups of animals, there is no clear concept explaining animals' preference to some tastes and their rejection of others. It is believed that the palatability of substances might be related to the features of the metabolic processes in the animal; with that, it is noted that this relation is very indirect (Kassil', 1972). According to our results, among the amino acids stimulating consumption of food there are none belonging to the group of the so-called essential amino acids, for which an increased nutrient requirement is observed in carp and in other fish (Millkin, 1982). At the same time, all the amino acids (except serine) unattractive for carp are among the essential acids. According to the general rules laid down on the basis of numerous observations on the feeding of different animals (Bronshstein, 1950; Kharborn, 1985) the phytivorous animals show a preference in sugars. Proceeding from these conceptions, an indifferent response of the carp to saccharose may be attributed to a carnivorous type of carp nutrition. At present it is difficult to connect the high palatability of citric acid and cysteine for the carp with features of its nutrition or metabolism.

The tasks of this investigation did not include any detailed study of the relationship between the structural peculiarities of amino acids and their gustatory effectiveness. Carrying out a comparative analysis of the effectiveness of amino acids becomes is complicated because of the fact that, because of their different water solubility, some of them underwent gustatory testing at different concentrations in the agar-agar pellets. Nevertheless it is possible to note some peculiarities, since a concentration of most of the amino acids (16 from 21) in the pellets was equal and amounted to 0.1 M. Thus, the polar (hydrophobic) and nonpolar (hydrophilic) amino acids were equally represented among the gustatory stimulating, deterrent and indifferent amino acids. Both acidic amino acids, glutamic and aspartic, were in the group of stimulating amino acids while the negatively charged, basic amino acids were either neutral (histidine and lysin) or unattractive (arginine). Among the stimulating amino acids there were no aromatic compounds, however there was proline, which has a molecule with a heterocyclic structure. The groups of amino acids of different palatability equally included amino acids with different numbers of carbon atoms in the molecule. So, the stimulating amino acids were not only those with three–four carbon atoms (cysteine, alanine, aspartic acid) but also the amino acids with five carbon atoms (proline, glutamic acid, glutamine). Among seven deterrent amino acids only two, threonine and serine, had four and three carbon atoms, respectively. The remaining deterrent amino acids had five–six carbon atoms and more. Thus, the obtained data do not support the idea based on the results of the electrophysiological investigation of the taste responses of channel catfish *Ictalurus punctatus*, that the most effective gustatory stimulants for fish are predominantly

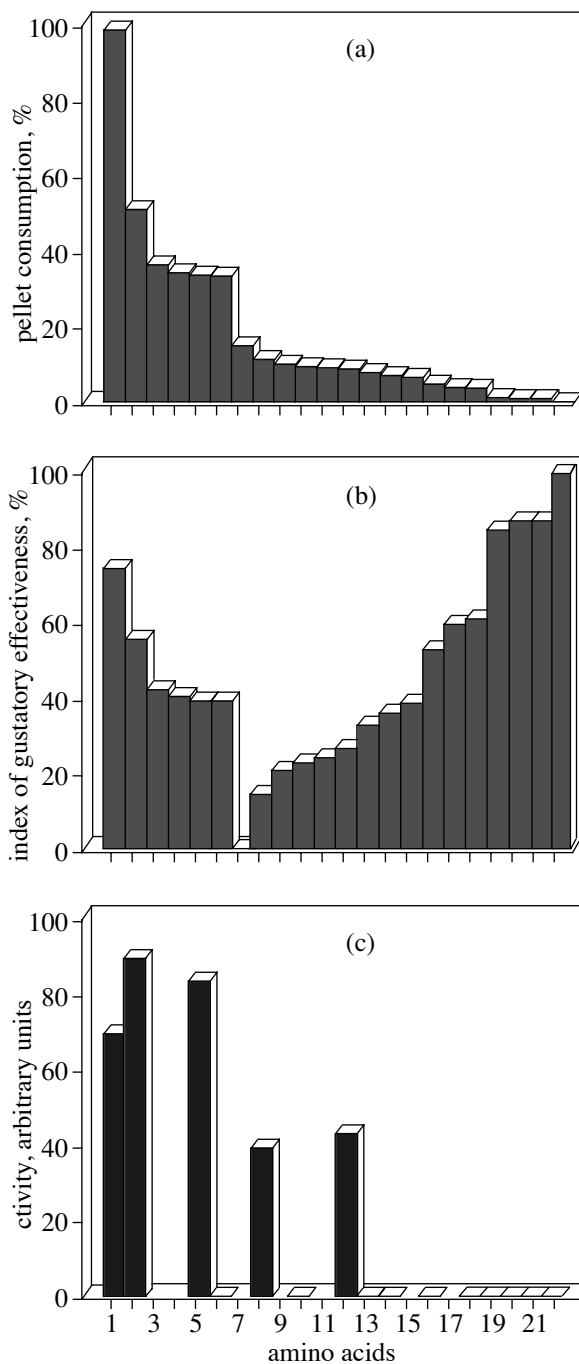


Fig. 2. Ranked series of free amino acids: a—their palatability; b—by index of gustatory effectiveness; c—value of activity induced in facial nerve of carp (according to data by Marui *et al.*, 1983b; arrows indicate the amino acids which were not tested).

