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# GUSTATORY RESPONSE OF COMMON CARP *CYPRINUS CARPIO* TO VARIABLE CONCENTRATIONS OF TWO STIMULATORY AMINO ACIDS

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**ABSTRACT**

Common carp possesses a highly evolved gustatory system that is stimulated by a narrow range of free amino acids including L-cysteine and L-proline. A synergetic effect on the gustatory response of a combination of these two substances has not previously been demonstrated. In this study, groups of common carp were randomly presented with different concentrations of L-cysteine (0-0.1 M) and L-proline (0-0.05 M) in agar pellets in one minute trials. First retention duration, total retention duration during each trial and the number of ingestions (pellet acceptances) were recorded for each of a total of 690 trials. Palatability, pellet consumption rate and average pellet acceptances were calculated for each pellet type. It was shown that L-cysteine was more highly stimulatory than L-proline but no synergism between the two regarding the gustatory response was observed. The results are relevant for the formulation of aquafeeds and angling baits for carp.

## INTRODUCTION

Common carp *Cyprinus carpio* has experienced a long history of cultivation on several continents and is one of the species produced in the highest volumes with a total of 2.9 million tonnes cultivated worldwide (FAO, 2009). Additionally, the species is of importance for recreational fishing and a popular target for anglers particularly in Europe and more recently the United States (Rapp et al., 2008) where carp angling has become an economically important activity and a commercially attractive alternative for water bodies (Arlinghaus and Mehner, 2003).

The main objectives of the activities described above are the efficient cultivation and capture of the fish respectively. In order to optimize these, knowledge of the fish biology is of vital importance. Among different aspects of the biology of carp, the chemosensory system plays a particularly important role because it is connected to the regulation of the feeding behavior of the fish (Pavlov and Kasumyan, 1990). Although feeding behavior is influenced by both exogenous and endogenous factors (Lamb, 2001), an optimum feeding response depends in part on the ability of a fish to locate food, which is often a combination of visual and chemosensory methods (Kasumyan and Døving, 2003).

In both culture and in the wild, carp uses a system of chemoreception composed of olfaction and gustation to optimize the search for food. The structures of major importance in the gustatory response are the taste buds (Sibbing, 1988). Among teleosts, common carp has been shown to possess a particularly highly evolved chemosensory system due to the high number of taste buds present and their location on both the intra- and extraoral sur-

faces of carp (Devitsina, 2006). Due to its preference for waters with high suspended solids and the fact that it feeds both day and night on the bottom of its habitat, it is understandable that the chemoreceptory system should be more highly developed and used as the main way for the fish to find food using chemical signals.

Chemical signals from food are responsible for causing changes in carp movement, increased or decreased appetite and ingestion of greater or lesser quantities of food as a consequence (Atema, 1980), as is the case in other species (Kasumyan et al., 1995; Dias et al., 1997). According to the studies in this area (Kasumyan and Døving, 2003), it has been shown that important differences exist between substances regarding palatability and responses to stimuli among species (Kasumyan and Prokopova, 2001) and geographical location (Kasumyan and Sidorov, 2005). Among the substances studied in fish, the majority of these have included representatives of the four classical taste substances (sweet, salty, sour and bitter) and more recently umami (Roper, 2007). Other substances include organic acids (Kasumyan and Prokopova, 2001), nucleosides and nucleotides (Carr et al., 1996) and betaine (Barnard, 2006). However, regarding taste preference and palatability, amino acids have been shown to be particularly stimulatory in carp (Kasumyan and Morsi, 1996).

General findings in fish indicate that only $\alpha$-amino acids are highly stimulatory, that L-isomers are always more stimulatory than D-isomers and that the stimulatory effectiveness is not directly related to their essentiality (Kasumyan and Døving, 2003). A study by Marui et al., (1983) led to the classification of carp as a fish with a narrow range regarding the number of amino acids that cause positive stimulation. Responses to specific amino acids show no clear relationship with the natural diet of species of fish even though in the natural situation amino acids are important indicators of the presence of food (Carr et al., 1996).