1. cysteine, 0.1 M; 2. proline, 0.1 M; 3. glutamic acid, 0.01 M; 4. aspartic acid, 0.01 M; 5. alanine, 0.1 M; 6. glutamine, 0.1 M; 7. CONTROL; 8. histidine, 0.1 M; 9. lysin, 0.1 M; 10. leucine, 0.01 M; 11. tyrosine, 0.001 M; 12. glycine, 0.1 M; 13. asparagine, 0.1 M; 14. isoleucine, 0.01 M; 15. norvaline, 0.1 M; 16. tryptophane, 0.01 M; 17. arginine, 0.1 M; 18. threonine, 0.1 M; 19. methionine, 0.1 M; 20. phenylalanine, 0.1 M; 21. serine, 0.1 M; 22. valine, 0.1 M; Concentration of the stimulant solutions of free amino acids in the work by Marui *et al.*, 1983b is in mM.

uncharged amino acids with three–four carbon atoms (Caprio, 1978).

Previously the effectiveness of free amino acids for the carp gustatory receptors was assessed by a method of recording the responses in n. facialis and r. mandibularis (Marui *et al.*, 1983b). A comparison of these data with the results of the behavioral tests shows (Fig. 2) that the amino acids causing strong electrophysiological responses have, as a rule (except glutamine), attractive tastes for carp, while practically all the deterrent amino acids but serine were ineffective as stimulants in electrophysiological tests. Some of the amino acids neutral for the carp, glycine and histidine, caused noticeable electrophysiological responses. The effectiveness of the amino acids is considerably different in the electrophysiological and behavioral tests performed in the Arctic char *Salvelinus alpinus* (Hara *et al.*, 1993; Kasumyan, Sidorov, 1995) and rainbow trout (Adron, Mackie, 1978; Marui *et al.*, 1983a). At the same time, according to other authors' data, a strong correlation between the amino acid series ranked by gustatory effectiveness obtained by electrophysiological and behavioral methods is noted for rainbow trout (Jones, 1989) as well as channel catfish, Japanese eel, *Fugu pardalis*, *Chrysophrys major* (See review by Marui, Caprio, 1992).

The statistical calculations revealed a presence of a significant, positive correlation between the palatability of amino acids (pellet eating rate, %) and the intensity of the electrophysiological gustatory responses caused by them (Marui, *et al.*, 1983b) ($r=0.68$; $P < 0.01$). This correlation, as seen in Fig. 2, is determined by the stimulating amino acids. However any connection fails to be revealed ($r = -0.11$; $P = 0.05$) if the index of palatability (Ind_{pal}), estimated by the analogy with the index of electivity introduced by Ivlev (1977) for quantitative estimation of feeding selectivity, and used in the experimental investigations of taste reception and trophology of fish, is used for estimating the behavioral effectiveness of amino acids (Goh, Tamura, 1980; Takeda *et al.*, 1984; Gore, 1984). As shown in Fig. 1, an index of palatability well reflects the fish's response to taste. At the same time use of the absolute values of the index of palatability allows us to quantify the effectiveness of a taste substance without regard to the specifics of the behavioral response caused by it. We called this index an index of gustatory effectiveness of substance (Ind_{eff}). Using these relative indices for the estimation of fish's response to taste is, as we believe, a good practice, particularly providing that the responses in fish of the same species and age to the control group of pellets cannot be the same in the different series (See Tables 1, 3–5) (Kasumyan, Sidorov, 1992).

The absence of a connection between the amino acid palatability expressed by palatability index and the value of the induced electrophysiological responses (Marui *et al.*, 1983) can be caused by the fact that the induced activity was recorded in one of the facial nerve

rami (n. facialis r. mandibularis) innervating only those intraoral gustatory buds, which are in the front palate of carp. At the same time the other taste receptors located in the rear palate on the tongue, gill arches, and in the pharynx also participate in the process of gustatory testing the caught pellet. These gustatory buds are supplied by other facial nerve ramus, n. facialis and r. palatinus, as well as n. glossopharyngeus (IX), and n. vagus (X). The analysis of the electrophysiological and behavioral data suggests that in the carp the taste receptors responsible for sensing the different types of taste are located in the different zones of the oral cavity. Apparently, the response of rejecting the unpalatable pellets is based on the function of the taste receptors located in the rear of the oral cavity, the pharynx and the branchial arches innervated by the pairs IX and X of the cerebral nerves. The taste receptors at the front of the oral cavity, innervated by n. facialis r. mandibularis, are responsible for sensing the palatable substances, at least among free amino acids.

The existence of similar functional topography of the taste receptors is shown in humans, in which, in particular, sensing bitter substances is ensured by the gustatory buds located at the rear of the tongue, while the gustatory buds at the tip of the tongue respond to sweet and salty substances (Kassil', 1972; Sheperd, 1987). Perhaps, a vector of gustatory bud distribution in an oral cavity from the buds adjusted to sensing the palatable substances at the front of the oral cavity up to those removed from the entry of the oral cavity, responsible for the reception of deterrent substances, is common for all animals. The position of the gustatory buds of the "deterrent" type at the end of intraoral gustatory sensory canal undoubtedly has an exceptionally important role in the gustatory system in the feeding behavior of animals. This sensory system is, as I.P. Pavlov emphasized (1951), borders a definition as being of the external or internal environments of an organism, and is aimed toward the maintenance of its homeostasis. The investigations on sturgeons has demonstrated that the behavioral gustatory responses mediated by different gustatory buds (extraoral and intraoral) to the same substances can differ essentially, as well as with only some substances (citric acid, alanine) and can be completely opposite (Kasumyan *et al.*, 1991; Kasumyan, Kazhlaev, 1993; Kasumyan, Sidorov, 1994; Kasumyan, 1992).

Threshold concentrations were determined for the citric acid, calcium chloride and cysteine which had high level of palatability. For cysteine this concentration was 10^{-2} M, for citric acid 5×10^{-3} M and for calcium chloride, 0.9 M. Proceeding from the size of pellet and its volume, we calculated the absolute amount of the substance contained in it at the threshold concentration. For cysteine and citric acid these amounts were 4.27 and 3.39 μg , or 3.53×10^{-10} and 1.77×10^{-10} M. The content of such an amount of a highly effective substance in pellet is sufficient to cause a significant taste response in fish. Undoubtedly, in reality this

amount is far lower because only the external surface of the pellet comes in contact with the fish's gustatory receptors, while the substance part inside the pellet is unavailable to the gustatory receptors. With reference to *Lepomis macrochirus* it was previously found (Gerchart *et al.*, 1991) that presence of 2×10^{-6} M or 660 g active substance (deoxycorticosteroid) in food results in its significant gustatory rejection.

There are only several works using the behavioral testing methods which give the data on the determined levels of gustatory sensitivity in fish to the substances. A comparison of these data shows that the threshold concentrations for carp and other fish species are close. So, in the Caspian *Salmo trutta* the threshold concentrations of palatable amino acids are in the range of 10^{-2} – 10^{-3} M (Kasumyan, Sidorov, 1994b), in *Fugu pardalis* is 10^{-1} – 10^{-2} M (Hidaka, 1982). For the intraoral gustatory sensitivity of the Siberian sturgeon the threshold concentration of citric acid was 5×10^{-2} M, that of calcium chloride— 9×10^{-2} M and sodium chloride—1.7 M (Kasumyan, Kazhlaev, 1993). The lowest threshold concentration is determined in rainbow trout for proline— 10^{-4} M (Jones, 1989).

In the electrophysiological experiments the threshold concentrations of amino acids are by many orders of magnitude lower and reach 10^{-7} – 10^{-9} M and less (Marui *et al.*, 1983a, 1983b; Kiyohara *et al.*, 1981; Caprio, 1982, 1984). An abrupt discrepancy between the threshold concentration values determined by the behavioral and electrophysiological methods can be connected, as noted previously, with the functional interaction of intraoral gustatory and mechanosensory systems. In the process of gustatory testing the caught prey by fish not only the gustatory chemoreceptors and mechanoreceptors are stimulated. As is known, food texture has a certain importance in the feeding of fish and in their selection of food objects (Pavlov, Kasumyan, 1990; Atema, 1980; Hunter, 1980). Because of the increased toughness of external covering of the food organisms, the existence of spikes and barbs in them, or because of the excessive hardness of the artificial food pellets, they may be rejected by fish (Hunter, 1980; Lemm, 1983; Stradmeyer *et al.*, 1988). The intraoral chemoreceptors and mechanoreceptors of fish have a common somatotopic representation in the brain centers (Marui *et al.*, 1988; Kanwal, Caprio, 1988; Hayama, Caprio, 1990), however the features of the functional interaction of these sensory system are still inadequately studied. It is also out of question that a discrepancy between the threshold concentration values obtained in the behavioral and electrophysiological experiments could have been caused by the fact that, in the former case, a highly limited number of taste receptors was stimulated while in the electrophysiological investigations a significant part of gustatory receptive field was irritated. In the latter case a stimulant solution action itself is also essentially longer.