The detection thresholds of fish species to different amino acids are highly variable and so are the intensities of responses to stimulation that have been reported using different methods e.g. olfactometer (Saglio et al., 1990) and impregnated agar pellets (Kasumyan and Morsi, 1996). Several other methods have been utilized for the experimental determination of fish preference to different taste stimuli including electrophysiology (Funakoshi et al., 1981).

Mixtures of amino acids have been shown to have a synergistic or antagonistic effect and combinations of several amino acids have resulted in increased stimulation in other species (Carr, 1976; Takeda, 1984). However, in common carp, with the exception of the study by Saglio et al. (1990) which involved amino acid mixtures to the olfactometer water flow and therefore evaluated olfaction rather than gustation, a gustatory synergistic effect of different concentrations of two highly stimulatory amino acids in food has not been demonstrated.

The incorporation of substances that have been shown to be stimulatory to the chemosensory system of carp is a possible way to increase feed intake and provide benefits for both aquaculturists and anglers. The economic importance of aquaculture is well known. Recreational fishing is a separate, huge industry in itself responsible for anglers spending US$ 42 billion in 2006 alone (U.S.F.W.S. 2006). The formulation of more attractive baits through greater knowledge of amino acid interactions and their relationship with the gustatory system of carp may lead to advances in the formulation of carp aquafeeds and various benefits for anglers, fishery owners, bait manufacture and the environment.

The goal of this study was to demonstrate the gustatory response of common carp to combinations of L-cysteine and L-proline in agar pellets and if possible show the existence of synergic effects of one or more combinations.

## MATERIALS AND METHODS

### *a) Fish and tank setup*

We conducted the experiment on 15 common carp of mean weight 92.1±12.0 g and mean length of 17.3±0.7 cm. The fish were captured using rod and line from Laguna Esmeralda, Región Metropolitana, Chile (S33°38′44″, W71°16′3″). Fish were transferred to the wet lab stock tanks and allowed to acclimatize for a two week period. Fish were maintained on a 12L:12D cycle with lights on at 8.00 a.m.

Following acclimatization, the fish were weighed, measured and transferred to 5 glass aquaria of 70 liters containing dechlorinated tap water in groups of three fish (mean TW 276.2 ± 1.7 grams). The bottoms of each tank were painted white externally. Filtration was achieved using a 1500 liter/hour matured biological filter (Atman, China) which provided water movement and oxygenation by way of a venturi. Water quality was monitored during the experiment. Mean dissolved oxygen was 8.9 ± 0.22 mg/L and mean temperature was 20.3 ± 0.78 °C. Total ammonia was kept below 0.22 ± 0.05 mg/L and mean pH was 7.6±0.11. Each aquarium included a hole in the cover glass through which fish could be fed. Fish were fed a carp diet manufactured on-site (44% protein, 29% carbohydrate, 18%

lipid) once per day at a level equivalent to 1% BW. This diet had the following wet weight composition: Soy protein isolate (380 g/kg), cornmeal (250 g/kg), fish oil (150 g/kg), egg albumin (190 g/kg), vitamin and mineral supplement (30 g/kg).

### *b) Pellet manufacture and amino acid content*

Pellets were manufactured from agar powder (Merck Chile, Santiago). To prepare the gel, 100 ml of water was boiled and agar added to it in a proportion of 5%. It was immediately agitated until it was completely dissolved with two drops of Ponceau R4 stain to give the mixture a red color. The correct quantity of pharmagrade L-cysteine and/or L-proline (Merck Chile, Santiago) was weighed using a precision balance and added to the mixture according to the proportions shown in Table 1. The mixture was then poured into a labelled petri dish to a depth of 8 millimeters. The procedure was repeated for the other amino acid combinations. All the mixtures were transferred to the refrigerator and left overnight to enable complete gelling.