The study of dose-effect relationship, which we conducted with reference to two substances (See Fig. 1), showed that this relationship can have a form similar to a linear (cystedine) or parabolic (citric acid, Ind_{pal}) function in a semilogarithmic coordinate system. A clearly pronounced decrease in the substance effectiveness with increases in its concentration was not observed previously in the electrophysiological investigations (Marui, Caprio, 1992). At the same time, the experiments with the carp fingerlings from the current year and pigmented juvenile eel *Anguilla anguilla* indicated reducing a food palatability with increasing the content of some substances (sodium chloride, quinine-hydrochloride, citric acid) in food (Appelbaum, 1980).

When studying the traits of carp'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, cysteine and proline during the trial reached 15 or more seconds, which distinguishes the carp from such fish as the Caspian *Salmo trutta* or keta whose time of retention of the pellets in the oral cavity was some times shorter (Kasumyan, Sidorov, 1992, 1993, 1994b). It is obvious that this feature reflects an ecological specificity in carp feeding. In distinction from the Caspian *Salmo trutta* and keta the carp is typically bottom-feeder characterized by its consumption of a great amount of detritus along with food objects. As special investigations indicate (Sibbing *et al.*, 1986; Sibbing, 1991) the process of separating the food objects from detritus in benthophage carps is rather complicated and needs time.

Another feature of the taste response of carp is the number of repeated acts of catching the pellet, which is greater compared to that the number in the above mentioned salmonids (Kasumyan, Sidorov, 1992, 1993, 1994b). The carp chiefly inhabits the slow-flowing or stagnant waters. Under these conditions rejecting the food object in order to catch it again does not entail a great probability of losing the food because of its rapid drift downstream, as it occurs in the salmonid, which inhabits rivers with high speed of water flow.

One more distinction of the taste response of carp is a high positive correlation between pellet eating rate and time of their gustatory testing in the oral cavity. Unlike the Caspian *Salmo trutta* in which a positive correlation between these parameters of taste response was also revealed (Kasumyan, Sidorov, 1994b), in the carp the time of gustatory testing the most palatable substances was longest. One can suppose that the different time of gustatory identifying the palatable and deterrent substances by fish is connected with the biological consequences behind swallowing or rejecting the caught prey by animals. In the first case, if an animal makes a wrong decision the negative consequence will be by far more serious and dramatic. Swallowing a harmful food object can cause deep disturbances and

failures in metabolic processes, resulting in injury and sometimes death. Thus, the mass mortality of juvenile *Pagrus major* and *Engraulis japonicus* (White *et al.*, 1989) from eating the dinoflagellates *Gonyaulax excavata* containing toxins was observed. Apparently, just therefore the sure and correct palatable substance identification, accompanied, perhaps, by multiple repeated gustatory tests of pellets and the triggering of the reflex act of swallowing food based on this information requires more time. More rapid rejection of the food with deterrent taste properties can be explained from the positions of the theory of optimum foraging and minimizing unproductive time expenditure when feeding. However these suggestions are not universal, because, for example, a relationship between the time of gustatory testing the food and its palatability has a dependence differently in keta than in carp and Caspian *Salmo trutta* (Kasumyan, Sidorov, 1992). Additional investigations are needed to obtain clearer knowledge of regularities of gustatory responses of the fish having different feeding habits and needs.

Carp belongs to the traditional and important creatures of freshwater aquaculture. Its rearing is based on the broad use of artificial feeds. Our data can be used for increasing the palatability of feeds, carrying out the works for correction of their formulas by including special substances with highly stimulating effects, or by excluding the components containing deterrent compounds from the food composition. This will allow not only the reduction of direct losses of artificial feeds but also will ensure the more effective conversion of food for fish growth, because it is known (Takeda, Takii, 1992) that consumption of the chemosensory attractive feed is accompanied in fish by the more intensive secretion of digestive enzymes.

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SPELL: specificity, proline, serine, facialis, mandibularis, branchial, salmonid