After gelling had occurred, a plastic tube of 4 millimeter internal diameter was used to remove cylindrical-shaped agar pellets from each gel. The pellets had a generally soft texture and very slightly rubbery. Care was taken not to crush them when handled. The pellets were transferred to hermetic containers and kept refrigerated at 4 °C between trials. On each fourth day, a new batch of pellets was produced.

Although the trials were based on the experiment used by Kasumyan and Morsi (1996), some changes were made to the experimental design. Whereas other authors used farmed fish of 12-15 centimeters, we used larger fish because of the size of wild fish available at the time of the experiment. Carp are not farmed in Chile. The size of the agar pellets used was increased accordingly. Kasumyan and Morsi (1996) used pellets of 2.5 millimeters in length and 1.5 millimeters in diameter. We also used three carp per tank while other authors used individual fish for the trials. This was because common carp are shoal fish (Billard, 1999) and in our opinion, the presence of conspecifics more closely represented the natural situation.

Additionally, other authors used a control pellet without any amino acids incorporated. This was because they were evaluating both stimulatory and deterrent amino acids. In our case, since L-cysteine and L-proline had both already been established as stimulatory in earlier experiments and L-cysteine had already been found to be more stimulatory than L-proline, our control for comparisons between combinations and determination of synergistic effect was the pellet with the lowest concentration of L-cysteine (pellet %Cys:Pro 0:100).

### *c) Gustatory response trials*

Response trials began 15 minutes after the fish had received their daily food ration. One pellet type was randomly selected and a pellet added to the first tank. Several parameters were recorded: 1) the number of times that the pellet was ingested (captured) by the fish during a period of 60 seconds, 2) the length of time that the pellet was kept in the fish's mouth following initial ingestion, 3) the total duration of the pellet within the mouths of the fish for the 60 second trial, and 4) the consumption rate of pellets, meaning whether the pellet was swallowed or not during the trial. A digital stopwatch (Samsung, Japan) with a lap time function was used to register times and periods. The results of the visual observations were recorded on a spreadsheet. Following completion of the first trial, we continued with the other tanks in turn, returning to the first tank to carry out the second trial with another pellet type and repeating the sequence until each tank had received one pellet of each of the six types.

The percentage of eaten pellets was determined according to the total number of pellets swallowed compared with the total number presented for each pellet type. We also calculated the index of palatability using the same formula used by Kasumyan and Morsi (1996):

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

Table 1. Proportions of L-cysteine and L-proline included in each gel. All values are in M as concentrations of the agar gel

| Stimulant | % Cys:Pro | | | | | |
|---|---|---|---|---|---|---|
| | 200:0 | 100:0 | 75:25 | 50:50 | 25:75 | 0:100 |
| **L-cysteine** | 0.1000 | 0.0500 | 0.0375 | 0.0250 | 0.0125 | 0 |
| **L-proline** | 0 | 0 | 0.0125 | 0.0250 | 0.0375 | 0.0500 |
| **Total** | 0.1000 | 0.0500 | 0.0500 | 0.0500 | 0.0500 | 0.0500 |

Where $Ind_{pal}$ is the index of palatability of the substance (in this case, the combination of amino acids), R is the consumption of pellets with the substance (%), C is the consumption of control pellets (%).

The end of pellet retention in the oral cavity was determined by completion of mastication. For us to record swallowing, the completion of mastication had to occur within the 60 second period. Also, in the event of a pellet not being ingested by a fish during the 60 seconds, the trial was not considered in the consumption calculation. In other words, only pellets that were ingested at least once during the trial were considered in the calculation of consumption rate. Uneaten pellets were removed from the tank at the end of each 60 second trial.

### *d) Statistical analysis*

For the statistical analysis of the results we used the Statgraphics Centurion XV v. 15.1.02 (manufactured by StatPoint Inc., USA). The total number of trials was 690 over a period of 23 days.

For the calculation of average number of ingestions, average duration of the first ingestion and average total duration of ingestions in 60 seconds, we carried out two-tailed t-tests at the 95 and 99% confidence intervals between the control values and each of amino acid combinations.

## RESULTS

### *a) Ingested and non-ingested pellets*

The general results of the 690 trials are shown in Table 2 indicating the total number of trials carried out and the number of trials that resulted in ingested and non-ingested pellets for each pellet type.

Table 2. General results of the gustatory response trials for the six pellet types used

| Pellet (% Cys:Pro) | Parameters of taste response | | |
|---|---|---|---|
| | Total trials carried out | Non-ingested pellets | Number of trials with ingested pellets |
| **200:0** | 115 | 5 | 110 |
| **100:0** | 115 | 7 | 108 |
| **75:25** | 115 | 7 | 108 |
| **50:50** | 115 | 9 | 106 |
| **25:75** | 115 | 10 | 105 |
| **0:100** | 115 | 14 | 101 |
| **Total number (%)** | 690 (100) | 52 (7.5) | 638 (92.5) |

Of the total number of 690 trials, 7.5% of trials (52) resulted in the pellets not being ingested at any time during the 60 second periods. The number of non-ingested pellets increased as the concentration of cysteine in the pellet decreased and the concentration of proline in the pellet increased. The highest rate of non-ingested pellets was for the pellet absent of L-cysteine and containing only L-proline, corresponding to 12.2% of the total number of pellets used.

### *a) Number and duration of ingestions per trial*

The total numbers of ingestions for all the trials using each pellet type are shown in Table 3. Also shown are the average number of ingestions calculated by dividing the total number of ingestions by the number of trials where ingestion occurred. For the average number of ingestions, the average duration of the first ingestion and the average total duration of ingestions, values that are significantly different from those of the control are expressed using superscript.

With the exception of pellet type 100:0, both, the total number of ingestions and the average number of ingestions per trial, decreased in a directly proportional manner with increased concentration of L-cysteine and reduced concentration of L-proline in the pellet. The type 100:0 pellet was t-tested and shown not to be significantly different to the 200:0 and 75:25 pellets regarding average number of ingestions per trial. The highest number of ingestions per trial was found to be 12, corresponding to both the 0:100 and 25:75 pellets. The lowest value for the highest number of ingestions per trial was 5 for the 100:0 pellets. All the pellets were found to be significantly different from the control at the 99% confidence level regarding the average number of ingestions per trial.

The highest retention time for the average first ingestion was for that of the 100:0 pellet. The retention times for the three pellet types with the highest concentrations of L-cysteine and lowest concentrations of L-proline (200:0, 100:0 and 75:25) were significantly higher than those of the other pellets. The lowest average first retention was for the pellet with the highest concentration of L-proline (control). In general, the average first retention time decreased with decreasing incorporation of L-cysteine and increasing incorporation of L-proline, although there is a significantly greater difference between the reaction of carp regarding the initial ingestion of the 200:0, 100:0 and 75:25 pellets compared with those of the 50:50, 25:75 and 0:100 pellets.

A similar pattern can be observed with the results of average total duration of pellets in the oral

Table 3. Total and average number of ingestions, average duration of first ingestion and average total duration of ingestions for each pellet type, showing significant differences where appropriate.

| Pellet (% Cys:Pro) | Parameters of taste response | | | |
|---|---|---|---|---|
| | Total number of ingestions | Average number of ingestions per trial | Average duration of first ingestion (secs) | Average total duration of ingestions per trial (secs) |
| **200:0** | 191 | 1.74[b] | 34.70[b] | 47.46[b] |
| **100:0** | 174 | 1.61[b] | 39.11[b] | 45.29[b] |
| **75:25** | 208 | 1.93[b] | 32.65[b] | 45.34[b] |
| **50:50** | 246 | 2.32[b] | 24.90 | 35.92[a] |
| **25:75** | 234 | 2.23[b] | 24.94 | 37.79[b] |
| **0:100 (control)** | 329 | 3.26 | 19.52 | 29.64 |
| **Total** | 1382 | - | - | - |

[a] significantly different at 95% confidence level with respect to the control
[b] significantly different at 99% confidence level with respect to the control

cavities during 60 seconds. The duration is shorter for lower L-cysteine and higher L-proline incorporation. All pellets show average total durations that are significantly different from those of the control and four out of five pellets are significantly different at the 99% confidence level. In all three parameters measured, the pellet without L-cysteine indicated the lowest taste preference of carp.

### *c) Pellet consumption*

The consumption of pellets followed a similar trend to the previous parameters. Pellet consumption, as a percentage of the total number of pellets ingested, decreased from 82.7% in the case of the 200:0 pellet to 41.6% in the case of the control pellet. However, the relationship between amino acid combination (expressed as L-cysteine concentration) and consumption rate was only partially linear, as can be seen in Figure 1.

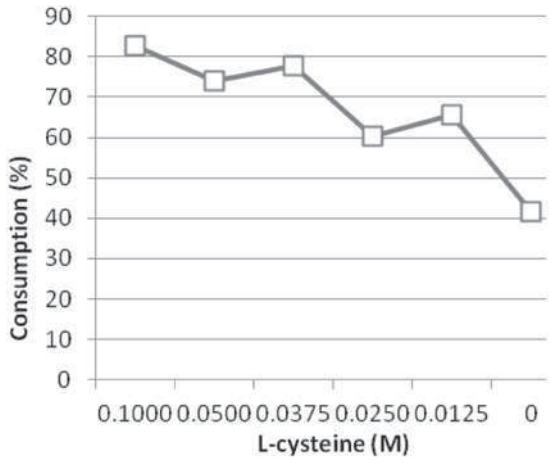


Figure 1. Relationship between L-cysteine concentration of pellets and consumption rate.

### *d) Palatability*

Palatability was determined using the formula presented by Kasumyan and Morsi (1996). The results are shown in Table 4.

Table 4. Palatability indices of the pellets compared with the control pellet (0:100)

| Pellet (% Cys:Pro) | Palatability index (%) |
|---|---|
| **200:0** | +33,1 |
| **100:0** | +28,1 |
| **75:25** | +30,3 |
| **50:50** | +18,4 |
| **25:75** | +22,5 |
| **0:100** | 0 |

Palatability followed the same tendency shown in Figure 1 for consumption rate. The lowest value apart from the control was that of the 50:50 pellet, whereas the highest palatability was that of the 200:0 type pellet which contained the highest concentration of cysteine of all the pellets used.

## DISCUSSION AND CONCLUSIONS

Cysteine and proline have received attention in studies on attraction and taste preference in carp and both have been found to show high stimulatory effectiveness in the species using electrophysiological methods (Marui et al., 1983) and impregnated agar pellets (Kasumyan and Morsi 1996). Invasive electrophysiological methods have also been used on other species (e.g. rainbow trout *Oncorhynchus mykiss*, Yamashita et al., 2006). Non-invasive methods appear to be more effective than invasive techniques in evaluating stimulatory substances on the chemosensory system with the use of pellets caus-

ing intraoral rather than extraoral responses that can be measured using behavioral techniques. The methods used in this study were effective in showing the gustatory rather than olfactory response to the two free amino acids used and the sensitivity of that response to different concentrations of those substances.

Based on the trials carried out here, it is clear that gustatory system of carp is especially sensitive to amino acids. This has been demonstrated previously with several species of fish including cyprinids (Kasumyan and Døving, 2003). Goh and Tamura (1980) showed that the amino acid spectrum of fish able to cause stimulation is highly species specific. Marui et al. (1983) found that, due to the limited number of amino acids that the authors found to be stimulatory, the taste receptors in carp showed a more narrowly-tuned chemical spectrum than the olfactory system in the same fish. In that study, the authors determined that these were proline, alanine, cysteine, glutamate and glycine. In addition, betaine (trimethylglycine) was considered to be stimulatory. A later study by Saglio et al. (1990) showed the effect of different combinations of amino acids on attraction and exploration by carp. In this study, basic amino acids (histidine, arginine and lysine) and polar, uncharged amino acids (glycine, serine, threonine, tyrosine, asparagine and glutamine) did not cause attraction but did increase exploration by the fish. Acidic amino acids (aspartic and glutamic) did not produce significant activity. However, non-polar amino acids (alanine, valine, leucine, isoleucine, phenylalanine and methionine) caused significant effects on both attraction and exploration. Also, different pairs (combinations) of acid groups were mixed and tested and the most effective of these were shown to be a combination of non-polar and polar uncharged amino acids with the simplest combination being alanine, valine and glycine.

Proline is a neutral and non-polar amino acid and cysteine is neutral and slightly polar. Although a combination of these two amino acids was used in our study, none of the concentration combinations were more effective than L-cysteine alone. In the cases of pellets containing both amino acids (75:25, 50:50, 25:75), with a reduced concentration of L-cysteine and increased concentration of L-proline, a lower rate of pellet consumption and lower first and total retention times during the trials were observed.

Other species have been found to be stimulated by a greater number of amino acids. For example, in a review by Kasumyan (1997) the results of oral taste responses of a series of experiments with a total of 21 fish species indicated that the number of stimulant amino acids ranged from 0 to 13. Even between closely related species, there can be considerable differences in preferences. In the same series of experiments, tench (*Tinca tinca*), a closely related cyprinid, was found to be stimulated by 12 different amino acids including cysteine and proline and deterred by none, whereas common carp was shown to be stimulated by 6 amino acids (cysteine, proline, glutamic acid, aspartic acid, alanine and glutamine) and deterred by 7. Among the amino acids which most frequently acted as stimulants for these fish are L-alanine, L-cysteine and L-serine. These studies show that amino acids are more often indifferent taste substances than they are stimulatory.

Palatability in common carp has been evaluated for a range of taste substances including amino acids (Hara, 2006). Although dissolved amino acids have been shown to elicit a stimulatory gustatory response via the water in contact with the fish through olfaction (e.g. Saglio et al. 1990), some studies have demonstrated the stimulatory capacity of both cysteine and proline to cause increased consumption of pellets and increased retention time through their action by direct contact with intraoral taste buds (Kasumyan and Morsi 1996) and to cause locomotor activity and searching behavior as well as "pecking" in some cyprinids (e.g. goldfish, Hara 2006). The study by Kasumyan and Morsi (1996) was particularly extensive regarding the number of amino acids tested in common carp and palatability was determined for a total of 21 amino acids plus the classic taste substances. L-cysteine and L-proline (both 0.1 M) were determined to have palatabilities of 74.1% and 55.7%, respectively and consumption rate of pellets of 99.0 ± 1.0% and 51.6% ± 5.2%, respectively. In our case, we compared palatability and consumption rate not with a control absent of amino acids but instead with the pellet with the lowest concentration of L-cysteine and the highest concentration of L-proline, which, we were correct in supposing, would result in the lowest stimulation and act as an effective control. This explains our relatively lower palatability indices. The palatabilities of cysteine:proline mixtures increased as the concentration of L-proline was reduced and that of L-cysteine increased, with the pellet with the highest concentration of L-cysteine (200:0, 0.1M) resulting in the highest consumption rate, palatability and average total retention times per trial and the lowest number of non-ingested pellets. Kasumyan and Morsi (1996) used the same concentration of cysteine and found pellets containing L-cysteine alone to show the highest consumption rate, palatability and first retention duration but not the

longest average total retention time per trial, which was found to be proline in their case. However, Kasumyan and Morsi (1996) found the average number of ingestions (referred to as "catching acts" by the authors) to be 4.9 for proline compared with 1.0 for cysteine. We found something similar with an average number of 3.26 for proline and 1.74 for cysteine (200:0, 0.1M).

Threshold levels have similarly been examined for gustatory system of common carp using electrophysiology (e.g. Marui et al. 1983) and agar pellets (Kasumyan and Morsi 1996) for both L-cysteine and L-proline. Marui et al. (1983) found proline to be the most effective stimulus tested, averaging $10^{-8.5\pm0.9}$ (S.D.) M. Thresholds for other stimulatory substances were in the range of $10^{-4} - 10^{-8}$ M, with that of cysteine to be $10^{-4.8\pm0.4}$ (S.D.) M. These were obtained by adding the test substances to the water flowing through the mouth and out of the gills of paralyzed carp and the stimuli produced by each substance recorded. Averaged gustatory neural activity was shown to increase with logarithmic increase in stimulus concentration over a wide concentration (6-7 log units). The authors also indicated a tendency for saturation at concentrations equal to or above $10^{-3}$ M.

Kasumyan and Morsi (1996) determined threshold concentrations L-cysteine. For cysteine, the concentration at which the consumption of pellets significantly exceeded the level of the control pellet consumption was found to be 0.01 M, or $10^{-2}$ M. According to the size of the pellet used in this study, the absolute amount of the substance contained in it was 4.27 μg or $3.53 \times 10^{-10}$ M. However, they suggested that in reality, the amount is much lower because only the external surface of the pellet comes in touch with the gustatory receptors of the fish and is therefore responsible for acceptance or rejection of the pellet. In our study, the larger size of pellet used meant that the amount of L-cysteine that it contained was 22.8 times higher than the highest concentration used (0.1 M), which meant an absolute amount considering the volume and not only the surface of the pellet of 97.4 μg or $8.05 \times 10^{-9}$ M.

Examples of synergistic gustatory responses have been observed in other species, for example, in pigfish (*Orthopristis chrysopterus*). A synthetic shrimp extract was manufactured from 19 amino acids and was found to be as stimulatory as a natural shrimp filtrate (Carr, 1976). In European eel (*Anguilla anguilla*), a mixture of neutral and acidic amino acids and neutral amino acids were shown to be the most palatable combination (Mackie and Mitchell, 1983). Also, synergistic effects were observed when 20 amino acids were mixed with 6 nucleotides and presented to jack mackerel (Ikeda et al., 1988).

Despite the importance of common carp for both aquaculture and recreational angling, little is understood regarding synergistic effects of using more than one amino acid in a feed or bait. According to the results obtained in this study, L-cysteine is clearly more stimulatory than L-proline and decreasing the amount of L-cysteine in a diet or bait would reduce the stimulatory effects of the amino acid. Replacing the concentration of L-cysteine with L-proline reduces rather than enhances the stimulatory response. The opposite is true if amounts of L-proline were to be replaced by L-cysteine. Here, the preference for L-cysteine over L-proline was demonstrated by the reduced number of ingestions (fewer rejections) of pellets with higher concentrations of L-cysteine and corresponding longer retention times and consumption rate for these combinations.

It has been suggested that the inclusion of free amino acids that are not stimulatory for a species may mask the stimulatory effects of the amino acids or other substances that are (Kasumyan and Døving, 2003). In culture as in the wild, carp uses a system of chemoreception to optimize the search for food and so the taste buds are of major importance (Sibbing, 1988). Carp uses the chemosensory system as its principal way of locating food and making decisions with regard to its handling. Its preference for turbid waters containing high levels of suspended solids and the fact that it feeds both during the day and at night would suggest that carp depends greatly on chemoreception rather than alternative methods of food location such as visual (e.g. bass and trout), electrodetection (e.g. sharks and rays) and mechanoreception (e.g. eels) (Kasumyan and Døving, 2003). It is understandable that the chemosensory systems should be more highly developed and evolved and used as the main way for the fish to find food using chemical signals as previously demonstrated (e.g. Sibbing, 1988). This has been attributed to both the structure (Toyoshima et al., 1984) and distribution and density of taste buds which are present both intra- and extraorally in high densities particularly on the barbels of the fish and on the palatal organ (Devitsina, 2006). The taste buds in these regions are responsible not only for determining the palatability of the food but also its acceptance or rejection, also aiding in the separation of food from non-food before posterior processing occurs (Callan and Sanderson, 2003).

Chemical signals from food items are responsible for changes in carp movement, increased or decreased appetite and greater of lesser amounts of food as a consequence (Atema, 1980). According to studies in this area, it has been shown that important differences exist between substances regarding

palatability and responses to stimuli (Kasumyan and Døving, 2003). This is relevant for both carp cultivation and angling. It is in the interest of the feed producer to provoke a positive response in the way of increased food acceptance and consumption as well as higher growth as a result. In the case of bait manufacture, a positive stimulus will result in a higher probability of bait ingestion and subsequent capture of the target fish. For these reasons it is important to know which substances and combinations are the most effective to elicit the most stimulatory response.

The results of studies with taste substances such as amino acids and organic acids (Kasumyan and Prokopova, 2001) have shown high variability between even closely related species. The evidence also suggests that the gustatory response to different substances is genetically specific with low plasticity. This is supported by studies between different geographical locations of fish (e.g. brown trout *Salmo trutta*, Kasumyan and Sidorov, 2005) and comparative analysis between species living in the same body of water and sharing the same food sources in cyprinids (Giles et al., 1990; Adámek et al., 2003) and salmonids (Ringler, 1985; Bridcut and Giller, 1995).

It is particularly interesting that different fish species display extremely different responses to specific amino acids. In the natural situation, amino acids are important indicators of the presence of food (Carr et al., 1996). However, there seems to be no clear relationship between the amino acid profiles of natural diets of fish species and the responses of those species to specific amino acids. Neither is there a clear relationship between trophic classification (in the case of common carp, omnivore) and the response to different amino acids in the way of active preference or rejection (Kasumyan and Døving, 2003). This would suggest that each species possesses a positive and negative preference for each substance and it is therefore necessary to carry out research with each species and not by family or trophic classification.

Recent research has examined the gustatory response at a higher organizational level (Døving et al., 2009). Greater comprehension of the mechanisms which determine acceptance by fish may lead to increased food consumption, lower feed loss and reduced environmental impact in aquaculture.

The results of this study provide evidence that common carp is able to distinguish between low concentrations of stimulatory amino acids and express preference between combinations of each. No evidence was found that L-cysteine and L-proline act synergistically in the gustatory response produced in the species.

It remains to be shown if synergism can be demonstrated using a small number of stimulatory amino acids in the species.

*Sažetak*

## OKUSNA REAKCIJA ŠARANA *CYPRINUS CARPIO* NA PROMJENJIVE KONCENTRACIJE DVIJU STIMULATIVNIH AMINOKISELINA

Šaran ima visoko razvijeni okusni sustav kojeg stimulira uski raspon slobodnih aminokiselina uključujući L-cistein i L-prolin. Sinergijski učinak kombinacije ovih dviju tvari na okusnu reakciju nije ranije dokazivan. U ovoj studiji različite koncentracije L-cisteina (0-0,1 M) i L-prolina (0-0,05 M) nasumce su davane testnim skupinama šarana u peletama agara tijekom jednominutnih pokusa. Trajanje prvog zadržavanja i ukupno trajanje zadržavanja tijekom svakog pokusa te broj gutanja (uzimanja) pelete zabilježeni su za svaki od ukupno 690 testova. Za svaki tip pelete izračunata je okusna reakcija, broj konzumacija i prosječno prihvaćanje od strane šarana. Pokazalo se da L-cistein znatno više stimulira konzumaciju od L-prolina te između njih nije uočen sinergizam. Rezultati su važni za pripremanje riblje hrane i ribičkih mamaca za šarane.

**Ključne riječi:** šaran, aminokiseline, L-cistein, L-prolin, okusni sustav

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